# Characterising trait anxiety in the common marmoset (*Callithrix jacchus*):

Investigations into behavioural, psychophysiological and cognitive phenotypes

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*To my grandparents,*

*Tashichi and Fumi Shiba*

## Preface

The following work was carried out at the Department of Physiology, Development and Neuroscience, University of Cambridge, during the years 2008 to 2012, under the supervision of Dr Angela C. Roberts.

I hereby declare that this dissertation has not been submitted, in whole or in part, for any other degree, diploma or qualification at any other university. This dissertation is the result of my own work and includes nothing that is the outcome of work done in collaboration. I have attempted to reference appropriately any idea or finding which is not my own.

This dissertation does not exceed the limit of length specified by the Degree Committee of Biology, as stated in the Memorandum to Graduate Students.

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## Abstract

The major aim of my thesis project has been to develop a non-human primate model of trait anxiety, using a new world monkey, the common marmoset. The first step was to identify animals high or low in trait anxiety. Based on the findings that high trait-anxious individuals display over-generalization of fear responses, a pathogenic marker of elevated trait anxiety in humans, a new aversive discriminative conditioning paradigm was designed. Testing a normal cohort of marmosets revealed that 26% of the animals displayed both behavioural and physiological signs of fear generalization, i.e. failure to discriminate safety from danger cues ('failed' group). The remaining 74% showed successful discrimination ('passed' group). Additional regression analysis on several behavioural and physiological responses early in training revealed two potential biomarkers of high trait anxiety in marmosets: suppressed baseline blood pressure, indicative of contextual effects, and hyper cue-specific vigilance. These measures predicted the animal's likelihood of passing or failing the discrimination. The finding that the 'failed' group showed intact discriminative performance in the appetitive domain rules out an interpretation of the results in terms of a general impairment in learning, per se. To further determine whether these hypothetically high trait-anxious animals would display enhanced anxiety-related responses in more classical primate models of anxiety, human intruder and rubber snake tests were performed on a large sample of marmosets. Principal component analysis on multiple behavioural measures revealed two components underlying performance: 'emotionality' and 'coping strategy'. Although no difference was found in the human intruder test, the 'failed' group displayed significantly elevated levels of 'emotionality' in comparison to the 'passed' group in the rubber snake test. Moreover, the two biomarkers of fear over-generalisation also reliably predicted the 'emotionality' scores. Finally, having developed a marmoset model of trait anxiety, investigations into the neural underpinnings, especially prefrontal involvement in trait anxiety mechanisms, were carried out by testing the animals on two cognitive flexibility tests: an orbitofrontal cortex (OFC)-dependent incongruent object discrimination test and a lateral prefrontal cortex (lPFC)-dependent detour reaching rule transfer test. Whilst group differences did not reach significance, the two biomarkers of fear over-generalisation, the suppressed baseline blood pressure and hyper cue-specific vigilance, were inversely and differentially correlated with perseverative performance on the two tests, the lPFC- and OFC-dependent tests, respectively. This not only indicates that high trait anxiety can lead to improvements in certain aspects of prefrontal cognitive function but also suggests that changes in the activity of at least two distinct prefronto-subcortical neural circuits, a cue-sensitive amygdala-OFC and a context-sensitive hippocampus-lPFC circuit, may contribute to trait anxiety.

## Contents

# Chapter 1

## General introduction

Have you ever wondered why you are taller or shorter than your siblings? Why the colour of your eyes, hair and skin differ from your friend's? And, why you are so shy while your friend is much more outgoing? These characters that make you unique among fellow humans are called traits. A global definition of traits encompasses a wide range of biological factors and their related processes, from molecular elements to behavioural patterns. You are born with a unique set of genes. The genetic makeup of an individual consists of unique combinations of distinct variants of specific genes. When one talks about an individual's genotype or genetic trait, it usually refers to the specific variant of a particular gene that the individual is carrying. Genes give rise to proteins that are involved in an organism's internal biochemical processes. Individual's biochemical processes differ from those of others depending on his/her genotypic traits; therefore, these unique biochemical mechanisms are also part of the trait. The expression of genes and biochemical processes are under the influence of unique environmental pressures to which an individual is subjected. Through cascades of biochemical operations occurring at cellular to system levels, this gene × environment interaction eventually results in observable differences in structure, function and behaviour of an individual, that is called a phenotypic trait or phenotype. Therefore, phenotypic traits not only refer to visible body parts such as height and eye colours but also mental functions such as emotion and cognition.

Psychological traits are defined as "generalized and personalized determining tendencies—consistent and stable modes of an individual's adjustment to his/her environment" and have been used to characterise individual differences in personality (H. Eysenck, 1991; John & Srivastava, 1999). Personality psychologists have been trying to identify such traits through lexical searches (classifying adjectives describing personality), experimental approaches (mainly using personality questionnaires) and statistical techniques (factor analysis). One of the most influential personality models is the big-five trait taxonomy, which identifies five major bipolar dimensions that compose a personality. These are 1) extraversion vs. introversion, 2) agreeableness vs. antagonism, 3) consciousness vs. lack of direction, 4) neuroticism vs. emotional stability, and 5) openness vs. closedness to experience (John & Srivastava, 1999). The neuroticism dimension reflects negative emotionality and is associated with anxiety, anger, depression, shyness and impulsiveness, which have been subjects of interest especially among psychiatrists and neuroscientists. Trait anxiety is a related facet of neuroticism (M. Eysenck, 2000). Since trait anxiety and neuroticism are highly correlated with each other (H. Eysenck, 1991; Hansell et al., 2012), these terms are often used exchangeably; though the term trait anxiety tends to appear more frequently in

neurobiology and genetic literatures whilst neuroticism is favoured in the contexts of personality psychology.

Trait anxiety has attracted interests of psychiatrists and neuroscientists mainly because it is considered to be a risk factor for developing mood/affective disorders, especially anxiety disorders. Considerable evidence has been accumulated that demonstrates the association between elevated levels of trait anxiety and vulnerability to anxiety disorders (M. G. Calvo & Cano-vindel, 1997; Susan Mineka & Zinbarg, 2006; Sandi & Richter-Levin, 2009). Recent research has focused on the underlying mechanisms of trait anxiety. As explained above, visible phenotypes are the end-products of complex interactions between the internal and external systems, occurring from molecular level to the systems level. At each step, individuals differ from one another; therefore, each process represents a unique trait. Unravelling the complex mechanisms that are eventually manifested as individual differences in trait vulnerability to anxiety will require considerable devoted efforts from numerous scientists in the fields of genetics, biochemistry, pharmacology, neurobiology, neuropsychology and behavioural neuroscience, but the implications will have multiple significance for human society. At a clinical level, the obtained information can be used to develop more effective pharmacological and non-pharmacological treatment, e.g. cognitive behavioural therapy, more accurate diagnostics for the classification of anxiety disorders, and improved preventative measures for those that are identified as vulnerable to anxiety disorders. At an educational level, understanding the environmental impacts on vulnerability to anxiety benefits school teachers and parents so that they can create not only preventative but also optimal environments for the better development of children at schools and in the home. Understanding individual differences in a broader sense, that is, “what makes one differ from others” may also provide insights into the fundamental philosophical question of personal identity and self.

Although investigations into the neural mechanisms of human trait anxiety have benefitted from recent development and refinement of neuroimaging technologies, spatial and temporal resolutions have not yet been as accurate as to clearly elucidate the exact neural circuits involved. It must also be remembered that such studies are correlative and do not demonstrate cause and effect. On the other hand, studies in experimental animals can help us to determine cause and effect and identify the neural systems that underlie trait anxiety. Indeed, much of our knowledge about the mammalian neural systems that are involved in processing threatening stimuli and expressing fear/anxiety responses have stemmed from research in animals, especially rodent models (Fendt & Fanselow, 1999; P. J. Lang, Davis, &

Ohman, 2000; Millan, 2003). Granting that understanding human anxiety is the goal of scientific investigations, animal models are empowered by empirical advantages such as controllable environmental conditions and use of pharmacological and neurobiological manipulations. Non-human primate models, because of their phylogenetic closeness and morphological similarity to humans, are expected to serve as a bridge, making the basic findings in rodent models applicable to the observations in humans. The possession of a well-developed prefrontal cortex that is implicated in higher order executive functions makes non-human primates particularly valuable in the investigation into the neural systems that underlie the regulation of fear and anxiety. Thus, the major aim of my research project, described in this thesis, has been to develop a non-human primate model of trait anxiety, using a new world monkey, the common marmoset.

## 1.1 Trait Anxiety: Definition

The concept of trait anxiety was originally suggested by Freud (Freud, 1921) who distinguished between neurotic (trait) anxiety and objective anxiety. According to psychoanalytic theory, trait anxiety is a signal that unconscious material is threatening to enter consciousness. When childhood Oedipal conflicts and associated sexual guilt are unresolved, a large portion of one's psychotic energy is committed to repressing these traumas. With little psychic energy left for everyday functioning, many situations pose a threat of a breakdown in defences. Anxiety is created to signal the threat of a defence breach; that the person is in danger of re-experiencing a repressed psychological trauma. The result is a general tendency to perceive threat and respond anxiously to many stimuli. In contrast, objective anxiety is an adaptive response to realistic dangers, such as a physical attack or a robbery.

In order to develop more objective measurements of anxiety, Spielberger (C. D. Spielberger, 1985; C. Spielberger, 1975) defined trait anxiety from a more operational point of view. His trait-state anxiety theory distinguishes between state and trait anxiety. State anxiety is defined as "a transitory emotional state or condition characterised by subjective feelings of tension and apprehension and activation of the autonomic nervous system. It varies in intensity and fluctuates over time as a function of the amount of stress that impinges upon an individual." On the other hand, trait anxiety refers to "individual differences in anxiety proneness as a relatively stable personality trait. It is not necessarily directly manifested in behaviour, per se, but may be inferred from the frequency that a person experiences elevation in state anxiety over time. Persons who are high in trait anxiety are more vulnerable to stress and respond to a wider range of situations as dangerous or threatening, often, but not necessarily with greater intensity of state anxiety." Whilst state anxiety is an observable response, trait anxiety cannot directly be observed but is manifested as state anxiety when stress is experienced. Trait anxiety is the idea that future anxiety propensities can be inferred from past experiences, by the assumption of a continuity in the frequency and the intensity of anxiety behaviour from past to future.

Based on this conceptual hypothesis, Spielberger (C. D. Spielberger, 1985) developed the state-trait anxiety inventory, which consists of two, 20-item self-report scales for measuring state and trait anxieties. Items for trait anxiety include scales for somatic anxiety, fear, shyness, worry, lack of self-confidence and sadness. These are proposed as measurable

components of anxiety proneness. The inventory is one of the most widely used psychometric instruments in basic, applied and clinical anxiety researches. In addition to these conceptual formulations, examining the aetiological basis of state and trait anxiety from a behavioural genetic approach has also further validated the distinction between these two aspects of anxiety. By testing a large sample of twins on the state-trait anxiety inventory and comparing genetic and environmental sources of influence, Legrand and colleagues (Legrand, McGue, & Iacono, 1999) showed that trait anxiety symptoms were moderately heritable and partly influenced by individual specific environments whilst state anxiety was largely influenced by both non-shared and shared environmental factors. Another twin study (Lau, Eley, & Stevenson, 2006) also reported a large environmental influence on state anxiety whilst moderate genetic and individual specific environmental effects on trait anxiety.

The concept of trait anxiety as an enduring psychological entity is consistent with an evolutionary perspective, according to which the anxiety response is part of an evolved defense mechanism that helps organisms to keep away from dangerous situations (Marks, Nesse, & Arbor, 1994). As an adaptive response protecting against survival threats, anxiety should mobilize most of ones metabolic, behavioural and mental resources in a coordinated manner, to provide energy and prepare for vigorous and potentially hazardous actions. Four ways in which anxiety can provide protection have been proposed: 1) Escape (flight) or avoidance (pre-flight) involve individuals creating or maintaining distances from potential or specific threats; 2) Aggressive defense (anger, clawing, biting or spraying with noxious substances) is an attempt to forcibly remove the source of the danger; 3) Freezing/immobility may benefit individuals in a) locating the source of threat or assessment of the danger, b) concealment, and c) inhibiting the predator's attack reflex; and 4) Submission/appeasement can be useful in intra-group conflicts (Marks et al., 1994). Considering that these defensive responses are essential for survival, it is plausible that the psychological function (i.e. anxiety) and related neural mechanisms driving the behaviours have been shaped and maintained through natural selection as a heritable trait.

In English, a word "fear" is also used to describe negative emotion or apprehensive feeling when one is under threat. In everyday language, anxiety and fear are often used as synonyms. In fact, one of the definitions given to fear in the Oxford English Dictionary is "a feeling of anxiety concerning the outcome of something or the safety of someone." Even in scientific literatures, many researchers conceptualize anxiety and fear as largely or entirely interchangeable (Barlow, 2001; Sylvers, Lilienfeld, & LaPrairie, 2011). However, there have been some attempts to differentiate the two psychological concepts. A recent meta-analysis

(Sylvers et al., 2011) suggested several factors that may differentiate between trait anxiety and trait fear. Where stimuli are concerned, fear is defined as an aversive reaction elicited by the perception of a specific and often physical threat stimulus, whilst anxiety refers to emotional responses to a diffuse threat, where danger is not clearly imminent, or, to uncertain situations. These different types of stimuli cause different sets of autonomic and behavioural defensive reactions (Sylvers et al., 2011). The fearful stimuli cause arousal responses that tend to be short lived (Michael Davis, Walker, Miles, & Grillon, 2010) and behavioural responses characterised as fight, flight and freezing. Neurobiological substrates of these behaviours implicate the amygdala, especially the central nucleus of the amygdala (CeA: medial division), as a lesion of this structure disrupts freezing responses both in rats (J. E. LeDoux, Iwata, Cicchetti, & Reis, 1988) and non-human primates (Kalin, Shelton, & Davidson, 2004). Anxiety-related arousal, in contrast, is long-lived (Michael Davis et al., 2010) and is associated with prolonged hypervigilance. Neurobiologically, in addition to the CeA (lateral division), the bed nucleus of the stria terminalis (BNST) has been implicated in anxiety responses since an inactivation of the BNST specifically reduced sustained light-enhanced startle response in rats (Michael Davis et al., 2010). Therefore, trait fear describes an organism chronically engaging in fight, flight or freezing behaviours due to perceiving specific environmental cues as threatening, whilst trait anxiety describes an organism in a chronic state of hypervigilance due to the anticipation of a generalized threat. However, despite the efforts of elucidating the difference, there has not been any consensus among researchers. In order to avoid the complication, literatures often also describe related behaviours as anxious/fearful or anxiety-related, fear-related responses (Williamson et al., 2003). Although the aim of the present project is not to define the difference between anxiety and fear, the issue is further discussed in relation to the obtained experimental results in Chapter 3.

## 1.2 Anxiety Disorders

There is considerable evidence that enhanced trait anxiety is associated with higher risk of developing affective/mood disorders including anxiety disorders and depression disorder (Clark, Watson, & Mineka, 1994; Sandi & Richter-Levin, 2009; Zinbarg & Barlow, 1996). Trait anxiety can be conceptualized as lying on a continuum. One end of the continuum represents a low amount of anxiety, the middle represents a higher level of anxiety and the other end of the continuum represents a severe level of anxiety. There are positive features of anxiety. Anxiety acts as a warning signal for impending danger, or harm. Anxiety can also induce motivation. Some anxiety is therefore adaptive and this level of anxiety represents the lower end of the continuum. A moderate level of anxiety represents the middle of the continuum. At the higher end of the continuum lie various anxiety disorders representing a severe amount of anxiety that interferes with daily functioning and is highly maladaptive (Sylvers et al., 2011). The Diagnostic and Statistical Manual of Mental Disorders (DSM) published by the American Psychiatric Association provides a common language and standard criteria for the classification of mental disorders. The current version is DSM-IV-TR (fourth edition, text version) produced in 2000. Another commonly used diagnostic manual for mental disorders is the International Statistical Classification of Diseases and Related Health Problems (ICD), produced by the World Health Organization (WHO). The ICD was developed alongside the DSM and therefore the two manuals share most of the same codes. Although both manuals are used widely worldwide, an international survey of psychiatrists from 66 different countries across the world comparing the use of the two manuals found that the ICD-10 was more frequently used and more valued for clinical diagnosis and training, and that the DSM-IV was more valued in research communities (Mezzich, 2002). To be diagnosed with an anxiety disorder, a patient must meet a certain number of the criteria for that disorder. If the patient does not meet enough of the criteria, the patient is not diagnosed with the anxiety disorder. Below describes main disorders categorised under anxiety disorder dimension in the DSM (Susan Mineka & Zinbarg, 2006), the diagnostic symptoms for adults and association studies, if available, for trait anxiety measured with psychological inventories.

**Specific Phobia (SP)**

Individuals with specific phobias show intense and irrational fears of particular types of objects or situations. They usually go to great lengths to avoid such situations, but when they do happen to encounter them, they feel intense fear and anxiety, even panic. Typical specific

phobias include fear of snakes, spiders, dogs, cats, flying, heights, water, blood, needles and dental procedures. Almost anything can be the object of a phobia. About 11% of people experience SP. Illness phobia are more common in men, and animal phobia are more common in women (Hyman & Pedrick, 2006). The diagnostic criteria for specific phobia outlined by the DSM-IV-TR include:

A. Marked and persistent fear that is excessive or unreasonable, cued by the presence or anticipation of a specific object or situation.
B. Exposure to the phobic stimulus almost invariably provokes an immediate anxiety response, which may take the form of a situationally predisposed panic attack.
C. The person recognizes that the fear is excessive or unreasonable.
D. The phobic situation is avoided or else is endured with intense anxiety or distress.
E. The avoidance, anxious anticipation, or distress in the feared situation interferes significantly with the person's normal routine, occupational (academic) functioning, or social activities or relationships, or there is marked distress about having the phobia.
F. In individuals under age 18 years, the duration is at least 6 months.
G. The anxiety, panic attacks, or phobic avoidance associated with the specific object or situation are not better accounted for by another mental disorder.

There are few association studies of SP for trait anxiety. Test anxiety has been proposed as a type of SP. It is characterised by extreme fear of poor performance on tests and examinations. King and colleagues (N. King & Mietz, 1995) screened a large sample of 9$^{th}$ and 10$^{th}$ grade students for test anxiety with a self-report test (Test Anxiety Scale for Children) and identified top and bottom 5% individuals on the distribution of the test scores as high- and low-test-anxious groups, respectively. 61% of the high-test anxious students were also diagnosed for phobic disorder with the DSM-III. Their scores on the state-trait anxiety inventory for children (C. D. Spielberger, 1973) revealed that high-test-anxious students showed higher scores on the trait anxiety scale but not on the state anxiety scale. A recent meta-analysis (Kotov, Gamez, Schmidt, & Watson, 2010), however, reported that whilst all anxiety disorders were related to neuroticism, the personality dimension that is highly correlated with trait anxiety, SP was found to have only a modest association with the trait. The authors noted that a specific phobia is generally considered to be one of the least severe disorders among the DSM categorised anxiety disorders.

**Social Anxiety Disorder (SAD, Social Phobia)**

With more than 13% of people developing social phobia in their lifetime, this is the most common anxiety disorder. Also, it is more prevalent in females than males by a ratio of 2 to 1 (Hyman & Pedrick, 2006). Individuals with SAD show excessive fear of situations in which they might be evaluated or judged by others, and they either avoid such situations or endure them with marked distress. The diagnostic criteria for SAD by the DSM-IV outline:

A. A marked and persistent fear of one or more social or performance situations in which the person is exposed to unfamiliar people or to possible scrutiny by others. The individual fears that he or she will act in a way that will be humiliating or embarrassing.
B. Exposure to the feared social situation almost invariably provokes anxiety, which may take the form of a situationally bound or predisposed panic attack.
C. The person recognizes that the fear is excessive or unreasonable.
D. The feared social or performance situations are avoided or else are endured with intense anxiety or distress.
E. The avoidance, anxious anticipation, or distress in the feared social or performance situation interferes significantly with the person's normal routine, occupational (academic) functioning, or social activities or relationships, or there is marked distress about having the phobia.
F. In individuals under age 18 years, the duration is at least 6 months.
G. The fear or avoidance is not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition and is not better accounted for by another mental disorder.
H. If a general medical condition or another mental disorder is present, the fear in Criterion A is unrelated to it.

Topcuoglu and colleagues (Topçuoğlu, Fistikci, Ekinci, Gimzal Gönentür, & Cömert Agouridas, 2009) compared state-trait anxiety scores for 36 social phobias diagnosed by the DSM-IV criteria with 36 control subjects and found that the two groups significantly differed on both trait and state anxiety scales, but the difference was larger for the trait anxiety scale. Muris and colleagues (Muris, Merckelbach, Schmidt, & Tierney, 1999) assessed a large sample of primary school children on both a measure of disgust sensitivity, the trait anxiety version of the state-trait anxiety inventory for children, and measures for DSM-defined anxiety disorder symptoms, including social phobia. There was a significant positive correlation between trait anxiety scores and scales for social phobia. Although disgust sensitivity was also significantly correlated with the social phobia scale, the significance disappeared after controlling for trait anxiety. In contrast, trait anxiety still remained highly

significantly correlated with the social phobia scale, when controlling for disgust sensitivity, indicating a substantial effect of trait anxiety on social phobia symptoms.

**Panic Disorder (PD) With and Without Agoraphobia**

People with PD experience recurrent unexpected panic attacks that occur without their being aware of any cues or triggers, and they must also experience worry, anxiety, or behavioural change related to having another attack. Around 5% of people are affected by PD. Most experience their first panic attack in their early twenties, very rarely before age 16 or after age 45. The number of women with PD outnumbers that of men by a ratio of 2 to 1 (Hyman & Pedrick, 2006). Many, but not all, people with PD also go on to develop some degree of agoraphobic avoidance of situations in which they perceive that escape might be either difficult or embarrassing if they were to have a panic attack. Many of these situations are the commonly observed ones such as shopping malls, driving, standing in line, sitting in a theatre, and so forth. The DSM criteria for PD outlines:

A. Both (1) and (2):
    1. Recurrent unexpected Panic Attacks
    2. At least one of the attacks has been followed by 1 month (or more) of one (or more) of the following:
        a. Persistent concern about having additional attacks
        b. Worry about the implications of the attack or its consequences(e.g., losing control, having a heart attack, “going crazy”)
        c. A significant change in behaviour related to the attacks
B. The presence (or absence) of Agoraphobia
C. The Panic Attacks are not due to the direct physiological effects of a substance or a general medical condition (e.g., hyperthyroidism).
D. The Panic Attacks are not better accounted for by another mental disorder.

A number of studies have reported an association between PD and trait anxiety. Monkul and colleagues (Monkul et al., 2010) subjected PD patients, their healthy first-degree relatives and healthy controls to a 35% carbon dioxide challenge and measured their baseline anxiety with the state-trait anxiety inventory. The results revealed that the PD group was significantly higher in both state and trait anxiety scales than the healthy relative and non-related control groups. Whilst trait anxiety scores predicted $CO_2$-induced panic in female PD patients, state anxiety scores did not. In female relatives, state anxiety scores predicted $CO_2$-induced panic. Plehn and Peterson (Plehn & Peterson, 2002) conducted a longitudinal study by surveying a

large sample of young people for anxiety sensitivity, another proposed dimension of anxiety that refers to individual differences in the fear of anxiety sensations (Reiss, 1997), using the anxiety sensitivity index (Reiss & Peterson, 1986), trait anxiety with the state-trait anxiety inventory and diagnostic for PD with the DSM criteria at two different time points with an interval of 11-13 years. At both first and second time points, anxiety sensitivity and trait anxiety were significantly positively correlated with the prevalence of PD. However, more importantly, a logistic regression analysis revealed that whilst trait anxiety scores at the first time point predicted the development of PD at the second time point, anxiety sensitivity did not. Tanaka and colleagues (Tanaka et al., 2012) also reported a significantly higher trait anxiety in PD patients, compared to healthy controls.

**Generalised Anxiety Disorder (GAD)**

About 3% of people have GAD (Hyman & Pedrick, 2006). People with GAD are characterised, primarily, by chronic excessive worry about a number of events or activities for at least six months, and the worry must be experienced as being difficult to control. In addition to anxiety and worry, the patients feel restless, keyed up, on edge, fatigued, or irritable. They can also have difficulty concentrating, experience muscle tension, and sleep disturbances. The DSM provides the criteria for GAD as:

A. Excessive anxiety and worry (apprehensive expectation), occurring more days than not for at least 6 months, about a number of events or activities (such as work or school performance).
B. The person finds it difficult to control the worry.
C. The anxiety and worry are associated with three (or more) of the following six symptoms (with at least some symptoms present for more days than not for the past 6 months).
    1. Restlessness or feeling keyed up or on edge
    2. Being easily fatigued
    3. Difficulty concentrating or mind going blank
    4. Irritability
    5. Muscle tension
    6. Sleep disturbance (difficulty falling or staying asleep, or restless unsatisfying sleep)
D. The focus of the anxiety and worry is not confined to features of other anxiety disorders.

E. The anxiety, worry, or physical symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.
F. The disturbance is not due to the direct physiological effects of a substance) or a general medical condition and does not occur exclusively during a Mood Disorder, a Psychotic Disorder, or a Pervasive Developmental Disorder.

A relatively small number of studies have been conducted to determine an association between GAD and the trait anxiety measure. Gomez and Francis (Gomez & Francis, 2003) examined how trait anxiety was related to the presence and severity of GAD. Groups of GAD patients diagnosed with the DSM-IV criteria and healthy controls were assessed with the state-trait anxiety inventory for trait anxiety. Comparison revealed significantly higher trait anxiety scores in the GAD than control group. Regression analysis revealed trait anxiety as a significant predictor of GAD severity. Hishinuma and colleagues (Hishinuma et al., 2001) assessed a large sample of adolescents with the state-trait anxiety inventory and for a range of anxiety disorders including GAD, social phobia and over-anxious disorder. The subjects were assessed for the two measures either on the same day (concurrent) or with a time interval (non-concurrent). The results revealed that whilst state anxiety was a better predictor of current anxiety disorders, trait anxiety was the best predictor of the development of anxiety disorders in the future.

**Post-Traumatic Stress Disorder (PTSD)**

The symptoms of PTSD include re-experiencing the trauma, passively avoiding the reminders of the trauma, numbing of affect, and heightened general arousal. Traumatic events such as sexual, physical, and emotional abuse, rape, assault, and even witnessing disasters or death can lead to development of PTSD. The symptoms of PTSD most often occur immediately after a trauma, but occasionally surface years later when a person is under further stress. PTSD affects almost 8% of people (Hyman & Pedrick, 2006). The patients tend to have a higher prevalence of depression, PD, GAD, social phobia, substance abuse and suicidal tendencies. However, many people experience horrendous traumatic events and do not develop PTSD. It appears that many factors, environmental and genetic, contribute to an individual's vulnerability to PTSD symptoms. The diagnostic criteria for PTSD in the DSM are:

A. The person has been exposed to a traumatic event in which both of the following were present:

1. The person experienced, witnessed, or was confronted with an event or events that involved actual or threatened death or serious injury, or a threat to the physical integrity of self or others
2. The person's response involved intense fear, helplessness, or horror.

B. The traumatic event is persistently re-experienced in one (or more) of the following ways:
   1. Recurrent and intrusive distressing recollections of the event, including images, thoughts, or perceptions.
   2. Recurrent distressing dreams of the event.
   3. Acting or feeling as if the traumatic event were recurring (includes a sense of reliving the experience, illusions, hallucinations, and dissociative flashback episodes, including those that occur on awakening or when intoxicated).
   4. Intense psychological distress at exposure to internal or external cues that symbolize or resemble an aspect of the traumatic event
   5. Physiological reactivity on exposure to internal or external cues that symbolize or resemble an aspect of the traumatic event

C. Persistent avoidance of stimuli associated with the trauma and numbing of general responsiveness (not present before the trauma), as indicated by three (or more) of the following:
   1. Efforts to avoid thoughts, feelings, or conversations associated with the trauma
   2. Efforts to avoid activities, places, or people that arouse recollections of the trauma
   3. Inability to recall an important aspect of the trauma
   4. Markedly diminished interest or participation in significant activities
   5. Feeling of detachment or estrangement from others
   6. Restricted range of affect (e.g., unable to have loving feelings)
   7. Sense of a foreshortened future (e.g., does not expect to have a career, marriage, children, or a normal life span)

D. Persistent symptoms of increased arousal (not present before the trauma), as indicated by two (or more) of the following:
   1. Difficulty falling or staying asleep
   2. Irritability or outbursts of anger
   3. Difficulty concentrating
   4. Hypervigilance
   5. Exaggerated startle response

E. Duration of the disturbance (symptoms in Criteria B, C, and D) is more than 1 month.

F. The disturbance causes clinically significant distress or impairment in social, occupational, or other important areas of functioning.

Weems and colleagues (Weems et al., 2007) compared pre-disaster trait anxiety scores (the state-trait anxiety inventory for children) and the symptom severity of PTSD (based on DSM-IV) for 52 children who experienced traumatic events during hurricane Katrina. The results revealed not only a significant positive correlation between pre-disaster trait anxiety scores and post-disaster PTSD symptoms, but also the former significantly predicted the latter. Another study (Paparrigopoulos et al., 2006) examined an association between trait anxiety and the development of PTSD symptoms for the first-degree relatives of patients treated in the intensive care unit (ICU). The results showed that trait anxiety measured by the state-trait anxiety inventory at the patient's admission to the ICU was a significant predictor of the PTSD symptom measured at the discharge of the patients from the ICU, suggesting increased risk for developing PTSD for individuals high in trait anxiety. Sinici and colleagues (Sinici, Yildiz, Tunay, Ozkan, & Altinmakas, 2004) compared state and trait anxiety levels measured by the state-trait anxiety inventory of war-veterans with PTSD and found that trait anxiety levels were significantly higher than state anxiety levels among the patients.

**Obsessive-Compulsive Disorder (OCD)**

The central features of OCD are unwanted and intrusive thoughts, impulses, or images that cause marked anxiety or distress; these are usually accompanied by compulsive behaviours or mental rituals that are performed to neutralize or prevent the distressing thoughts or images. Even normal people experience occasional cognitive intrusions that do not differ in content from those seen in OCD (Rachman & Silva, 1978). What distinguishes people with OCD is that clinical intrusions/obsessions are (a) associated with greater distress, (b) more frequent, and (c) more strongly resisted. About 2.5% of the population have OCD. It usually begins before the age of 30, in childhood or adolescence, but can also have a later onset (Hyman & Pedrick, 2006). The DSM provides the diagnostic criteria for OCD as:

A. Presence of obsessions, compulsions, or both:

Obsessions as defined by:

1. Recurrent and persistent thoughts, impulses, or images that are experienced, at some time during the disturbance, as intrusive and inappropriate and that cause marked anxiety or distress
2. The thoughts, impulses, or images are not simply excessive worries about real-life problems

3. The person attempts to ignore or suppress such thoughts, impulses, or images, or to neutralize them with some other thought or action
4. The person recognizes that the obsessional thoughts, impulses, or images are a product of his or her own mind (not imposed from, as in thought insertion)

Compulsions as defined by:

1. Repetitive behaviours (e.g., hand washing, ordering, checking) or mental acts (e.g., praying, counting, repeating words silently) that the person feels driven to perform in response to an obsession, or according to rules that must be applied rigidly
2. The behaviours or mental acts are aimed at preventing or reducing distress or preventing some dreaded event or situation; however, these behaviours or mental acts either are not connected in a realistic way with what they are designed to neutralize or prevent or are clearly excessive

B. At some point during the course of the disorder, the person has recognized that the obsessions or compulsions are excessive or unreasonable.
C. The obsessions or compulsions cause marked distress, are time consuming (take more than 1 hour a day), or significantly interfere with the person's normal routine, occupational (or academic) functioning, or usual social activities or relationships.
D. If another psychiatric disorder is present, the content of the obsessions or compulsions is not restricted to it (e.g., preoccupation with food in the presence of an Eating Disorder; preoccupation with drugs in the presence of a Substance Use Disorder; preoccupation with sexual urges or fantasies in the presence of a Paraphilia; or guilty ruminations in the presence of Major Depressive Disorder).
E. The disturbance is not due to the direct physiological effects of a substance or a general medical condition.

There have been relatively fewer studies reporting an association of trait anxiety with OCD than with other anxiety disorders. Aldea and colleagues (Aldea, Geffken, Jacob, Goodman, & Storch, 2009) reported a significant positive correlation between trait anxiety level assessed by the state-trait anxiety inventory and symptom severity of OCD patients. Karadag and colleagues (Karadag, Oguzhanoglu, Ozdel, Atesci, & Amuk, 2005) assessed state and trait anxiety levels (the state-trait anxiety inventory) of OCD patients diagnosed with the DSM-IV criteria and healthy controls, and reported significantly higher scores on both state and trait anxiety scales in the OCD group than in the control group.

**Major Depressive Disorder (MDD)**

Although depressive disorders are categorised separately from anxiety disorders, considerable co-morbidity between them suggests a common underlying factor (R. Kessler, Berglund, & Demler, 2003; Sandi & Richter-Levin, 2009). People with MDD experience recurrent major depressive episodes. A major depressive episode is characterised by a combination of symptoms that interfere with a person's ability to work, study, sleep, eat and enjoy pleasurable activities. These symptoms include a very low mood, a lack of interest in activities normally enjoyed, changes in weight and sleep pattern, fatigue, feelings of worthlessness and guilt, difficulty concentrating and thoughts of death and suicide. If a person has experienced the majority of these symptoms for longer than a two-week period, they may be diagnosed as having had a major depressive episode. Prevalence of MDD ranges from 8-12% and is almost twice as common in women as in men (Andrade et al., 2003). The diagnostic criteria for MDD and major depressive episode provided by the DSM are:

A. Presence of two or more Major Depressive Episodes.

   Major Depressive Episode is defined as:

   a. Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.
      1. Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g., appears tearful).
      2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day
      3. Significant weight loss when not dieting or weight gain, or decrease or increase in appetite nearly every day.
      4. Insomnia or hypersomnia nearly every day
      5. Psychomotor agitation or retardation nearly every day
      6. Fatigue or loss of energy nearly every day
      7. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick).
      8. Diminished ability to think or concentrate, or indecisiveness, nearly every day
      9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide
   b. The symptoms do not meet criteria for a Mixed Episode.
   c. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

d. The symptoms are not due to the direct physiological effects of a substance or a general medical condition.
e. The symptoms are not better accounted for by Bereavement.

B. The Major Depressive Episodes are not better accounted for by Schizoaffective Disorder and are not superimposed on Schizophrenia, Schizophreniform Disorder, Delusional Disorder, or Psychotic Disorder Not Otherwise Specified.
C. There has never been a Manic Episode, a Mixed Episode, or a Hypomanic Episode.

Kennedy and colleagues (Kennedy, Schwab, Morris, & Beldia, 2001) compared trait anxiety levels measured by the state-trait anxiety inventory between the patients diagnosed with either MDD, PD, GAD, SP, OCD or mixed anxiety and depression (MAD) and healthy controls. The trait anxiety scores of the patients for all the disorders, both independently and together, were significantly higher than those of the controls. However, trait anxiety scores did not clearly differentiate anxiety disorders from depressive disorders, suggesting trait anxiety may be the common underlying factor. A long-term follow-up study (Chambers, Power, & Durham, 2004) of the patients with anxiety disorders including GAD, PD and social phobia, or MDD, reported high trait anxiety levels for those who continued to show the disorder symptoms. Not only was MDD highly co-morbid with other anxiety disorders but also trait anxiety was unable to distinguish between them. Given the high co-morbidity between anxiety disorders and MD and the finding that the development of anxiety disorders tends to precede the onset of MD (Bittner et al., 2004), Sandi and colleagues (Sandi & Richter-Levin, 2009) have proposed a model linking high trait anxiety with anxiety disorders and MDD. According to their hypothesis, individuals high in trait anxiety are prone to develop anxiety disorders when exposed to stressful life events. Anxiety disorders are associated with malfunctioning neural systems that bias cognition, attention and memory to negative stimuli and mood. Sustained stress exposure caused by this negative bias produces a spiral of progressively enhanced dysfunctions that eventually lead to the development of MDD.

## 1.3 Animal Models of Trait Anxiety

A range of different experimental paradigms have been used to study trait anxiety. Very often more than one paradigm is used in order to determine how stable the trait is across different contexts. When characterising trait anxiety, two different approaches are used. 1) Those individuals, that are either very high or very low responders, are separated into two groups, and subsequently the groups are directly compared to one another. 2) Overall anxiety levels are correlated with other variables of interest. In some cases, selective breeding of the high and low responder groups is performed to create strains or lines. Subsequent investigations into the underlying neurobiological or genetic mechanisms of these traits can then be conducted. Behavioural paradigms used to study trait anxiety fall into two main categories, conditioned or unconditioned (Rodgers, Cao, Dalvi, & Holmes, 1997). The following paragraphs describe the major behavioural paradigms used to study trait anxiety in rodents and non-human primates.

**Elevated Plus-Maze Test**

One of the most widely used anxiety tests that measure an animal's unconditioned behaviour is the elevated plus-maze (EPM) test (Rodgers et al., 1997). The elevated plus-maze consists of two open and two enclosed arms that form a plus-shaped platform elevated 40-70cm above floor level. The model depends upon the induction of a conflict between the aversion of being exposed to an open and elevated platform on the one hand and the motivation to explore the new environment on the other. As a consequence, the less anxious the individuals are, the more they explore the open arms. This is indicated by an increase in the proportion of time spent in the open arms as compared to the closed arms and an increase in the proportion of entries into the open arms. The converse pattern indicates higher anxiety. The validity of the EPM as an anxiety test has been derived mainly from the repeated demonstration that performance is sensitive to anxiolytic and anxiogenic compounds. Whilst conventional anxiolytics used for the treatment of humans, such as chlordiazeproxide, diazepam and phenobarbitone increased the time spent on, and the number of entries into the open arms, anxiogenic agents such as yohimbine, caffeine and amphetamine reduced these parameters (Lister, 1987; Pellow, Chopin, File, & Briley, 1985). However, the effects of other anxiolytic agents such as serotonin specific reuptake inhibitor (SSRI) have been more variable (Handley, McBlane, Critchley, & Njung'e, 1993).

One of the aspects of high trait anxiety is the tendency to respond anxiously in a wide range of stressful situations (C. D. Spielberger, 1985). In order to examine if the behaviour observed in the EPM were a trait-like phenotype, Henniger and colleagues (Henniger et al., 2000) tested two strains of rats that had been selectively bred for high or low anxiety behaviours in the EPM on other types of anxiety tests: the black-white box and the social interaction test. The difference in anxiety-related behaviour between the two strains was highly consistent in both tests of unconditioned anxiety. In the black-white box paradigm, the animals in the high anxiety-related behaviour (HAB) line entered the brightly lit white compartment less often, and spent less time in it, than their counterparts in the low anxiety-related (LAB) line. Likewise, in the social interaction test, the HAB rats spent less time in active social interaction than the LAB rats. Thus, regardless of the type of tests, the HAB rats behaved more anxiously than the LAB rats. This consistency in behavioural patterns observed across different anxiety tests suggest that the individual differences elicited in the EPM reflect stable emotional traits. Although it has been well accepted that the EPM is sensitive in detecting trait-like differences in anxiety-related behaviours (e.g. Duvarci et al. 2009), some studies argue that the observed behaviours may reflect more of a state-like anxiety than a trait-like anxiety based on the EPM's poor test-retest reliability (Andreatini & Bacellar, 2000) and poor correspondence with the free-exploratory paradigm, another rodent anxiety model (Goes, Antunes, & Teixeira-Silva, 2009). The elevated zero-maze is a modified version of the EPM, which has also been shown to be sensitive to both anxiolytics and anxiogenics (Shepherd, Grewal, Fletcher, Bill, & Dourish, 1994).

**Open Field Test**

Another anxiety test in rodents that is often used in combination with the EPM is the open field test (OFT). The procedure consists of subjecting an animal to an unknown environment from which escape is prevented by surrounding walls. The shape of the environment may differ, being circular, square or rectangular, and it may contain objects such as platforms, columns, tunnels, etc. (Goes et al., 2009). As in the EPM, the OFT is based on a test-induced conflict between a rodent's spontaneous preference for the periphery of the apparatus, called thigmotaxis, and the motivation to explore the new environment. The animal is placed in the centre or close to the walls and behaviours such as locomotion, frequency of rearing or leaning, grooming and defecation are measured for a period of 2 to 20min. Increase of time spent in the centre, a ratio of centre/total locomotion and the decrease in the latency to enter the centre are usually used as indicators of low anxiety. Jakovcevski and colleagues (Jakovcevski, Schachner, & Morellini, 2008) examined whether

trait-like anxiety identified on the OFT correlates with the long-term behavioural and neuroendocrine changes induced by an acute stressor. A large sample of mice was tested on the OFT and categorised into high or low trait-anxiety groups based on their latency to enter the open field. Five days after the OFT, the animals from both groups were randomly assigned to an acute stress exposure, namely forced encounter with a rat, or a control condition. Subsequently, the animals were tested on the EPM. The results showed that those in the high trait-anxiety group that experienced the stressful event displayed significantly enhanced anxiety-related activities in the EPM, which were accompanied by enhanced hypothalamic-pituitary-adrenal (HPA) axis activity and increased messenger RNA (mRNA) expression for glucocorticoid and mineralocorticoid receptors in the hippocampus. No effect of acute stress was observed in the animals in the low trait-anxiety group. Moreover, the high trait-anxious group showed increased basal levels of hippocampal mRNA for the glucocorticoid receptor under unstressed condition, indicative of high anxiety (Wei et al., 2004), suggesting that the OFT is sensitive to trait anxiety. As for the effects of anxiolytics on the OFT performance, a literature review (Prut & Belzung, 2003) indicates that whilst conventional anxiolytics such as benzodiazepine receptor agonists or 5-$HT_{1A}$ receptor agonists decreased anxiety-related behaviours, newer anxiolytic compounds such as alprazolam and SSRI that have been effective in anxiety disorders such as PD, OCD, social phobia and PTSD were poorly effective as anxiolytics in the OFT. Given this observation, the authors suggested that the OFT may be a model of normal anxiety, sensitive to classical anxiolytics but not a model of pathological anxiety.

**Black/White Box Test**

Another commonly used rodent model of anxiety is the black/white box test, also known as dark/light box test. As with the EPM and OFT, this test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour in response to mild stressors, i.e. novel environment and light. The apparatus consists of two inter-connected compartments. One compartment is coloured white, brightly lit and can be smaller than the other, which is coloured black and dimly illuminated. A greater proportion of time spent in the white, than in the black compartment, and a greater number of transitions between the compartments, without an increase in spontaneous locomotion, are considered to reflect low anxiety. A literature review (Bourin & Hascoët, 2003) on the effects of anxiolytics on the black/white box test shows that it is sensitive to both conventional anxiolytics such as benzodiazepines as well as to newer anxiolytic compounds such as SSRI's. The consistency with other types of anxiety paradigms has also been demonstrated.

In particular, the animals that are identified high or low trait-anxious in the two-way active avoidance acquisition test and the EPM have been shown to display responses in the same direction to the black/white box test (Henniger et al., 2000; Landgraf & Wigger, 2002; Thierry Steimer & Driscoll, 2003).

**Holeboard Test**

Another popular but less widely used test of unconditioned anxiety in rodents is the holeboard test. An animal is tested in an enclosed arena with holes in the floor into which the animal can poke its head (head-dipping). The test assesses a rodent's natural tendency to explore the new environment. The exploration behaviour is seen by way of the animal plunging its head in and out of the hole a few times and then moving on to the next hole. Whilst a high frequency of head-dipping is considered as a sign of low anxiety, a low level of head-dipping is usually indicative of high anxiety that prevents the animal from exploring. The holeboard test has shown to be sensitive to the effects of various anxiogenic and anxiolytic compounds including benzodiazepine and 5-$HT_{1A}$ receptor agonists (Redfern & Williams, 1995; Takeda, Tsuji, & Matsumiya, 1998). Escorihuela and colleagues (Escorihuela et al., 1999) tested the RLA/Verh and RHA/Verh rat lines on the holeboard test, the EPM and the OFT. The RLA/Verh and RHA/Verh rat lines had been selectively bred for high or low anxiety phenotypes, respectively, based on the performance on the two-way active avoidance acquisition test (see below for description). As expected, the results showed that the high anxious RLA/Verh rats displayed significantly fewer numbers of head-dipping behaviour than the low anxious RHA/Verh rats. This difference between the strains was consistent with their behavioural patterns observed in the EPM and OFT, namely that the RLA/Verh rats showed enhanced anxiety-related behaviours compared to the RHA/Verh rats. This consistency across different anxiety paradigms indicates that the holeboard test is a sensitive test of trait anxiety.

**Hyponeophagia Test**

Relatively less widely used test of anxiety in rodents is the hyponeophagia test. When encountering a novel food, mice and rats tend initially to consume only small amount. This behaviour is called "bait shyness" (Deacon, 2011) and is thought to be because the rodents are unable to vomit therefore liable to poisoning. The amounts ingested gradually increase until the animal has determined whether the substance is safe and nutritious. In a typical test setting, a highly palatable but novel substance is offered to the animal in a novel situation,

such as a new cage. Longer latency to eat and smaller amount of food ingested are related to high anxiety phenotype. As in other anxiety tests described above, the sensitivity to anxiolytic and anxiogenic compounds has been demonstrated (R. A. Shephard & Broadhurst, 1982; R. Shephard & Estall, 1984). For instance, the administration of diazepam anxiolytic dose-dependently reduced the eating latency whilst this effect was cancelled with the administration of anxiogenic *d*-amphetamine (R. A. Shephard & Broadhurst, 1982). The RLA/Verh and RHA/Verh rat lines were also tested on the hyponeophagia test (T Steimer, Escorihuela, Fernandez-Teruel, & Driscoll, 1998). Similarly to the findings for the other anxiety tests, the anxious RLA/Verh rats displayed significantly longer eating latency, the sign of high anxiety, than the less anxious RHA/Verh rats.

**Social Interaction Test in Rodents**

The social interaction test in rodents is thought to simulate human social behaviours that are often disrupted in anxiety disorders, especially in social phobia (Kaidanovich-Beilin, Lipina, Vukobradovic, Roder, & Woodgett, 2011). The rodent social interaction test is based on spontaneous social interaction behaviours such as sniffing, following and grooming. In the test, two animals are placed into an arena and the time spent by the pair in social interaction is scored. A longer time spent in social interaction is a sign of low anxiety or indicative of an anxiolytic effect, whereas a specific decrease in social interaction indicates an anxiogenic effect. As with other anxiety tests, the social interaction test has also been validated by its sensitivity to various anxiolytic and anxiogenic compounds (File & Seth, 2003; Yasumatsu, 1995). In order to examine whether the behavioural pattern observed in the social interaction test is related to trait-like phenotype identified in other anxiety tests, the HAB and LAB rat lines that had been selectively bred for high and low anxiety behavioural phenotypes, respectively, on the EPM were subjected to the social interaction test (Henniger et al., 2000). A pair of rats that both belonged to the same line but were unknown to each other was placed in the centre of the arena facing each other. Their behaviour was observed for 10min. The results revealed a significant difference between the strains; the high anxious HAB rats spent significantly less time in the social interaction than the less anxious LAB rats, suggesting that the social interaction test may be sensitive to trait anxiety.

**Social Interaction Test in Marmosets**

The social interaction test has been used not only in rodents but also in non-human primates. Non-human primates are a valuable animal source for studying anxiety due to similarities

with humans in their physiological and behavioural responses to anxiety-inducing situations (Newman & Farley, 1995). The common marmoset (*Callithrix jacchus*) is a New World primate species native to the dense tropical forests of Brazil. Because of their small body size, relatively high reproductive rate for primates and the similarity in brain morphology to humans, they have been used as an experimental model in neuroscience research (Yamazaki & Watanabe, 2009). Marmosets are highly social animals living in stable extended family groups, and have thus developed a complex social/emotional behaviours including facial expressions, body postures and vocalizations (Stevenson & Poole, 1976). Cilia and Piper (Cilia & Piper, 1997) developed the social interaction test for marmosets and examined the effect of the anxiolytic, diazepam, on the animal's social behaviours. In the test, following an acute administration of either vehicle or diazepam, two pairs of male/female marmosets, that had no previous auditory or visual contact with one another, were placed in adjacent observational cages that were temporarily separated by an opaque barrier. The removal of the barrier allowed visual contact between the pairs. The animals' behaviours were monitored for a period of 10min. The behavioural parameters included the frequency of aggressive behaviours (i.e. anogenital presentation, slit-stare and piloerection), the frequency of anxiety-related behaviours (i.e. scent marking, head-body bobbing and wet-dog shake), the frequency of scratching and the frequency of allogrooming. The results revealed the significant anxiolytic effect of the compound on the specific behaviours. In comparison to the control animals, the diazepam administered animals showed a significant reduction in aggressive behaviours, anxiety-related behaviours and scratching, the latter being considered an anxiety-related displacement activity (Marilia Barros et al., 2007). On the other hand, the allogrooming, which was considered as an affiliative behaviour, was increased in the anxiolytic group. Given the consistency of the results with the anxiolytic effect of diazepam in humans, the authors argued that the social interaction test in marmosets was a non-human primate model of anxiety.

**Marmoset Predator Confrontation Test**

Marmosets suffer one of the highest rates of predation among primates, which may have led them to develop a variety of defensive behavioural strategies, many of which seem to persist even among captive and captive-born individuals (Marilia Barros, Boere, Mello, & Tomaz, 2002; Caine, 1998; Cheney & Wrangham, 1987). Barros and colleagues (M Barros, Boere, Huston, & Tomaz, 2000) developed the marmoset predator confrontation test (MPCT) that simulates such fear/anxiety provoking encounters with a predatory threat. In the test, a marmoset is placed in a figure-8 maze with a taxidermized wild cat, a natural predator of

marmosets, positioned in the corner of the maze. This setting simulates the encounter with a predator through casual and spontaneous exploration that would occur under natural situations. Various anxiety-related behaviours were measured, including the proximity to the predator (frequency and time spent in the closest section to the predator), exploratory activity (sniffing or licking any part of the apparatus), scratching, scent marking, tsik-tsik vocalization (warning call), vigilant scanning and locomotion. Marked changes were observed in these behaviours upon encountering the predator, i.e. reduction in the proximity to the predator, locomotion, exploratory activity and increase in scratching/scent marking, the tsik-tsik vocalizations and vigilant scanning (Marilia Barros, De Souza Silva, Huston, & Tomaz, 2004). Pharmacological validations of the test confirmed that these behavioural parameters were sensitive to a variety of anxiolytic compounds. Anxiolytics, such as the benzodiazepine receptor agonist, diazepam, and the serotonergic 5-$HT_{1A}$ receptor partial agonist, buspirone, reversed the predator-induced proximic avoidance and scratching/scent marking responses, while increasing exploratory activities (M Barros et al., 2000; M Barros, Mello, Huston, & Tomaz, 2001). These changes were considered as anxiolytic-like effects. Similar results were observed with the use of the neuropeptide substance P and the selective 5-HT1A receptor antagonist WAY 100635 (Marilia Barros, De Souza Silva, Huston, & Tomaz, 2002; Marilia Barros et al., 2003). Not only the MPCT but also other versions of predator confrontation paradigms have been widely used as an ethologically-based test to measure anxiety and fear-induced behaviours in non-human primates including rhesus monkeys. Human intruder and snake stimulus are the most frequently employed types of predators. Pharmacological and neurobiological studies involving these stimuli are reviewed in relation to the current project in Chapter 3.

Ethologically relevant anxiety tests such as above described paradigms form the backbone of preclinical research on the neurobiology of psychiatric disorders, and are employed both as screening tools in the search for novel therapeutic agents and as simulations for studies on underlying mechanisms (Rodgers et al., 1997). The validity of these paradigms as anxiety-sensitive tests has been provided mainly by testing the effects of known anxiolytics and anxiogenics on the observed parameters. However, whether these paradigms are differentially sensitive to state or trait anxiety has not been clear. It has been proposed that the paradigms that involve forced encounter with anxiogenic stimuli (e.g. open space, brightly lit box etc.) induce state anxiety, whereas 'free' exploratory response to novel objects or space reflect levels of trait anxiety (Griebel, Sanger, & Perrault, 1996). Accordingly, the EPM, OFT and black/white box test can be considered the tests of state anxiety. On the other hand,

the significant effects of anxiolytics that have been used to treat anxiety disorders, psychopathological development of high trait anxiety, on the observed behaviours suggest that these tests are sensitive to trait anxiety. Other ways of assessing the validity of these paradigms as tests of trait anxiety are to examine correlations across different types of paradigms and test-retest reliability. Trait anxiety is defined as an enduring tendency to display anxiety-related responses to a wide variety of environmental stimuli. Accordingly, individuals high in trait anxiety should exhibit anxiety-related behaviours across different paradigms and to a repeated exposure to the same paradigm. Evidences from multiple testing of high and low anxious strains of rats on the EPM, OFT, holeboard test and hyponeophagia test indicated that the strains differed in the behavioural responses measured in the former three tests, i.e. the high anxious rats showed greater anxiety-related responses than the low anxious ones, suggesting that these paradigms are sensitive to trait anxiety (Escorihuela et al., 1999; Landgraf & Wigger, 2002). Another study reported a significant correlation in anxiety-related behaviours between the EPM and hyponeophagia test (Trullas & Skolnick, 1993). However, there have been reports for a poor correspondence between the EPM and free-exploratory paradigm or OFT (Goes et al., 2009; Schwarting & Pawlak, 2004). Carola and colleagues (Carola, D'Olimpio, Brunamonti, Mangia, & Renzi, 2002) attributed this discrepancy to the conventional temporal and spatial parameters, such as the duration and number of open/closed arm entries, not being able to fully characterise the complexity of anxiety-related behaviours. Therefore, the authors compared mice responses between the EPM and OFT in a variety of ethological parameters, such as rearing, stretching, head-dipping, grooming and sniffing, in addition to the conventional measures, and found a substantial correlation in the anxiety-related behaviours between the tests. Although the test-retest reliability of the EPM has been examined, the results have not been conclusive (Andreatini & Bacellar, 2000; Schwarting & Pawlak, 2004). Despite a few contradictory reports, overall evidences from the multiple-testing studies, especially with anxiety high/low inbred strains, support the validity of these ethologically relevant unconditioned anxiety paradigms as measures of trait anxiety. However, it should also be emphasized that when aiming to measure individual differences in trait anxiety, it is important to test the subjects on not only one but multiple different paradigms to produce reliable results. This is because, firstly, specific behavioural responses observed in the different types of tests may reflect different aspects of anxiety, and secondly, trait anxiety is reflected in the tendency to display anxiety responses to a wide range of situations.

Besides the above described individual differences in unconditioned behaviours (or innate behaviours) used to define trait anxiety, other tests have been used to characterise other forms of negative emotional traits, based on conditioned fear responses. In some cases, their relationship to unconditioned anxiety behaviour has been measured. Fear conditioned responses have traditionally been used in the investigation of neural mechanisms underlying the processing of aversive stimuli (J. LeDoux, 2000; Phelps & LeDoux, 2005); thus, the paradigms did not necessary differentiate trait from state anxiety. However, in the view of clinical psychology, conditioned fear responses have been associated with the aetiology of anxiety disorders (Susan Mineka & Oehlberg, 2008; Susan Mineka & Zinbarg, 2006). For instance, social phobia develops after one experiences or observes others undergoing a fear conditioning event. Experience of a traumatic conditioning event is a necessary criterion for PTSD diagnostics. Social phobia can also arise as a result of direct traumatic conditioning such as public humiliations and embarrassments. Panic disorder is hypothesized to develop through the association between initial panic attacks and initially neutral internal and/or external cues. These cues become conditioned stimuli, which lead to agoraphobic avoidance and sustained apprehensive state described as “fear of fear”. High trait anxiety is considered as a vulnerability factor for developing anxiety disorders, affecting the speed and strength of conditioning (Susan Mineka & Zinbarg, 2006). Therefore, those high in trait anxiety are more easily fear-conditioned upon encountering a traumatic event than low trait-anxious individuals; this may account for why not everyone who experiences a traumatic event develops anxiety disorder.

**Fear Conditioning Paradigm and Fear-Potentiated Startle**

One of the most well established paradigms for conditioned responses is Pavlovian fear conditioning that measures the freezing response to a conditioned stimulus. Fear conditioning in rats has been the primary tool in the investigation of neural pathways involved in processing, storage and expression of fear (Phelps & LeDoux, 2005). Freezing is defined as the cessation of all movement with the exception of respiration-related movement and non-awake or rest body posture (Bush, Sotres-Bayon, & Ledoux, 2007). In a fear conditioning paradigm, an emotionally neutral conditioned stimulus (CS) such as a tone, is presented together with an aversive unconditioned stimulus (US), usually an electric foot shock. After repeated presentations an animal learns to associate the CS with the US. Once the association is formed, even when the animal is presented with the CS alone, it shows a coordinated conditioned behavioural response, such as freezing, together with autonomic and endocrine activations. However, if the CS is presented alone repeatedly without any

further external aversive stimulus, gradually the animal dissociates the CS from the US and the conditioned responses dissipate. This process is called extinction.

In order to investigate individual differences in the responses to fear conditioning and fear extinction, Bush and colleagues (Bush et al., 2007) tested a large number of outbred rats to take advantage of the natural variability that occurs in such populations. The rats were exposed to repeated presentations of a neutral audio, CS, paired with an aversive foot-shock, US. Their freezing responses during the CS presentations were scored. Subsequently, the rats were categorised into high or low reactivity phenotype groups. In order to examine if these behavioural patterns were persistent traits, two days after the conditioning session both groups were presented with the CS alone in the same context as in the conditioning session; then, one day later they were given four CS presentations alone in a context distinct from the one used in the conditioning session. The freezing behaviour was scored in both sessions. The results showed a clear difference in the freezing behaviour between the high and low reactivity phenotype groups. Not only did the rats in the high reactivity phenotype group displayed greater freezing than did the ones in the low reactivity phenotype group, the behavioural pattern persisted throughout the subsequent CS alone sessions both in the same and the different contexts from the original fear conditioning context, suggesting that those distinctive fear reactivity patterns were relatively stable.

In order to see if there were similar trait-like phenotypes in how one recovers from conditioned fear, the authors (Bush et al., 2007) trained another set of rats (that had already acquired a fear conditioned response) on extinction trials. Based on the rate of reduction in their freezing response to CS alone presentations, the rats were then divided into fast recovery or slow recovery groups. Despite the difference in the rate of reduction, at the end of the extinction training both groups appeared to have extinguished the fear response. In order to examine if the tendency to recover quickly from fear was associated with more stable fear recovery across time, the animals were subjected to a later test for the retrieval of extinction learning. The results showed that the slow recovery group displayed significantly greater freezing and slower retrieval of extinction than the fast recovery group, suggesting that these distinct recovery phenotypes were stable.

To establish the relationship between fear conditioning and trait anxiety Lopez-Aumatell and colleagues (López-Aumatell et al., 2009) compared fear-potentiated startle, elicited by the exposure to a fear conditioned stimulus, between Roman High-(RHA/Verh) and Low-(RLA/Verh) Avoidance rats. These strains of rats were created by selectively breeding the

animals that showed good vs. poor acquisition of two-way active avoidance (explained below). Compared to RHA/Verh rats, RLA/Verb rats display higher anxiety or emotional reactivity in a variety of unconditioned behavioural paradigms that subject them to stressful or conflict conditions (Escorihuela et al., 1999). In the experiment, the rats were first exposed to a startle noise. Subsequently, the animals received fear conditioning sessions, in which they were conditioned to an acoustic CS paired with a foot-shock US. After the acquisition of the fear conditioned response, they were re-exposed to the startle noise preceded by the CS. The difference in the freezing response between baseline startle and the startle during the presence of the CS is considered a measure of fear-potentiated startle. The results showed that the RLA/Verh rats not only displayed higher baseline startle responses but also displayed a markedly enhanced fear-potentiated response, even after adjusting for the difference in the baseline, as compared to the RHA/Verh rats. These studies indicate that differences in fear reactivity can accompany individual difference in trait anxiety.

**Two-Way Active Avoidance Acquisition Test**

In contrast to the Pavlovian or classical fear conditioning paradigms that measure involuntary responses to a CS, the two-way active avoidance acquisition test is an instrumental or operant conditioning paradigm, which involves the animal modifying its voluntary behaviour as a result of fear learning. In the typical two-way active avoidance acquisition test, a shuttle box, which is divided into two equally sized compartments, is used. An animal is placed in one of the compartments, in which a CS, usually a light or tone, is presented, followed by an US, usually a foot-shock. The CS/US presentation is terminated when the animal crosses to the other compartment. If the crossing occurs during the CS (before US onset), it is considered an avoidance response. Based on good vs. poor acquisition of the active avoidance, the two strains of rats, namely the RHA/Verh and RLA/Verh rats respectively, have been selectively bred since 1972 (Escorihuela et al., 1999; Thierry Steimer & Driscoll, 2003).

The RHA/Verh and RLA/Verh strains have been implicated in trait anxiety since they show differential responses in a variety of unconditioned anxiety response paradigms including the EPM, OFT, holeboard test and black/white box test; the former strain having been associated with a low trait anxiety phenotype whilst the latter with a high trait anxiety phenotype (Escorihuela et al., 1999; Thierry Steimer & Driscoll, 2003). The difference in the behavioural phenotypes between the strains is supported by the differential anxiety-related endocrine responses to stressful conditions. When exposed to environmental and/or psychosocial

stressors the high trait-anxious RLA/Verh rats display increased stress-induced prolactin reactivity and hyper-activation of the hypothalamus-pituitary-adrenal (HPA) axis, i.e. increased stress-related hormones such as corticosterone and corticotropin secretion, compared to the less trait-anxious RHA/Verh rats (Thierry Steimer, 1997).

Conversely, the validity of the two-way active avoidance acquisition test as a sensitive paradigm for trait anxiety has also been examined by testing the animals that have been identified either as high or low anxious on other unconditioned anxiety tests. Schwarting and Pawlak (Schwarting & Pawlak, 2004) divided a cohort of normal rats into high or low anxiety groups based on their repeated performance on the elevated plus maze. The groups were subsequently tested on the two-way active avoidance acquisition test. The results showed that the rats in the low anxiety group displayed superior avoidance behaviour to the high anxious rats, suggesting that enhanced emotionality in the high anxious rats may impair acquisition of avoidance learning (Schwarting & Pawlak, 2004) or that enhanced trait anxiety is associated with a reactive rather than a proactive coping style (Thierry Steimer & Driscoll, 2003).

As mentioned earlier, fear conditioning paradigms along with a range of other unconditioned anxiety tests have been extensively utilized in the neurobiological investigations elucidating a network of structures that are involved in the processing and expression of fear and anxiety. Decades of investigation have identified some key structures in the network, which will be described in the following section.

## 1.4 Neurobiology of Anxiety

Anxiety and fear normally comprise adaptive responses to threat or stress. Neural processing for these adaptive responses begins when visual, auditory or olfactory information of threat-related stimuli are carried to the brain from sensory organs. The first step in the processing is to evaluate the emotional salience of the stimuli, which involves appraisal of its valence, its relationship with previous conditioning and behavioural reinforcement experiences, and the context in which it arises (Charney & Drevets, 2002). Neurobiological investigations, especially using Pavlovian fear conditioning and fear potentiated startle, have identified the amygdala, almond-shaped groups of nuclei located deep within the medial temporal lobe, as the central structure in this process (J. E. LeDoux, 2000). Differential role of the bed nucleus of the stria terminalis, which sits on the major output pathway of the amygdala, from the amygdala in fear conditioning suggests that different neural circuits are involved in the processing of fear- vs. anxiety-related stimuli (Michael Davis et al., 2010). The hippocampus has been implicated in fear conditioning to contextual stimuli (Phillips & LeDoux, 1992). The expression of fear and anxiety conveys the range of behavioural, endocrine and autonomic responses, which are mediated by the hypothalamic and brainstem regions that receive projections from the limbic structures. The regulation of these emotional responses have implicated the prefrontal control, especially medial prefrontal cortex as evidenced by its influence in the extinction of conditioned fear (Gregory J Quirk, Garcia, & González-Lima, 2006). Modulatory role of the orbitofrontal cortex for fear and anxiety responses has also been suggested based on its connections with sensory cortical regions and major limbic structures (Kringelbach & Rolls, 2004) as well as on the empirical evidences from conditioned and unconditioned anxiety tests (Agustín-Pavón et al., 2012; Lacroix, Spinelli, Heidbreder, & Feldon, 2000). Following sections describe the role of each of those structures in the processing of fear and anxiety in more detail.

**Amygdala: Central structure mediating the expression of fear**

The amygdala has long been the target of research investigating emotional processing in the brain. An extensive amount of work especially on fear conditioning indicates that the amygdala plays a crucial role in associating sensory information from various modalities and initiating the production of autonomic and behavioural responses to environmental threat (J. E. LeDoux, 2000). Briefly, conditioned fear is mediated by the transmission of information about the conditioned stimulus (CS) and unconditioned stimulus (US) to the amygdala, and by the control of fear reactions through output projections from the amygdala to the

behavioural, autonomic and endocrine response control systems located in the brain stem. The amygdala consists of several interconnecting nuclei, each of which receives from and projects to other distinct brain regions. The lateral nucleus (LA) is typically viewed as the sensory interface of the amygdala since it is the main entry site of sensory information coming from sensory thalamus and cortical regions (Phelps & LeDoux, 2005). Lesions of LA have shown to disrupt both behavioural and autonomic conditioned responses to a cued CS paired with foot-shock in rats (Ledoux, Romanski, & Xagoraris, 1990). Also, nociceptive stimulation activates the cells in LA, which indicates that LA may be the site of the convergence of CS and US information and thus involved in the formation of CS-US association (J. LeDoux, 2000). It has been suggested that the two sensory input routes to LA may play different roles in fear processing. The transmission through the thalamic pathway is rapid but the quality of the information is crude and therefore is mainly involved in the processing of simple sensory stimuli. On the other hand, the cortical pathway is slower but carries more accurate information; therefore, it is required for processing of more complex stimuli. Over-activation of the former may be associated with inappropriate anxiety responses to harmless stimuli that share some features with real threat (Phelps & LeDoux, 2005). This point is further described in Chapter 2. In addition to LA, the basal (B) and accessory basal (BA) nuclei are considered as the entry sites of sensory information. However, since these nuclei mainly receive projections from the areas of the ventral hippocampus (Canteras & Swanson, 1992), which is involved in the integration of contextual information (Phillips & LeDoux, 1992), this input route is implicated in processing contextual fear. Rats with selective excitotoxic lesions of BA exhibited disruption of performance in the contextual, but not cued auditory fear conditioning (Onishi & Xavier, 2010).

LA, B and BA all directly project to the central nucleus (CE) of the amygdala. CE in turn projects to hypothalamic and brain stem areas that mediate expression of fear responses. Therefore, damage to CE should interfere with the expression of conditioned fear responses. For instance, bilateral electrolytic lesions of CE in rabbits abolished conditioned heart rate response to a CS paired with electric shock (Gentile, Jarrell, Teich, McCabe, & Schneiderman, 1986). Excitotoxic lesions of CE in rats disrupted both arterial pressure and freezing responses in fear conditioning (Iwata, LeDoux, Meeley, Arneric, & Reis, 1986). Similarly, localized cooling of CE in cats attenuated conditioned blood pressure and respiratory responses in fear conditioning (Zhang, Harper, & Ni, 1986). Rodents are nocturnal and are naturally afraid of bright light. Acoustic CS presentations to rats placed in the brightly lit space induce greater startle response, which is considered as a measure of conditioned fear. Bilateral electrolytic lesions of CE completely blocked this fear-potentiated

startle response (Hitchcock & Davis, 1986). Similarly, excitotoxic lesions of CE in rats also abolished fear-potentiated startle response (Campeau & Davis, 1995). CE mediates not only conditioned fear responses but also unconditioned fear responses (M Davis, 1992). Excitotoxic lesions of CE in rhesus monkeys significantly attenuated fear-related behavioural responses to a snake and freezing behaviour when confronted by a human intruder (Kalin et al., 2004).

Though not to the same level of specificity as animal models, neuroimaging studies in humans also indicate the involvement of the amygdala in processing fear/anxiety information. An early study (LaBar & Gatenby, 1998) with functional magnetic resonance imaging (fMRI) tested normal humans in a fear conditioning task and showed an increased activation of the amygdala during conditioned fear acquisition and extinction. The extent of activation during acquisition was significantly correlated with autonomic indices of conditioning, i.e. skin conductance response. A more recent fMRI study (Indovina, Robbins, Núñez-Elizalde, Dunn, & Bishop, 2011) demonstrated that, in a fear conditioning task that involved both cue and context stimuli, individuals high in trait anxiety exhibited increased amygdala responsivity to the phasic cue and reduced ventral prefrontal cortical activity to both cued and contextual stimuli. The authors proposed that vulnerability to anxiety may be associated with either/both hyper-responsive amygdala or/and impoverished prefrontal regulatory mechanism. This point is further described in Chapter 4. Not only conditioned stimuli but also unconditioned stimuli such as emotional facial expressions, i.e. images of angry and fearful faces, induce increased activation of the amygdala, which has been shown to positively correlate with trait anxiety levels (M. B. Stein, Simmons, Feinstein, & Paulus, 2007). Increased amygdala response has also been reported for patients with anxiety disorders. For instance, when social phobias and healthy controls were presented with emotional facial expressions and scrambled image, the left amygdala, left insula and structures involved in face recognition showed stronger activation in the patient group than in the control group (Gentili et al., 2008). A recent systematic review on neuroimaging studies conducted on specific phobias (Linares & Trzesniak, 2012) also pointed out that the common structures greatly activated by phobia-related stimuli in patients across studies include the amygdala, insula, anterior cingulated cortex and prefrontal and orbitofrontal cortex. Patients with GAD were also reported to exhibit greater bilateral dorsal amygdala reactivity to cues preceding both aversive and neutral pictures than healthy controls, suggesting that enhanced anticipatory anxiety or worry experienced by the patients may be associated with increased amygdala responsivity (Nitschke et al., 2009). Another study (Brunetti et al., 2010) compared the amygdala reactivity to aversive and neutral pictures between the people who experienced traumatic

events and subsequently developed PTSD and the people whose traumatic experience did not lead to PTSD. Whilst the non-PTSD group showed enhanced amygdala response to only emotionally negative stimuli, the PTSD group displayed high amygdala reactivity to both emotional and neutral stimuli. The levels of the amygdala activation were positively correlated with the symptom severity. Contrary to the reports for other anxiety disorders, an fMRI study comparing the amygdala reactivity to emotional/neutral facial expressions between OCD patients and healthy controls reported attenuated amygdala activation across all face conditions in the OCD group in comparison to the controls (Cannistraro et al., 2004).

**Bed Nucleus of the Stria Terminalis: Possible dissociation between fear and anxiety**

The sensory interface LA of the amygdala projects not only to CE but also to the bed nucleus of the stria terminalis (BNST), specifically the fibres from LA go right through CE to BNST (M Davis & Whalen, 2001). Since BNST projects to the same target regions as CE, i.e. hypothalamus and brain stem areas, it had been hypothesized that lesions of BNST would produce the same disruptive effects in the expression of fear responses as do the lesions of CE (M Davis & Whalen, 2001). However, excitotoxic lesion of BNST in rats produced no effect on either conditioned freezing or arterial pressure responses to acoustic CS in fear conditioning (J. E. LeDoux et al., 1988). Also, electrolytic lesions of BNST did not block acquisition of fear-potentiated startle to explicit visual CS (Gewirtz, Mcnish, & Davis, 1998; Hitchcock & Davis, 1986). These evidences indicate that despite similar anatomical connections, BNST and CE are functionally dissociated (David L Walker, Toufexis, & Davis, 2003).

Walker and Davis (D L Walker & Davis, 1997) proposed a hypothesis that whilst CE mediates short-duration phasic responses to specific threat stimuli, BNST mediates long-duration sustained responses to continual or unpredictable presentations of threat-related stimuli. This functional difference between CE and BNST is paralleled by the difference in the conventional definition between fear and anxiety, respectively. Whilst fear is a short-lived sensation elicited by an acute presentation of specific objects, anxiety is a long-lasting sensation evoked by unspecific and unpredictable objects (David L Walker et al., 2003). This hypothesis has been tested with a light-enhanced startle paradigm, which exposes rats to either a dark or bright light condition alongside deliveries of acoustic startle stimuli. Rats are naturally averse to bright light; therefore, when placed in the light condition they remain in a state of sustained apprehension, which is expressed as a greater startle response, than in the dark condition. The anxiogenic nature of the light was attested by significant reduction of

the light-enhanced startle responses following the administration of benzodiazepine and non-benzodiazepine-related anxiolytics (David L Walker & Davis, 2002). Walker and Davis (D L Walker & Davis, 1997) selectively infused either AMPA receptor antagonist NBQX, which blocks glutamate activation of the receptor, or vehicle into CE, LA or BNST of rats and compared their startle responses in the light-enhanced startle test, which evokes sustained fear, and in the fear-potentiated startle test, which elicits conditioned phasic fear response. The results revealed that whilst the NBQX infusion to CE blocked the fear-potentiated but not the light-enhanced startle responses, the infusion to BNST blocked the light-enhanced startle but not the fear-potentiated responses. The infusion to LA disrupted both responses. This doubly dissociated outcome between CE and BNST infusions supported the hypothesis that whilst the former structure is involved in processing phasic fear cue, the latter is implicated in processing sustained anxiety-provoking stimuli. This differentiation between fear - specific cue - phasic response and anxiety - contextual stimuli - sustained response has also been tested in humans. Following an administration of benzodiazepine-related anxiolytics, subject's startle responses were compared on the fear-potentiated startle test and in the dark-enhanced startle test where darkness induces sustained apprehension in humans. The results revealed that whilst the anxiolytic compounds blocked the dark-enhanced startle and baseline startle responses of the fear-potentiated test, the cue specific startle response was insensitive to the anxiolytics (Johanna M P Baas et al., 2002).

Furthermore, BNST's involvement in processing long-duration cues as opposed to short-duration was examined by measuring fear-induced behavioural suppression (Waddell, Morris, & Bouton, 2006). Prior to receiving either BNST or sham lesions, rats were trained to lever-press for food delivery. Upon recovery, the rats were subjected to the same paradigm superimposed with the presentations of short (60s) or long (10min) acoustic CS's paired with foot-shocks. Both BNST and sham lesioned rats showed a significant behavioural suppression with the short CS, indicating successful fear conditioning. In contrast, to the long CS, whilst the sham lesion produced behavioural suppression, the BNST lesion had no effect, suggesting that BNST is essential for processing long-duration threat cue. The BNST's role in contextual conditioning has also been investigated. The same authors subjected the BNST and sham lesioned rats to a reinstatement paradigm. The reinstatement is a return of extinguished fear response that occurs when the subjects are placed back in the same context in which they had previously acquired the conditioned fear response. It is a measure of context conditioning since being placed into a different context does not reinstate the extinguished fear (Bouton & Bolles, 1979). Whilst the sham lesioned rats successfully reinstated the extinguished fear response, the BNST lesioned rats did not, suggesting that

the BNST has a critical involvement in contextual conditioning (Waddell et al., 2006). Another study (Sullivan et al., 2004) cross-compared the roles of CE and BNST on cued and contextual conditioning. Whilst lesions to CE in rats significantly attenuated the conditioned freezing and stress-induced corticosterone response to both cued and context CS's, lesions to BNST reduced these behavioural and endocrine responses to the context but not to the cue. This suggests the BNST's involvement is not only in the processing of contextual stimuli but also the expression of both behavioural and HPA axis responses. The baseline response in cued fear conditioning is also considered as reflecting a contextual conditioned response. In a fear-potentiated startle paradigm, whilst sham lesioned rats exhibited a gradual increase in the startle response during the baseline, this baseline startle response was blocked in the BNST lesioned rats, further supporting the BNST's crucial role in context conditioning (Gewirtz et al., 1998).

In addition, BNST's involvement has been reported not only in mediating conditioned but also unconditioned responses. The lesions to BNST in rats significantly attenuated anxiety-related responses in the EPM (Duvarci et al., 2009; Waddell et al., 2006). When exposed to a human intruder, rhesus monkeys displayed fear-induced freezing accompanied by significant activation of the brain area including BNST and the shell of the nucleus accumbens, as assessed by positron emission tomography (PET) (Kalin et al., 2004).

When tested on fear discriminative conditioning in which subjects are required to differentiate $CS^+$ paired with aversive US and $CS^-$ paired with absence of US, sham lesioned rats were able to discriminate the CS's, but the BNST lesioned rats could not (Duvarci et al., 2009). This over-generalization of fear response has been proposed as a pathogenic marker of enhanced trait anxiety in humans (Lissek et al., 2010). In a neuroimaging study with human subjects (Somerville, Whalen, & Kelley, 2010), increased activation of BNST is associated with higher trait anxiety level. In this study, healthy subjects with varying trait anxiety performed an environmental threat-monitoring task where a stimulus line continuously fluctuated in height, providing information relevant to subsequent risk for electric shock. The results showed that the individuals with greater trait anxiety displayed increased overall activation of BNST and exaggerated tracking of threat proximity correlated with BNST and insula activations. These activations were accompanied by an enhanced skin conductance response and exaggerated heart rate increase. Few studies have been conducted for BNST functionality among anxiety disorder patients. In a recent fMRI study (Yassa, Hazlett, Stark, & Hoehn-Saric, 2012), patients with GAD and healthy controls performed a gambling task with conditions of low or high uncertainty for monetary loss. Although the task did not involve

any explicit emotional stimuli, the condition of high uncertainty was intended to elicit a stressful response and sustained anxiety. The results revealed that, compared to the control subjects, the GAD patients demonstrated decreased activity in the amygdala and increased activity in the BNST during the condition of high versus low uncertainty. Given the results, the authors proposed that in GAD patients the amygdala may be engaged early in the course of a stressful or threatening event, but quickly disengages to allow BNST to maintain a continuous anxious state and that this process may be more exaggerated compared to non-anxious individuals.

**Medial Prefrontal Cortex: Role in the expression and recall of fear/anxiety memory**

A number of studies have been conducted to investigate a role of the prefrontal cortex (PFC) in fear and anxiety using rodent models. PFC is divided into several sub-regions that appear to be anatomically and functionally distinctive. The ventromedial prefrontal cortex (vmPFC) has been suggested to play an important role in affective processing through its ubiquitous connections with subcortical 'emotional' systems such as the amygdala and related structures (R. Davidson & Irwin, 1999). A number of studies using a fear conditioning paradigm showed vmPFC's involvement in the expression and recall of fear. For instance, the rats that had their vmPFC temporarily inactivated by infusions of tetrodotoxin could acquire conditioned fear responses but displayed significantly reduced freezing compared with the controls, suggesting that vmPFC may actively support the expression of fear (Sierra-Mercado, Corcoran, Lebrón-Milad, & Quirk, 2006). The pharmacological inactivation or permanent lesions of vmPFC impaired the recall of extinguished fear, suggesting that this region also play an important role in emotional regulation such as inhibition of fear responses (Morgan, Romanski, & LeDoux, 1993; Sierra-Mercado et al., 2006). The involvement of vmPFC in unconditioned anxiety responses has also been investigated. Mice that received pharmacological inactivation, i.e. infusion of $Na^+$ channel blocker lidocaine, of either ventromedial orbital cortex (vMO) or infralimbic (IL) vmPFC, both sub-regions of vmPFC, were subjected to a predatory threat in various situations. The results showed that whilst the inactivation of IL vmPFC significantly reduced anxiety-like defensive response in mice confronted with a hand-held anesthetized rat in comparison to both control and vMO inactivated mice, the inactivation of vMO significantly enhanced anxiety-like defensive response toward a barricaded live rat compared to the control and IL vmPFC rats. The authors suggested that although vmPFC is sensitive to threatening situations and events, the regions within vmPFC, especially IL and vMO may exert complimentary yet dissociable roles

in the processing of ethologically relevant threat stimuli (Wall, Blanchard, Yang, & Blanchard, 2004).

As noted in previous paragraphs, the studies for the role of BNST in emotional processing highlighted the difference between fear and anxiety, as fear may be associated with phasic response to threat cue stimuli whereas anxiety may be related to sustained response to threat-related context. The prefrontal involvement in context conditioning has been investigated in several studies. An earlier study (Morgan & LeDoux, 1999) demonstrated that the lesions to lateral PFC in rats reduced fear reactivity to contextual stimuli whilst the lesion did not affect the acquisition or extinction of cued stimuli. A more recent study (Resstel, Joca, Guimarães, & Corrêa, 2006) examined the effect of temporal inactivation of vmPFC on context conditioning. Prior to the pharmacological manipulation with the non-selective synapse blocker $CoCl_2$ or vehicle injection, all rats were conditioned to the context with foot-shock. The comparison of the manipulated and vehicle groups upon the re-exposure to the context revealed that the inactivation of vmPFC significantly reduced the conditioned freezing response and attenuated the mean arterial pressure and heart rate increases to the context compared to the vehicle group, suggesting an involvement of vmPFC in expression of not only behavioural but also cardiovascular fear responses to contexts.

As described above, animal studies indicate the importance of the medial prefrontal area, especially through its functional influence on subcortical emotional systems, in expression and regulation of emotional responses. A recent fMRI study (S. Lang et al., 2009) investigated the involvement of human medial prefrontal cortex (mPFC) in the acquisition and extinction of contextual conditioning. The acquisition of conditioned responses was accompanied by increased activations of the anterior cingulate cortex (ACC) which is located within mPFC, left hippocampus and amygdala. The extinction was accompanied by enhanced activation of dorsal ACC. Connectivity analysis revealed correlated activity between the dorsal ACC, left posterior hippocampus and amygdala during the acquisition. These findings imply that mPFC may exert an inhibitory influence on the amygdala and hippocampus that are activated by threat-related stimuli.

**Orbitofrontal Cortex: Regulation of fear/anxiety-related responses**

In primate brain, the orbitofrontal cortex (OFC) occupies the ventral surface of the PFC. The OFC receives inputs from all sensory modalities and shares bidirectional direct connections with the structure implicated in emotional processing such as the amygdala, hippocampus

and lateral hypothalamus (Kringelbach & Rolls, 2004; Milad & Rauch, 2007). The function of the OFC in anxiety/fear processing has been investigated in both rodent and non-human primate models of anxiety. The rats that received OFC lesion were significantly quicker to eat novel foods in the hyponeophagia test and more aggressive toward a conspecific in the social interaction test than the controls. As OFC is implicated in representing the values of goals or outcomes, the authors interpreted the results as that the lesioned rats were unable to integrate the potential risk of the anxiogenic nature of novel food or conspecific with the response outcomes, which led to the uninhibited behaviours (Rudebeck et al., 2007). Another study (Lacroix et al., 2000) exposed the rats that received lesions to either mPFC including ventral and medial OFC or lPFC including lateral OFC to the EPM, OFT and fear conditioning paradigm. Whist the former group showed significantly reduced anxiety-related responses in the unconditioned anxiety tests, the latter group showed enhanced fear-conditioned responses. Both groups developed stronger fear-conditioned responses to the context than did the controls. The results suggested not only the general role of OFC in fear and anxiety processing but also differential functions of its sub-regions, i.e. the medial portion may modulate the responses to threat-related environmental contingencies, whereas the lateral part may control the cue-related conditioned fear responses.

In non-human primate models, rhesus monkeys that received lesions to OFC were tested on the human intruder test (Kalin, Shelton, & Davidson, 2007). The paradigm consisted of three different conditions. In the alone condition, the animal was placed alone in a test cage. In the no-eye-contact condition, a human intruder enters the cage but does not make an eye contact. In the stare condition, the intruder makes a direct eye contact with the animal, which is known as highly offensive gesture to rhesus monkeys. Whilst in the stare condition the threat seems to be more imminent, greater uncertainty in the no-eye-contact condition may simulate anxiety rather than fear. The results showed that whilst the OFC lesion had no effect on both the alone and stare conditions, in the no-eye-contact condition the lesioned monkeys displayed significant reduction in the threat-induced freezing compared to the controls. When both groups were exposed to snake stimuli, the OFC lesioned animals displayed greater threat-induced behavioural inhibition than the controls. These responses were similar to the ones observed with the lesions of the CE of the amygdala (Kalin et al., 2004). However, whilst the CE lesions interfered with both the behavioural and endocrine, i.e. decreased HPA axis activity, responses, the OFC lesion blocked the behavioural response only, suggesting dissociable roles between CE and OFC in the expression of anxiety-related responses. A previous study demonstrated a significant activation of BNST in the rhesus monkeys when confronted with a human intruder (Kalin, Shelton, Fox, Oakes, & Davidson,

2005). Subsequently, Fox and colleagues (A. S. Fox et al., 2010) investigated the hypothesis that OFC may mediate anxiety-related behavioural response via modulating BNST activity. OFC lesioned monkeys were exposed to a human intruder and subsequently scanned for neural activities with PET. The results revealed that the OFC lesion not only reduced the freezing response in the no-eye-contact condition but also was associated with decreased activation in BNST, supporting the hypothesis that anxiety responses may be mediated by the OFC-BNST circuit. The investigations of prefrontal functions in the primate models of anxiety are further described in Chapter 3.

**Neurobiological underpinnings of the individual differences in trait anxiety**

Most of the animal models investigating the neurobiology of anxiety do not explicitly differentiate trait from state anxiety. One way of examining neural underpinnings of trait anxiety is to first observe the individual differences on the anxiety spectrum, then compare or neurobiologically manipulate those that show extreme phenotypes. Duvarci and colleagues (Duvarci et al., 2009) demonstrated that the abnormality in BNST may contribute to enhanced trait anxiety. When tested for an ability to discriminate safety from danger cue, non-lesioned control rats exhibited a bimodal distribution: the individuals at one end showed differential responses between the cues, whereas at the other end were those with poor discrimination, a pathogenic marker of high trait anxiety (detail explained in Chapter 2). Subsequent analysis for context conditioning and testing on the EPM revealed that the poor discriminators showed greater conditioning to the aversive context and stronger anxiety-related behaviours than those with better discriminative ability. Interestingly, in those parameters the BNST lesioned rats were statistically indistinguishable from those with high discriminative ability showing less anxious phenotypes. Given the results, the authors suggested that abnormal excitability in BNST may underlie trait vulnerability to pathological anxiety.

In non-human primate model, Fox and colleagues (A. S. Fox, Shelton, Oakes, Davidson, & Kalin, 2008) demonstrated that increased responsivity of the amygdala and its related structures such as the hippocampus, BNST and periaqueductal grey (PAG) are associated with dispositional tendency to have anxious temperament, a risk factor for anxiety disorders in young developing individuals. The authors first screened a large sample of preadolescent rhesus monkeys for behavioural inhibition, a putative biomarker for anxiety vulnerability in human children, under the conditions of isolation and exposure to unfamiliar human intruder. The groups of high, middle and low responders were selected. After four months, they were

re-exposed to the same stressful conditions followed by a non-stressful condition while their brain activities were monitored with PET. The observed anxious temperaments of the individuals were stable across the two occasions. This trait-like anxious temperament was associated with an increased activity centred at the right amygdala and surrounding associated structures including bilateral amygdala, bilateral BNST, bilateral hippocampus and PAG. The brain activities associated with anxious temperament under a safe and familiar context may indicate trait vulnerability to pathological anxiety. The region where anxious temperament was significantly correlated with brain metabolism across the stressful and non-stressful conditions also included bilateral amygdala, bilateral hippocampus and PAG. More recent study investigated the heritability of the anxious temperament and its associated brain activities by analyzing pedigree relationships in a large sample of rhesus monkeys for those phenotypes. The analysis revealed that the anxious temperament as well as the associated activation in the hippocampus was significantly heritable. The amygdala activation, however, was not found to be significantly heritable.

Related to the heritability of anxious temperament, a similar genetic polymorphism to the human serotonin transporter (5-HTT) gene polymorphism, a genotypic variation associated with vulnerability to anxiety and depression, and increased amygdala responsivity to potential threat (detail described in next section), has been identified in rhesus monkeys (K. Lesch, Meyer, Glatz, & Flügge, 1997). Consistent with the functional effect of human 5-HTT gene polymorphism, the short allele (S) is associated with lower expression of the transporter protein than the long allele (L). When S and L carrier groups of rhesus monkeys were exposed to the isolation and unfamiliar human threat while their brain activities were monitored with PET, different brain regions were found to be activated between the genotypes for the different stressor conditions. The S carriers exhibited greater amygdala activation during the isolation condition and increased reactivity of BNST to the human threat in comparison to the L carriers. S carriers also showed greater activations in the insula, OFC and lateral prefrontal cortical regions during both conditions. These results suggest not only that the abnormal neurobiological activities of those brain structures may underlie the heritability of anxious temperament but also that different intermediate brain phenotypes responsive to different threat-related context may together or independently contribute to the trait anxiety (Kalin et al., 2008).

As suggested in the non-human primate model, individuals high in anxiety may be impaired with this prefrontal inhibitory control of subcortical emotional circuits. An fMRI study (Bishop, Duncan, Brett, & Lawrence, 2004) tested human subjects with varying levels of state anxiety

on an attentional task involving emotional distractors and found that highly anxious individuals displayed both generally lower levels of rostal ACC activity and reduced recruitment of the lateral PFC in response to threat-related distractors. Another more recent study (Indovina et al., 2011) reported that high trait anxious individuals showed increased amygdala reactivity to cues predicting an aversive event; these individuals also showed reduced ventral PFC recruitment in response to both threat-related cue and context. These findings led to a hypothesis that trait vulnerability to anxiety may be associated with the uncoupling of the sensory stimulus-driven mechanisms centred on the amygdala and the prefrontal top-down control mechanism. The influence of trait anxiety on the prefrontal cognitive functionality is further discussed in Chapter 4.

## 1.5 Genetics of Anxiety

### Family and Twin Studies

In the research for the aetiology of anxiety disorders, the heritability of anxiety has been a major focus of investigation. One of the means to examine whether anxiety is inherited is to look at the risk for anxiety disorders in twins and families.

***Panic Disorder*** In the family studies of heritable disorders, the odds ratio is used to describe the probability of developing the disorder for a person who has the patient in his/her immediate family. A family study (Fyer et al., 1996) looking at the risk of developing PD found the morbidity risk of 9.5% for the families of PD patients (n=220) and 3.0% for the unaffected families (n=231). The odds ratio is calculated by dividing the former by the latter. With statistical adjustments, the odds ratio turned out to be 3.4. That is, if one has a PD patient in his/her first-degree relatives, the risk of developing PD is 3.4 times greater than someone without any PD affected family members. The summary odds ratio of five independent studies (Fyer et al., 1996; Horwath et al., 1995; Maier, Lichtermann, Minges, Oehrlein, & Franke, 1993; Mendlewicz, Papadimitriou, & Wilmotte, 1993; R. Noyes et al., 1986) for PD was found to be 5.0, which strongly supports the familial component in liability to PD (Hettema, 2001).

In twin studies, the proband-wise concordance, which is the proportion of the twins whose both members are affected by the disorder among the twins who have at least one member affected, is used to describe the risk of being affected by the disorder given that co-twin is affected. One study (Perna, Caldirola, Arancio, & Bellodi, 1997) examined 60 twin pairs of mixed sex whose at least one member is affected by PD. The proband-wise concordance was found to be 73.0% among monozygotic twins, whereas among dizygotic twins, the measure was found to be 0.0%, suggesting very strong genetic influence for the occurrence of PD. Two more studies (Torgersen 1983, n=598; Skre et al. 1993, n=81) reported the proband-wise concordance for monozygotic twins to be 30.8% and 41.7%, and for dizygotic twins, 0.0% and 16.7%, respectively. These results indicate the strong influence of genetic factors in the aetiology of PD. Other twin studies with large samples (Kendler et al. 1993, n=2163 female; Scherrer et al. 2000, n=6724 male) investigated how genetic and environmental factors contribute to the risk. Similar results were obtained between the studies, that 30-40% of the variance in liability for PD was accounted by additive genetic factor, indicating moderate genetic influence in the aetiology of the disorder. The remaining

variance was due to individual-specific environment and common family environment played no role in the occurrence of PD among the twins.

***Generalized Anxiety Disorder*** A family study (R. J. Noyes & Clarkson, 1987) that compared 123 family members of GAD patients and 113 family members of unaffected controls reported the odds ratio of 6.6, that is 6.6 times greater risk of the disorder for someone with a GAD patient in family. Similarly, another study (Mendlewicz et al., 1993) examining 102 family members of the patients and 130 of controls reported the odds ratio of 6.1. These results indicate relatively high risk for the disorder among the families of GAD patients. When the data from two twin large samples (Scherrer et al. 2000, n=6724; Hettema 2001, n=6200) were analysed for genetic and environmental factors for the liability to the disorder, a best fitting model predicted 31.6% of the variance was attributed to additive genetic factors. The remaining variance was due to individual specific environment and only small portion of the variance in women was accounted by common familial environment. Both family and twin studies of GAD provide convincing evidence of genetic influence underlying GAD aetiology.

***Phobia*** The summary odds ratio across four family studies of phobic disorders including simple phobia, social phobia and agoraphobia (Fyer & Mannuzza, 1995; Mannuzza & Schneier, 1995; R. Noyes et al., 1986; M. Stein & Chartier, 1998) was calculated to be 4.1, which strongly supports a familial risk for phobic disorders (Hettema, 2001). The analyses of large twin samples with phobia patients including social phobia, agoraphobia, animal phobia and situational phobia (K S Kendler et al. 1992, n=2163 female; K SKendler et al. 2001, n=2396 male) reported that twin resemblance in the disorders was due solely to additive genetic factors for all but animal phobias, indicating substantial genetic influence.

***Obsessive Compulsive Disorder*** The data from five family studies (Black, Noyes, Goldstein, & Blum, 1992; McKeon & Murray, 1987; Pauls, Alsobrook, Goodman, Rasmussen, & Leckman, 1995; Rasmussen, 1993) of OCD indicated strong familial aggregation of OCD. The summary odds ratio across these studies was 4.0, which suggests high risk for developing the disorder among those with OCD patients in their immediate families (Hettema, 2001).

Overall, the data from family and twin studies provide strong evidences for familial aggregation in anxiety disorders. The summary odds ratios were similar across different types of anxiety disorders, ranging from 4 to 6, suggesting that the major source of familial risk may be genetic. This was supported by the analyses that genetic factors accounted for

substantial proportion of the variance in liability to anxiety disorders together with other environmental factors.

**Candidate Gene Approach**

With supporting evidences from family and twin studies that genetic factors may underlie the differences in individual's basal anxiety level and the risk for anxiety disorder, researchers have turned to the search for specific genes related to anxiety phenotypes. Symptoms of anxiety are mediated by the actions of specific neurotransmitters and neuropeptides (Finn, Rutledge-Gorman, & Crabbe, 2003). Therefore, the genes involved in neurotransmitter pathways have been primary candidates for the genes regulating anxiety. The gene search that is based on the functional hypothesis of the proteins implicated in physiological processes of the phenotype is called candidate gene approach (Savitz & Ramesar, 2004; Tabor, Risch, & Myers, 2002).

***Dopamine D4 Receptor*** Dopamine is a neurotransmitter involved in various regulatory roles in behaviour and cognition such as voluntary movement, motivation, sleep, mood, attention and learning. Dopamine D4 receptors (DRD4) are expressed in the dorsolateral prefrontal and entorinal cortex, hippocampus, hypothalamus, globus pallidus, substantia nigra and dorsal-medial thalamus (Paterson, Sunohara, & Kennedy, 1999). The polymorphism was found in the repeated regions of an exon that codes for the portion of the receptor protein mediating intracellular signalling. The strategic position of this polymorphism suggests that DRD4 receptor variants are functionally distinctive (Asghari & Sanyal, 1995). Ebstein and colleagues (Ebstein, Novick, Umansky, & Priel, 1996) tested 124 Jewish healthy volunteers with mixed ethnics, age and sex on a personality questionnaire and examined the association with DRD4 polymorphism. The authors reported that the most commonly found genotypes were 4-repeat homozygous and 4/7-repeat heterozygous. The results revealed that individuals with the 7-repeat allele exhibited significantly elevated novelty seeking trait compared to those with other alleles. A meta-analysis of 29 association studies (Gestel & Broeckhoven, 2003) reported a significant relationship between the polymorphism and novelty seeking trait in only eight studies. The association with other personality traits has not been found. In addition to the repeat number polymorphism, a number of single-nucleotide polymorphism (SNP) has been identified in the promoter region of DRD4 gene (Mitsuyasu, Hirata, & Sakai, 2001). Okuyama and colleagues (Okuyama, Ishiguro, & Nankai, 2000) investigated an association between C/T polymorphism (-521C/T) and personality trait. The results revealed that the C allele was associated with higher novelty seeking trait and higher

gene expression than the T allele. A meta-analysis (Savitz & Ramesar, 2004) reported that four out of nine association studies found a significant relationship between the C allele and high novelty seeking trait. Although not conclusive, the studies so far suggest a possible functional association between DRD4 polymorphism and novelty seeking trait. As novelty seeking trait has been associated with altered anxiety temperament (Ballaz, Akil, & Watson, 2007; Stead et al., 2006), the polymorphism may also contribute to individual differences in anxiety trait.

***Dopamine D2 Receptor*** Since dopamine D2 receptor (DRD2) agonist-induced reactivity had been associated with the trait of positive emotionality, the DRD2 gene has also been considered as a candidate gene for personality modulator (Depue, Luciana, & Arbisi, 1994). Noble and colleagues (Noble et al., 1998) assessed 119 healthy volunteers for temperament traits and for DRD2 and DRD4 polymorphic genotypes. Novelty seeking trait was significantly associated with either three minor alleles (A1, B1, Intron 6 1) of DRD2 polymorphisms or the 7-repeat allele of DRD4 gene. Although the DRD2 and DRD4 polymorphisms were individually associated with the novelty seeking trait, when the two polymorphic effects were combined, the greatest contribution to the trait was observed. The DRD2 variants were also associated with reward dependence and persistent traits. No other studies have reported an association between DRD2 polymorphism and anxiety-related traits.

***Dopamine D3 Receptor*** The polymorphisms of dopamine D3 receptor (DRD3) gene have also been examined for the association with personality traits. Henderson and colleagues (Henderson et al., 2000) genotyped 2327 volunteers and assessed them for vulnerability to anxiety, depression and alcohol misuse. The sample was divided into two groups (862 and 1465) and analysed separately. The analysis of the first group revealed that one of the alleles (Ser) of the polymorphisms of DRD3 (Ser9Gly) exerted significant effect on neuroticism and behavioural inhibition. There was also a trend that this genotype was associated with depression and anxiety. However, when the analysis was extended to the second group, none of the associations maintained statistical significance.

***Dopamine Transporter*** Another candidate gene from the dopamine system is the dopamine transporter gene (DAT1). An association study on cigarette smokers (Sabol, Nelson, & Fisher, 1999) reported a significant effect of the polymorphism (DAT1*9) on cessation of smoking and low novelty seeking trait, suggesting that the altered dopamine transmission may reduce the need for novelty and reward by external stimuli, including cigarettes.

However, subsequent studies (Gestel & Broeckhoven, 2003; Jorm & Henderson, 2000) failed to replicate the findings or locate any association with trait anxiety.

***Catechol-O-Methyltranferase*** Since Catechol-O-methyltransferase (COMT) is a metabolic enzyme that degrades catecholamines such as dopamine, epinephrine and norepinephrine, COMT gene has been investigated as a candidate gene involved in personality traits. An association study on 2085 volunteers (Eley, Tahir, & Angleitner, 2003) found a weak effect of the COMT polymorphism on neuroticism only when females and males were analysed separately. Another study (Henderson et al., 2000) did not find any association with personality traits. In rodent models, however, a study with the gene knockout mice (Gogos & Morgan, 1998) reported the effect of the absence of COMT on neurotransmission to be brain region specific and sexually dimorphic. Compared to the wild-type, the gene knockout female mice showed significant reduction of dopamine in frontal cortex and enhanced anxiety-like trait on the dark/light box and open field tests. No difference between the wild-type and male knockouts was found, suggesting that COMT gene is expressed differentially between sexes.

***Serotonin Receptors (1A, 1B, 2C)*** Neurotransmission mediated by serotonin (5-HT) contributes to many physiologic functions such as motor activity, food intake, sleep and reproductive activity, as well as to cognition and emotional states (K. P. Lesch et al., 1996). There have been growing evidences that disturbances in the regulation of serotonergic neuronal activities underlie psychiatric disorders such as anxiety and depression (Piñeyro & Blier, 1999). More than 14 subtypes of 5-HT receptor have been identified (Finn et al., 2003). The polymorphisms of many of these receptor types have been investigated for possible connections with psychiatric traits in human association studies and genetic animal models.

A meta-analysis (Anguelova, Benkelfat, & Turecki, 2003) on 16 association studies that examined 20 different 5-HT receptor polymorphisms, most of them SNPs, for major depressive disorder reported inconclusive results; only few individual studies provided evidences for the association and most were non-significant. Since most of the individual 5-HT receptor loci were investigated by only one study, it was difficult to draw definite conclusions. More studies are needed.

In rodent model, Heisler and colleagues (Heisler & Chu, 1998) tested 5-HT-1A receptor knockout mice on a variety of anxiety tests including the elevated zero maze, open field test and novelty object test. Across the tests, the knockout mice consistently displayed increased anxiety-related behaviours compared to the wild-type mice. Two other studies (Parks &

Robinson, 1998; Ramboz & Oosting, 1998) using the 5-HT 1A deficient mice produced from different mice strains replicated the results that the lack of 5-HT 1A receptor led to heightened level of unconditioned anxiety responses. When the knockout mice were tested on fear conditioning paradigm, they exhibited greater freezing and increased tachycardia than the wild-type mice, supporting the involvement of the 5-HT 1A receptor in the regulation of anxiety responses (Gross, Santarelli, & Brunner, 2000).

In contrast to the 5-HT 1A studies, the mouse models of 5-HT 1B deficiency have produced inconsistent reports. When Zhuang and colleagues (Zhuang, Gross, & Santarelli, 1999) tested both 5-HT 1A and 1B receptor knockout mice on a variety of anxiety tests including the EPM, open field test and resident-intruder test, the opposite effect was observed between the two strains. Whilst the 5-HT 1A knockouts displayed increased anxiety and decreased aggression, the 5-HT 1B mice showed decreased anxiety and increased aggression. However, another study (Ramboz et al., 1996) reported that the 5-HT 1B mice displayed increased aggression and no difference in anxiety from the wild-types. It is suggestive that 5-HT 1B receptor may be involved more in the regulation of aggression than anxiety, but more studies are needed.

5-HT 2C receptor is a widely distributed postsynaptic receptor that has been implicated in the serotonergic regulation of feeding behaviour and anxiety state (Finn et al., 2003). When the 5-HT 2C receptor knockout mice were tested on a variety of unconditioned anxiety tests, they consistently displayed less anxious phenotype than the wild-type mice (Heisler, Zhou, & Bajwa, 2007). Another study (Kimura & Stevenson, 2009) tested the transgenic mice over-expressing the 5-HT 2C receptors in the cerebral cortex, hippocampus and amygdala on several anxiety tests and reported that the over-expression of the receptor led to increased anxiety-related behavioural responses. These findings suggest that 5-HT 2C receptors may be involved in the up-regulatory mechanisms of anxiety level.

***Serotonin Transporter*** The most well studied candidate gene from the serotonin system is the 5-HT transporter (5-HTT) gene, which is found on human chromosome 17 on location 17q11.1-q12 (K. Lesch & Mössner, 1998). Most studies focused on the length variation polymorphism that occurs in the promoter region of the gene (5-HTT-linked polymorphic region or 5-HTTLPR). The short variation has 14 repeats of a 44bp sequence whilst the long version has 16 repeats. The short (S) allele is transcribed less efficiently than the long (L) allele, which results in a decreased 5-HTT expression and 5-HT reuptake in the synaptic clefts (K. Lesch & Mössner, 1998).

Association was reported between the polymorphism and anxiety-related traits; carriers of the S allele had higher scores in neuroticism and harm avoidance personality traits than the L homozygous individuals (K. P. Lesch et al., 1996). Since this report, many studies have tried to replicate the results; however, there have been conflicting outcomes. A meta-analysis of 36 association studies (Savitz & Ramesar, 2004) reported 18 of these studies showed significant association between the polymorphism and anxiety-related personality traits. However, six of the 18 studies observed the association in the opposite direction, that is, the S allele was related to lower level of anxiety trait. Therefore, only 12 studies (33%) reported the association of the S allele with enhanced anxiety-related trait. These mixed results led researchers consider a possibility that environmental factors during the course of development may interact with the genetic makeup to produce psychological and behavioural endophenotype. Therefore, the genotype without taking account of the environmental variants does not necessary predict the variance in the phenotype. Related hypotheses are described in Chapter 4.

In support for the association with anxiety-related traits, an fMRI study (Hariri et al., 2002) reported that the S allele carriers exhibited increased reactivity in the amygdala in response to fearful stimuli compared to the L allele homozygous individuals. In addition, another fMRI study (Pezawas & Meyer-Lindenberg, 2005) reported reduced grey matter in perigenual anterior cingulated cortex and amygdala among the S allele carriers. These regions are critical for processing of negative emotion.

The second extensively studied polymorphism in 5-HTT is the variable number tandem repeat (VNTR) polymorphism in the second intron of the 5-HTT gene. Three allelic variations with 9, 10 or 12 repeats have been identified. A meta-analysis (Savitz & Ramesar, 2004) reported that three out of nine studies observed a significant association between the polymorphism and anxiety-related traits. Among these, Evans and colleagues (Evans, Battersby, & Ogilvie, 1997) reported high anxiety traits in the individuals with 9 or 10 repeats. Tsai and colleagues (Tsai, Hong, & Cheng, 2002) found the association between 10 or 12 repeat genotypes and high harm avoidance trait. Melke and colleagues (Melke & Landén, 2001) reported elevated anxiety trait in female 12 repeat carriers.

In transgenic rodent model, Holmes and colleagues (Holmes & Yang, 2003) created mutant mice whose 5-HTT gene was constitutively inactivated. The 5-HTT binding sites in brain were absent in 5-HTT homozygous (-/-) null mutant mice and were reduced 50% in 5-HTT

heterozygous (+/-) mutant mice. The -/- null mutant mice displayed robust phenotypic abnormalities on a variety of anxiety tests including the EPM, dark/light box and open field test. Across the tests, the -/- mice exhibited significantly enhanced anxiety responses in comparison to the +/- mice and +/+ controls, providing an evidence for the critical role played by 5-HTT in the regulation of anxiety.

**Quantitative Trait Loci Analysis**

Genetic differences related to anxiety are manifested phenotypically as a variation in behavioural patterns. This behavioural variation is a continuous trait that can be measured quantitatively. That is, within a population anxiety is not seen as an on/off trait. Rather, the differences in anxiety levels show continuous variation in a population, i.e. individuals are more or less anxious than others. Phenotype that is measured quantitatively is called a quantitative trait. Multiple genes, each of them have a small effect, contribute to the population variation for a quantitative trait. The locations of these genes on a chromosome are referred to as quantitative trait loci (QTL). Whilst candidate gene approach searches for a single gene whose variant may influence the workings of the encoded protein in the neural pathway of interest, QTL analysis tries to identify regions on chromosomes that contain multiple polymorphic genes modulating quantitative traits (Collard, Jahufer, Brouwer, & Pang, 2005).

The initial step of QTL analysis involves creating inbred strains of animals for a phenotype of interest (Wehner, 2001). For instance, rats are screened on a particular anxiety paradigm and selected into high or low anxiety groups based on their performances. Systematic mating of brothers and sisters within the same group for multiple generations generates an inbred strain. This inbreeding limits the number of alleles in the population and leads to genetic fixation, such that homozygosity, i.e. having two copies of the same allele, is produced at virtually all gene loci. The next step is to locate polymorphic genetic markers. Since QTL analysis involves a genome-wide analysis of multiple anonymous genes on different chromosomes, genetic markers are needed as ‘signs’ that divide the length of a chromosome into segments that may contain genes of interest. Genetic markers themselves do not necessarily affect the phenotype of interest; they can be located near or ‘linked’ to the genes controlling the trait. Polymorphic genetic markers are found by comparing the animals from different inbred strains and identifying genetic differences between them. Once genetic markers are identified, the animals from the inbred strains showing opposite phenotypes are crossed to produce F1 generation. Since both parents are homozygous, all F1 individuals are

genetically identical and show heterozygosity at all polymorphic alleles. The F1 individuals are then crossed to produce F2 generation. During prophase of meiosis in the F1 individual, paternal and maternal homologous chromosomes align together and portions of DNA strands are exchanged. In this crossover, generic markers are also transferred. The probability of two different genetic markers segregating together is high if they are located close together; however, the chance is low if they are far from each other. The markers that often segregate together are referred to as “linked”. Whilst linked markers indicate short distance between the markers, unlinked markers indicate long distance. By screening the recombinant chromosomes for the linkages between markers, researchers can construct a linkage map, which describes the position and relative genetic distances between genetic markers along chromosomes. Once a linkage map is established, the mapping population, i.e. F2 generation, is partitioned into different genotypic groups based on the presence or absence of a particular polymorphic marker or allele. These groups are then tested for the behavioural trait of interest, e.g. anxiety. If a difference is found between the groups, the marker is assumedly linked with a gene or QTL that regulates the phenotype. No difference between the groups indicates that the portion of DNA sequence linked with the marker does not contain any target gene.

A meta-analysis of seven published QTL studies on rats and mice (J Flint, 2002) reported that most of the QTLs most likely contain the target genes that modulate anxiety traits. Fifteen of the mouse’s 19 chromosomes were found to be implicated in influencing behaviours in at least one type of anxiety tests, and some chromosomal regions appear to influence almost a dozen different measures of anxiety. These include chromosomes 1, 10, 12 and 15. A target gene screen so far identified human chromosomes 1, 2, 6, 8, 10, 12, 18, 22 as homologous regions to the mouse chromosomes containing the QTLs associated with anxiety traits. An association study (Smoller & Acierno, 2001) suggested that a linkage for the vulnerability to anxiety (i.e. early onset susceptibility to anxiety disorders) is located on human chromosome 1q and 10q and that a linkage for panic disorder is located on human chromosome locus 12q13. Although the results were only suggestive at this point, the methodology has a potential for identifying human genes related to anxiety traits by using rodent QTL analysis.

Although individual findings may differ in details, growing evidences from different approaches strongly support the notion that individual differences in anxiety phenotype have genetic bases. Individuals are born with a particular set of gene variants or genotypes.

Genes provide potentials for developing specific phenotype. For instance, carrying the S allele of 5-HTTLPR may confer a risk for anxiety disorders. However, it is through the interaction with environmental pressures that genes give rise to neurobiological mechanisms. Therefore, unique set of genes interact with individual-specific environment producing distinctive neurobiological traits. For example, whilst growing up in a stressful environment may accelerate the adverse effect of the S allele leading to serotonergic system that is vulnerable to anxiety and depression, a supporting environment may modulate the expression of the S allele into the development of neurobiological system more resistant to stress. Unique neurobiological traits such as hyper responsive amygdala or reduced prefrontal functionality generate specific behavioural response to emotional stimuli, which may be diagnosed as the symptoms of anxiety disorder. The studies described in following chapters trace back the described path by first identifying the behavioural endophenotypes of trait anxiety and then investigating the neurobiological traits underlying the phenotypes. The studies do not extend to determine the genetic traits; however, the implication of genetic influences on the phenotypes is discussed across chapters.

As described in this introduction, high levels of trait anxiety have been viewed as a vulnerability factor for anxiety disorders (Bishop, 2007; Sandi & Richter-Levin, 2009). Despite recent developments and refinements of technologies, human imaging studies still lack the level of accuracy provided by lesion or electrophysiological studies in animals. Also, such studies are correlative and do not provide cause-effect mechanisms. On the other hand, rodent models have provided a basic understanding of the underlying neural mechanisms of fear and anxiety (Michael Davis et al., 2010; J. LeDoux, 2000), particularly at the subcortical level but our understanding of the cortical regulation of negative emotion is still relatively poor. Thus, there is a need for non-human primate models which can bridge the basic research in rodents with the observations in humans. Thus, the major aim of my research project, described in this thesis, has been to develop a non-human primate model of trait anxiety, which is expected to provide a foundation for further neurobiological and genetic research into trait anxiety. The model animal was the common marmoset (*Callithrix jacchus*). The starting point was to identify individual differences in the behavioural phenotype of trait anxiety in this species of monkey. The first step was to apply the findings from human anxiety studies, particularly with respect to the association between enhanced trait anxiety and over-generalisation of fear/anxiety responses. Having discovered that the over-generalisation of emotional responses similar to the findings in humans could be observed in a sample of the common marmoset, the second step was taken to investigate the

relationship between the over-generalisation and unconditioned anxiety-related responses to ethologically-relevant threatening stimuli. It was hypothesized that those that showed the over-generalization of emotional responses would display heightened anxiety/fear responses when encountering threatening stimuli such as a human intruder or model snake, the tests often used in non-human primate models of anxiety. Finally, having developed a marmoset model of trait anxiety with these tests, investigations on how anxiety trait would impact on cognitive functions, especially those associated with the prefrontal cortex was investigated. Two previously developed marmoset tests of cognitive flexibility shown to be dependent upon distinct regions of prefrontal cortex were used. Based on previous findings (Bishop, 2009; Indovina et al., 2011), those identified as high trait-anxious were hypothesized to show poor performance in these cognitive tests in comparison to the ones that were less anxious. These results would also provide us with possible insight into the neural underpinnings, especially of prefrontal involvement in trait anxiety mechanisms. Overall, this project aims to provide a successful new, non-human primate model of trait anxiety, which is expected to provide a foundation for further genetic, biochemical and neurobiological investigations.

# Chapter 2

## A novel test for assessing trait anxiety in marmosets: The aversive discriminative conditioning paradigm

## Abstract

Anxiety is an aversive emotional and motivational state occurring in threatening circumstances and is accompanied by changes in behavioural and physiological responses and cognitive processing (M. Eysenck, Derakshan, Santos, & Calvo, 2007). Individuals with high trait anxiety are more vulnerable to developing psychiatric mood and anxiety disorders (M. G. Calvo & Cano-vindel, 1997). However, its underlying neurobiological mechanism is not well understood. In order to forward the investigation in human anxiety, it is important to develop a reliable non-human primate model.

One of the key features of pathological / high-trait anxiety is the over-generalization of fear responses (Lissek & Grillon, 2010; Lissek et al., 2010). The aversive discriminative conditioning paradigm, in which subjects are required to discriminate a danger signal ($CS^+$) predicting an aversive outcome from a safety signal ($CS^-$), has been used to test fear discrimination in humans and rodents. Patients diagnosed with posttraumatic stress disorder and panic disorder displayed undifferentiated responses across a pair of CS's (Lissek & Grillon, 2010; Mauchnik, Ebner-Priemer, Bohus, & Schmahl, 2010) and high trait-anxious rats were poor at discriminating a safety from a danger signal (Duvarci et al., 2009).

In the current study, a cohort of marmoset monkeys was tested on the newly developed marmoset version of an aversive discriminative conditioning paradigm. The animals were presented with two auditory CS's, $CS^+$ paired with a loud noise and $CS^-$ paired with a very brief 'lights-off'. For the discriminative measure, both behavioural and cardiovascular components of the anxiety-related responses were measured.

The results revealed that seven out of the 27 animals tested failed to acquire discriminative conditioned responses between the CS's. Their successful discrimination on a subsequent appetitive discriminative conditioning task confirmed that the failure in the aversive discrimination was not due to an impaired general learning ability or impaired auditory perception. Two measures observed early on in the acquisition of the fear discrimination: heightened vigilance to the CS's and suppressed blood pressure in the baseline, predicted the animal's eventual failure on the discrimination. Since these measures appear indicative of high anxiety it is proposed that the animals that failed the discrimination were highly anxious and that these measures may be potential biomarkers of trait anxiety in marmosets.

## 2.1 Introduction

Anxiety is a mood state activated by distal or potential threat in the environment, and is associated with an adaptive defence response including arousal and vigilance (Michael Davis et al., 2010). Whilst state anxiety is experienced as a transitory emotional state that varies from moment to moment, trait anxiety is defined as a relatively stable feature of an individual (Belzung & Griebel, 2001; Gaudry, Vagg, & Spielberger, 1975). High trait anxiety can be detrimental to one's mental health since it increases the risk of anxiety disorders (M. G. Calvo & Cano-vindel, 1997).

High and low trait anxiety in humans is traditionally measured using questionnaires designed to detect individual differences in relatively stable tendency to experience more or less anxious situations. Performance on these questionnaires has subsequently been shown to reflect a variety of symptoms of high anxiety including over-generalization of fear responses (Dunsmoor, Prince, Murty, Kragel, & LaBar, 2011) and attentional bias to threat-related stimuli (E. Fox, 1994). Mice and rats have been the major experimental animal models in the field of anxiety research (Pawlak, Ho, & Schwarting, 2008). Most frequently used paradigms include the EPM, open-field arena, light-dark box and holeboard test. These tests have been used to evaluate the effect of anxiogenic and anxiolytic agents (Lister, 1987) as well as for selective breeding of high and low anxiety strains (Landgraf & Wigger, 2002; Yilmazer-Hanke, Wigger, Linke, Landgraf, & Schwegler, 2004) and detection of anxiety-related genetic variants (Jonathan Flint, 2003). In contrast, behavioural inhibition on the human intruder test has been used to characterise trait anxiety in monkeys (Kalin, 1993). This test was designed to mirror that used in children to measure extreme behavioural inhibition, a marker for an increased likelihood of developing affective disorders (Corcoran et al., 2012).

**Failure to acquire conditioned discriminative fear Is Associated with Pathological/High-Trait Anxiety**

Fear generalization theory postulates that the detrimental effect of high trait anxiety is manifested as over-generalization of fear. In healthy individuals, generalization of fear serves as an adaptive defence function (Dunsmoor, Mitroff, & LaBar, 2009; Dunsmoor, Prince, Murty, Kragel, & LaBar, 2011). When an individual encounters a novel stimulus that resembles a particular danger cue that they have already learnt is associated with an aversive event, it is sensible for that individual to display the same defence response to avoid the possible harm associated with the novel stimulus. However, displaying the defensive

behaviours towards a too broad a range of stimuli can be maladaptive (Dunsmoor et al., 2011). Such fear generalization has been associated with high anxiety and has been measured in the laboratory setting with the aversive discriminative conditioning paradigm.

The aversive discriminative conditioning paradigm has evolved from the simple aversive/fear conditioning paradigm, which has been widely used in the research of fear and anxiety. In the simple conditioning paradigm, the presentation of an emotionally neutral conditioned stimulus (CS) such as an auditory/visual cue is followed by an aversive unconditioned stimulus (US) such as electric foot-shock, loud noise and air puff. With repeated presentations of the CS-US pairings, the subject learns the CS-US association and develops a specific anticipatory response, such as freezing and increased vigilance, to the CS. In the discriminative conditioning paradigm, usually two CS's are presented. One of them ($CS^+$) is paired with an aversive US, so that it becomes a danger signal. The other CS ($CS^-$) is followed by either an absence of the US or a neutral stimulus, so that it becomes a safety signal. Repeated presentations of the CS-US pairs lead to the development of differential responses between the $CS^+$ and $CS^-$. The over-generalization of fear attenuates or eliminates this differential response. The subject develops similar level of anxiety-related response to both CS's.

By using the aversive discrimination paradigm, scientists have shown that fear generalization can be a robust marker of pathological anxiety, especially in relation to posttraumatic stress disorder (PTSD) and panic disorder (PD) (Lissek & Grillon, 2010). Grillon and Morgan (Grillon & Morgan, 1999) tested Gulf War veterans with or without PTSD diagnosis on the aversive discriminative conditioning paradigm and found that, in contrast to the non-PTSD group, the PTSD individuals failed to show differential anxiety responses (eye-blink reflex to a startle probe following CS) between the $CS^+$ and $CS^-$. Mauchnik and colleagues (Mauchnik et al., 2010) also reported that PTSD patients exhibited undifferentiated responses (skin conductance response: SCR) to both danger and safety cues in the discriminative conditioning task. These findings gave empirical evidence to the PTSD symptom, in which the patients tend to generalize fear across stimuli that resemble the cue associated with traumatic event.

Lissek et al. (Lissek et al., 2010), using a slightly different paradigm reported that when presented with a number of stimuli which differed in the degree of similarity to an actual $CS^+$, individuals with PD displayed a greater generalization of fear response (i.e. the responded stimuli included not only the ones that are similar but also ones less similar to the danger

cue) than non-PD subjects. The result supports the hypothesis that PD evolves through the fear generalization process in which actual stimuli that are present during panic attack proliferate its anticipatory anxiety property to a broader range of neutral stimuli.

As high trait anxiety is thought to be a vulnerability marker for pathological anxiety, it is perhaps not surprising that fear generalization is also prevalent among individuals with high trait anxiety in non-clinical samples. Baas and colleagues (J M P Baas, Van Ooijen, Goudriaan, & Kenemans, 2008) asked healthy subjects, after receiving an aversive discriminative conditioning, whether they were aware of the CS-US contingency. Half of the subjects, who reported being unaware of the CS-US contingency, not only showed undifferentiated anxiety-related eye-blink responses to $CS^+$ and $CS^-$, but also scored higher in trait anxiety scale than the aware group. Grillon (Grillon, 2002a) also reported that healthy subjects who failed to learn a CS-US association tended to score higher on trait anxiety.

In contrast to the number of clinical studies, far fewer studies have been conducted in animals investigating such generalisation. However, Duvarci and colleagues (Duvarci et al., 2009) tested rats on an aversive discriminative conditioning task, where auditory $CS^+$ was paired with foot-shock whilst $CS^-$ was a safety signal. They found that 18 out of 28 rats tested showed difficulty in discriminating the cues. The proposal that this may be due to heightened anxiety and fear generalisation was supported by the finding that the same rats, when tested on the elevated plus maze (EPM), showed less time spent in the open arms.

It should be noted though that some studies do not support the fear generalisation hypothesis of discriminative conditioning. It has been reported that individuals who are prone to pathological anxiety may be *more* conditionable and show hyper discriminability, relative to their non-anxious counterparts. Orr and colleagues (Orr et al., 2000) showed that, when tested on the aversive discriminative conditioning, the individuals with PTSD displayed a stronger anxiety response (SCR, heart rate and electromyogram) to the $CS^+$ than the $CS^-$, resulting in greater discriminability in comparison to healthy subjects. Michael and his colleagues (Michael, Blechert, Vriends, Margraf, & Wilhelm, 2007) also showed that PD patients not only discriminated the CS's but also they displayed persistent discrimination during extinction. These findings contradict to the fear generalisation hypothesis. Orr and colleagues (Orr et al., 2000) attribute the contradiction to the nature of response measurement. Whilst both studies reporting the enhanced conditionability among pathologically anxious subjects utilised SCR as the response measure, most of the studies reporting the fear generalisation used the eye blink startle reflex. It may be that autonomic

measures, particularly SCR, provide a more sensitive index of an aversive conditioning response in humans than does the eye blink startle magnitude. Alternatively, the autonomic measures and startle reflex may assess different emotional processes.

**The Merits of Aversive Discriminative Conditioning Paradigm to Measure Pathological/High-Trait Anxiety**

It is proposed here that the aversive discriminative conditioning paradigm has several merits over more conventional tests of anxiety for the detection of high and low trait anxious animals.

The conditioning paradigm provides anxiety/fear-related measures that are less confounded by undesirable behavioural measures. In the conventional anxiety tests, whilst ethologically relevant, i.e. they measure an animal's innate, unconditioned responses, the cause of the observed behaviours is not clearly defined (Jonathan Flint, 2003). For instance, an animal's activity rate measured in the EPM and open-field arena can be determined by the animal's exploratory tendency as well as its anxiety/fearfulness. However, there is no clear formulation of the relationship between exploration and anxiety (Jonathan Flint, 2003). This issue is less problematic in the paradigms that measure conditioned responses. In the conditioning paradigm, the subject's behaviour is specifically directed to the presented CS in anticipation of receiving the aversive US. Thus, the cause of the response is more apparent, and the behaviours exhibited are more specific.

In addition, the discriminative conditioning paradigm provides a clearer definition of high and low anxiety levels, in comparison to the conventional anxiety tests. The measures in the latter, such as the ratio of entries to open and closed arms in the EPM, the emergence time in the light-dark box and the number of head dippings in the holeboard test, are continuous variables and relative, that is, there is no clear-cut boundary that defines high and low anxiety levels. Thus, the cohort is often divided by an approximate measure such as median split or upper and lower quartiles (Pawlak et al., 2008). In contrast, the outcome of the discriminative conditioning paradigm is either a successful discrimination or a failure to display the differential response between the $CS^+$ and $CS^-$. This binary outcome shows when anxiety becomes detrimental but also provides the data that can be treated as categorical variable. Using the categorization criterion based on passing or failing the discrimination makes the grouping of high and low anxious individuals categorical and less arbitrary than using a median split or quartile divisions.

**The Aim of the Experiment**

The common marmoset (*Callithrix jacchus*) is a promising non-human primate model for research into the psychological and neural mechanisms underlying fear and anxiety (Marilia Barros & Tomaz, 2002; Yamazaki & Watanabe, 2009). Thus, it is important to develop an experimental method to reliably identify high and low trait anxiety in marmosets.

This chapter utilizes the aversive discriminative conditioning paradigm to identify high and low trait anxiety and to determine the merits of such an approach. With its powerful capability to elucidate the individual differences in trait anxiety and its statistical merit in grouping the individuals into the binary categories, the paradigm is expected to identify the animals on their trait anxiety spectrum. It is predicted that high trait anxious marmosets will fail to discriminate the CS's in contrast to the discriminative success of low anxious marmosets.

For the type of stimuli used as the aversive US, electric shock and air puff are among the frequently used stimuli. However, the use of electric shock on non-human primates should be avoided for ethical reason and the air puffing has a technical difficulty in the current study's setting which allows the animal free movement in the test chamber. Since loud noise has shown to be effective in inducing fear conditioned response both in humans (Peri, Ben-Shakhar, Orr, & Shalev, 2000) and marmosets (Mikheenko et al., 2010), the current study utilizes loud noise as the aversive US and non-aversive auditory stimuli as the CS's (details are described in 2.2.1.4 Behavioural Procedures). In line with the previous studies described above that used behavioural responses (e.g. freezing and fear potentiated startle) and physiological response (e.g. SCR, HR and electromyogram) as anxiety related responses, the current study also measures both behavioural (vigilant scanning) and physiological (HR) responses of the animals. In addition to the discriminative conditioning assessed by those measures, the present study investigates if there are any behavioural or physiological biomarkers that predict the discriminative outcome. Thus, the animals' responses during early sessions are also analysed (details are described in 2.2.1.5 Data Acquisition and Analysis).

One caveat of the discriminative conditioning paradigm is that the discrimination outcome, which is expected to reflect the subject anxiety level, can be confounded if the subjects are impaired in the general learning ability, that is, the impairment in visual/auditory perception and in the cognitive capability in the absence of threatening stimulus. Previous studies (M.-S. Man, Mikheenko, Braesicke, Cockcroft, & Roberts, 2011; Reekie, Braesicke, Man, & Roberts,

2008) have shown that, when tested on the non-stressful appetitive discriminative conditioning task, the healthy common marmosets are capable of discriminating the rewarded $CS^+$ from the non-rewarded $CS^-$.

Therefore, in order to rule out the possibility of general learning impairment, the animal that receive the aversive discriminative conditioning are subsequently tested on the appetitive discriminative conditioning using the same auditory cue stimuli (CS's), but now paired with food reward ($US^+$) or its absence ($US^-$) instead of the loud noise ($US^-$) or a neutral $US^-$. As in the aversive discrimination paradigm, both behavioural (appetitive head jerks) and physiological (blood pressure) responses are measured (details are described in 2.3.1.4 Data Acquisition and Analysis). As hypothesized, if the failure in the aversive discrimination is due to heightened trait anxiety, all animals, regardless of their performances in the aversive discriminative conditioning task, are expected to discriminate the CS's under the non-anxiety provoking condition.

## 2.2 Mild Aversive Pavlovian Discriminative Conditioning Paradigm

### 2.2.1 Methods and Materials

#### 2.2.1.1 Subjects

Twenty-seven healthy adult common marmosets (*Callithrix jacchus*, 14 females and 13 males, average age 2.3 years at the outset of testing) were used in this study (Table 2.1). All animals were experimentally naïve. All animals were born in my laboratory facility and maintained in the breeding pens with their respective family members including the parents and sibling until they were 18 months old (details of family record are given in Table 2.2). Subsequently, they were transferred to experimental cages and housed in male/female pairs in rooms with controlled humidity (50%) and temperature (24˚c) and with a 12-h light/dark cycle (maximum light intensity: 380lux). On weekdays, they were fed wholemeal bread, hard-boiled egg, marmoset jelly (Special Diet Services, Essex, UK) and a piece of fruit after testing. This diet was supplemented with additional fruit, eggs, bread, marmoset jelly (Special Diet Services) and peanuts on the weekends. Water was available ad libitum. All procedures were conducted in accordance with the project and personal licenses under the UK animals (Scientific Procedures) Act of 1986.

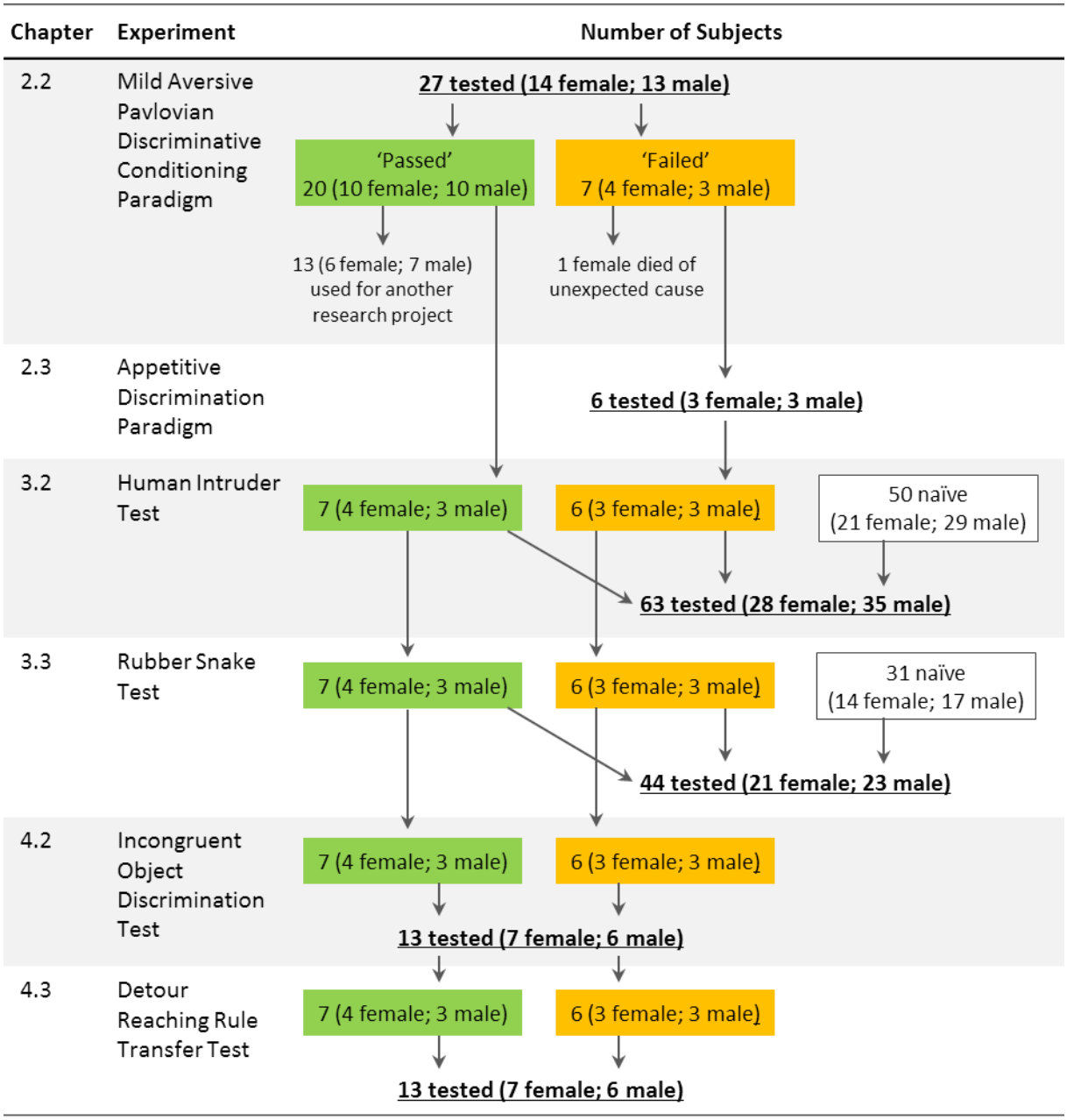
#### 2.2.1.2 Telemetry

##### 2.2.1.2.1 Telemetric Physiologic Monitors

To measure heart rate (HR) and blood pressure (BP) changes remotely in freely moving animals, a PhysioTel Telemetry System (Data Sciences, Inc. (DSI), St Paul, Minnesota, USA) was used. The system consisted of five basic components (Figure 2.1):

1) An implantable transmitter (TA11PA-C40, DSI) which continuously detected and transmitted BP from within the animal via radio-frequency signals (Figure 2.2);
2) A receiver (RPC-1, DSI) located underneath the behavioural testing box, which received the digitized information from the implanted transmitter and relayed the data for subsequent translation;
3) A calibrated pressure output adapter (R11CPA, DSI) with an ambient pressure reference monitor (APR-1, DSI) to convert the absolute pressure measured by the implanted transmitter into gauge pressure in millimetres of mercury (mmHg);

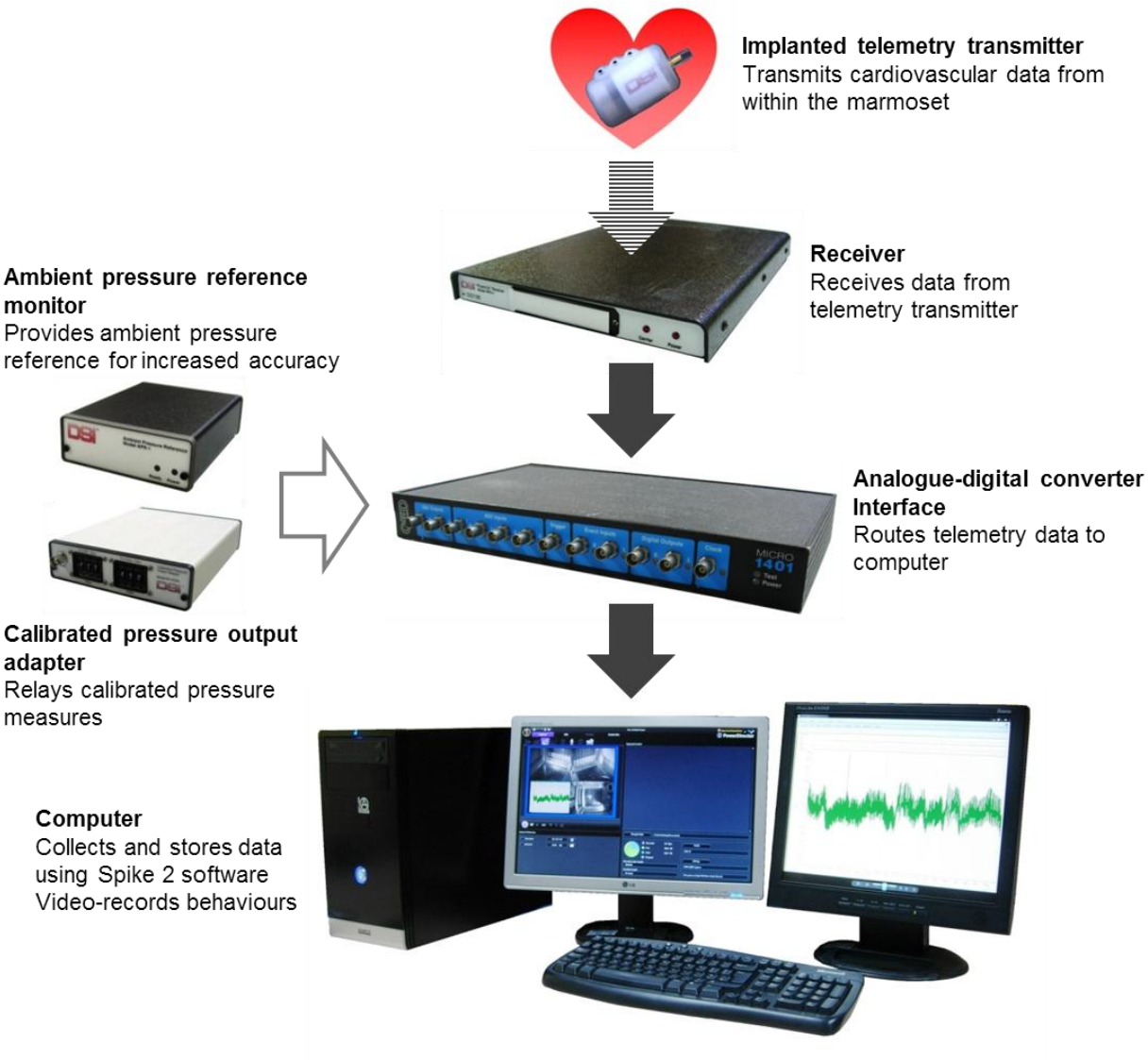
Figure 2.1 Telemetry Physiologic Monitoring System Setup.Table 2.1 The number of subjects tested on and carried through each experiment. The experiments are in chronological order from top to the bottom with their respective chapters indicated.




4) An analogue-digital converter (Micro 1401, Cambridge Electronic Design (CED), Cambridge, UK), which converted the digitized data into an analogue (continuous wave) signal; and
5) Data acquisition software (Spike2, Version 7.02, CED) for collection, analysis and storage of the accumulated data.

Table 2.2 Family information of 27 animals tested on the mild aversive Pavlovian discriminative paradigm including the parents and twin sibling. Some subjects were paired more than once therefore had more than one partner. Ticks indicate the experiments the subject was tested on. The animal numbers correspond to the numbers in Table 2.3, 2.4 and 2.5.

| | Animal Name | Sex | Date of Birth | Parents | Twin Sibling | Paired Partner | Experiment Subject Tested On | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Mild Aversive Pavlovian Discriminative Paradigm | Appetitive Discrimination Paradigm | Human Intruder Test | Rubber Snake Test | Incongruent Object Discrimination Test | Detour Reaching Rule Transfer Test |
| 1 | Jaguar | F | 06/11/2006 | Pin Plato | Caterham | Asti | ✔ | | ✔ | ✔ | ✔ | ✔ |
| 2 | Oyster | F | 15/06/2007 | Sharp Jan | Ceab | Lulu Morgan | ✔ | transferred to another research project | | | | |
| 3 | Chives | M | 17/12/2006 | Berg Star | no sib | Caterham Pin | ✔ | | ✔ | ✔ | ✔ | ✔ |
| 4 | Caterham | F | 06/11/2006 | Pin Plato | Jaguar | Chives | ✔ | transferred to another research project | | | | |
| 5 | Lulu | F | 16/07/2007 | Wally Wogan | Franky | Oyster Bovrit | ✔ | | ✔ | ✔ | ✔ | ✔ |
| 6 | Asti | M | 28/08/2006 | Nine Ten | Bordeaux | Jaguar | ✔ | | ✔ | ✔ | ✔ | ✔ |
| 7 | Bay | F | 20/01/2008 | Zinc Ace | Dill | Alfie | ✔ | | ✔ | ✔ | ✔ | ✔ |
| 8 | Excel | M | 29/03/2008 | Kaiser Orlaith | Eclat | Salt | ✔ | transferred to another research project | | | | |
| 9 | Salt | F | 19/03/2008 | Berg Star | Pepper | Bungle Excel | ✔ | transferred to another research project | | | | |
| 10 | Hallow | F | 20/07/2007 | Smithy Mathew | Stone | not paired | ✔ | transferred to another research project | | | | |
| 11 | Bovrit | M | 17/03/2008 | Pin Plato | Marmite | Lulu | ✔ | transferred to another research project | | | | |
| 12 | Seth | F | 26/07/2007 | Loocy Laburnam | no sib | Rupert | ✔ | transferred to another research project | | | | |
| 13 | Bart | M | 27/01/2009 | Sharp Jan | Lisa | Lisa | ✔ | transferred to another research project | | | | |
| 14 | Rhythm | M | 19/12/2007 | Booster Billy-Joe | Blues | Summer | ✔ | transferred to another research project | | | | |
| 15 | Alfie | M | 21/08/2007 | Zinc Ace | Summer | Bay | ✔ | | ✔ | ✔ | ✔ | ✔ |
| 16 | Noble | M | 20/10/2006 | Martyn Naville | Anna | Thyme | ✔ | transferred to another research project | | | | |
| 17 | Blues | M | 19/12/2007 | Booster Billy-Joe | Rhythm | Pernod Wacky Franky | ✔ | transferred to another research project | | | | |
| 18 | Sun | F | 30/08/2007 | Stable Elgar | Micro | Cumin | ✔ | transferred to another research project | | | | |
| 19 | Franky | F | 16/07/2007 | Wally Wogan | Lulu | Blues Rupert | ✔ | | ✔ | ✔ | ✔ | ✔ |
| 20 | Bolero | M | 14/10/2007 | Swiss Punch | Clio | Clio | ✔ | transferred to another research project | | | | |
| 21 | Stone | F | 20/07/2007 | Smithy Mathew | Hallow | Rosemary | ✔ | ✔ | ✔ | ✔ | ✔ | ✔ |
| 22 | Dani | M | 24/10/2007 | German Freeze | Rouge | Wacky | ✔ | ✔ | ✔ | ✔ | ✔ | ✔ |
| 23 | Morgan | M | 21/03/2007 | Zinc Ace | Bristol | Oyster | ✔ | ✔ | ✔ | ✔ | ✔ | ✔ |
| 24 | Thyme | F | 27/10/2006 | Stable Elgar | Basil | Noble | ✔ | ✔ | ✔ | ✔ | ✔ | ✔ |
| 25 | Hood | F | 24/05/2007 | German Freeze | Robin | Harry Rupert | ✔ | ✔ | ✔ | ✔ | ✔ | ✔ |
| 26 | Rupert | M | 11/10/2007 | Pin Plato | Pepi | Seth Hood Franky | ✔ | ✔ | ✔ | ✔ | ✔ | ✔ |
| 27 | Clio | F | 14/10/2007 | Swiss Punch | Bolero | Bolero | ✔ | died of an unexpected cause | | | | |


**Implanted telemetry transmitter**
Transmits cardiovascular data from within the marmoset
**Receiver**
Receives data from telemetry transmitter
**Ambient pressure reference monitor**
Provides ambient pressure reference for increased accuracy
**Analogue-digital converter Interface**
Routes telemetry data to computer
**Calibrated pressure output adapter**
Relays calibrated pressure measures
**Computer**
Collects and stores data using Spike 2 software
Video-records behaviours

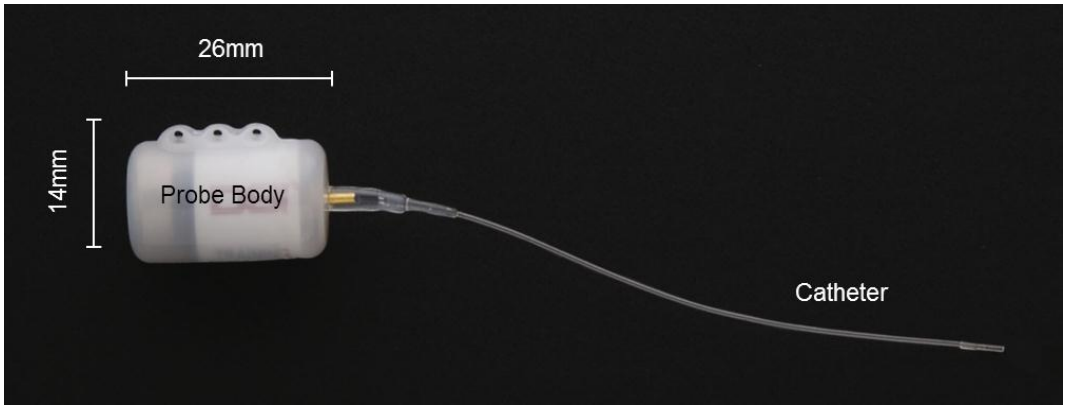



Figure 2.2 Photograph and dimension of the telemetry probe used for implantation.

**2.2.1.2.2 Implantation of Telemetry Transmitter**

***Pre-surgery Preparation*** One day prior to surgery, all animals received prophylactic antibiotic treatment: 0.25ml Flagyl-S (40mg/ml metronidazole; Winthrop Pharmaceuticals., Guildford, UK) and 0.25ml Synulox (50mg/ml clavlanate-potentiated amoxicillin; Pfizer Ltd., Kent, UK). On the day of surgery, the transmitter probes (TA11PA-C40) were immersed in sterile saline for up to an hour before implantation. This was to allow the catheters to reach osmotic equilibrium before introduction into the blood vessel and to decrease risk of thrombosis. Subjects were sedated with the anaesthetic drug, Vetalar (ketamine hydrochloride; 0.1ml of a 100mg/ml solution, i.m.; Pfizer Ltd., Kent, UK), given preoperative analgesia with the non-steroidal anti-inflammatory drug, Carprieve (carprofen; 0.03ml of 50mg/ml solution, s.c.; Norbrook Laboratories Ltd., Newry, UK) and administered atropine sulfate (0.1ml of 0.6mg/ml solution, s.c.; Animalcare Ltd., York, UK) for blood vessel dilation. The animal's abdomen was then shaved from the base of the rib cage to the pelvis in preparation for surgery and Betadine antiseptic (Animalcare Ltd., York, UK) was applied liberally to the entire area for sterility. Temperature was monitored via a rectal thermometer and, once anaesthetized, the animal was placed onto a heated mat to prevent heat loss. Blood oxygenation and heart rate were likewise monitored through the use of a pulse-oximeter, clipped either to a shaved hand or foot. The anaesthetic machine (Compact Anaesthesia Systems, VetTech Solutions Ltd., Cheshire, UK) with the inhalational anaesthetic, Isoflurane, and oxygen supply was set up. The face mask providing the Isoflurane (Flow rate 3%; IsoFlo, Abbot Laboratories, Berkshire, UK) and oxygen (0.5-1.0litre/min) was placed to cover the mouth and nose. Once the desired anaesthetic state was achieved (oxygen saturation 95-100%, pulse rate 200-250, body temperature 36-38˚c,

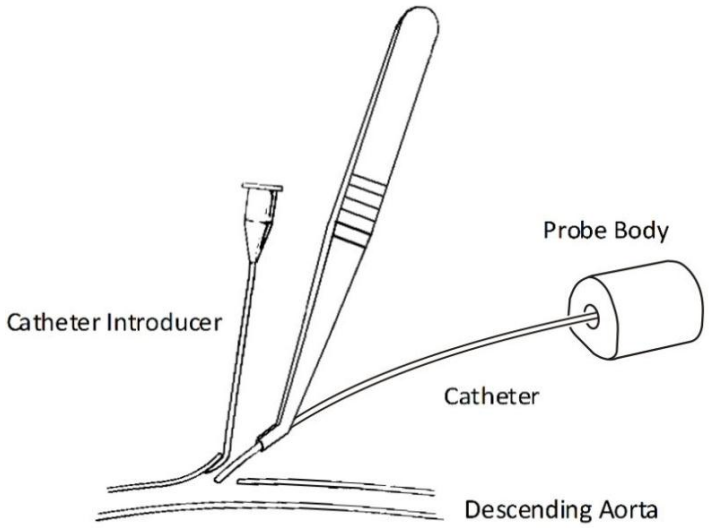



Figure 2.3 Insertion of the probe catheter into the descending aorta.

no pedal or eye reflex, regular unlaboured breathing), the animal was intubated by inserting the intra tracheal tube into the trachea. The gas supply was then switched from the face mask to the tube. Anaesthesia was maintained throughout surgery with Isoflurane concentration of 2-3% and oxygen flow of 0.3-0.4litre/min, any change of the animal's anaesthetic state or blood oxygen level resulted in the adjustment of the gas flow.

***Catheterisation Site Exposure*** The animal was placed in a supine position onto a sterile drape and the limbs were secured with masking tape to allow unrestricted access to the abdomen. A Steri-Drape (3M HealthCare; Borken, Germany) was then placed over the area to maintain a sterile surface. Under aseptic conditions, a 4-6cm midline abdominal incision was made using scissors, cutting through both the skin and muscle wall separately, to allow a clear view of the aorta from the upper portion of the vessel down to the bifurcation of the aorta to the renal arteries. The contents of the abdomen were exposed and using retractors, the intestines were held back to enable good visualization of the descending aorta. The aorta was then carefully dissected from the surrounding fat and connective tissue with the use of two soft cotton pressure swabs and all excess tissue was removed from the vessel. Once isolated, the lower portion of the aorta, just above the bifurcation, was lifted and a cotton thread, approximately 8cm in length, was passed underneath. The two ends were then clamped together with forceps to 1) lift the vessel for implantation and 2) to exert a small

amount of tension to prevent blood reflux after blood flow to the area was restricted during catheterisation.

***Catherterization*** Once the vessel was clear, to restrict blood flow, a finger was used to apply pressure to the upper most portion of the aorta and slight tension was placed on the thread at the base. Using a 23-gauge needle (bent at 60°, bevelled edge upwards), the vessel was punctured just above the bifurcation and the tip of the catheter was inserted using a catheter introducer (Figure 2.3). The catheter was then passed up the length of the vessel until approximately 125mm of the tubing was contained within the vessel. Once correctly positioned, the area was thoroughly dried and Vetbond (M3 Animal Care Products, Minesota, USA) tissue adhesive was applied to the puncture site. The glue was allowed to dry for approximately 15-30 seconds before tension at each end was slowly released and the site was monitored for leakage for a further a minute. After integrity of the seal was established, a cellulose patch was placed over the entry site and fixed in position with additional adhesive. Correct placement of the catheter could then be verified using an AM radio (tuned to 600Hz) and a magnet. The magnet was passed over the probe body to activate the devise and the radio was held nearby to pick up the blood pressure signal. Successful implantation was indicated by a fluctuating tone that corresponded to the cardiac cycle. Once verified, the probe was turned OFF again by passing the magnet back over the probe and was not switched ON again until the day before testing was restarted.

Following implantation, the thread and retractors were removed and the abdominal cavity was moistened with sterile saline. The intestines were gently replaced and the body of the transmitter was positioned on top, parallel to the long axis of the body with the catheter directed rostrally. The device body was then secured in position by incorporating the tabs on the implant into the muscle wall by using non-absorbable sutures (Ethilon 3-0 W; Ethicon Inc., Georgia, USA). After the closure of the muscle wall, the skin was closed using absorbable sutures (3-0 Vicryl W9444; Ethicon Inc., Georgia, USA) and Vetbond was applied to each stitch to ensure that the abdomen was completely sealed. After turning off the gas anaesthetic, 2.5ml of warmed glucose saline was administered (s.c.) to replace fluid loss. The abdominal incision site was cleaned with Betadine and a piece of melonin wound swab was bandaged to cover the site. Once the animal began to come around, the intra tracheal tube was removed and the animal was placed in the incubator with temperature control (Vetario Intensive Care Unit; Brinsea Products Ltd., Stanford, UK) to recover.

***Post-Operative Care*** Once the animal made a full recovery from the anaesthetic, 0.25ml each of Flagyl and Synulox was administered orally and they were allowed to return to their home-cage with free access to water and a full diet, including fruit, marmoset jelly, eggs, wholemeal bread, Farley's Rusk and peanuts for 10 days. Postoperative analgesia was maintained for three days with 0.1ml Metacam (1.5mg/ml meloxicam; Boehringer Ingelheim Vetmedica, Ingelheim/Rhein, Germany) given orally. Antibiotis, 0.25ml each of Flagyl and Synulox, were also administered orally for 10 days post-surgery to protect against intestinal infection. Bandage was removed 7-12 days after surgery. Testing started after a two-week recovery period.

#### 2.2.1.3 Mild Aversive Discrimination Test Apparatus

Upon commencement of the experiment, the experimenter went into the home room, in which the subject's home cage was housed, with a clear Perspex carrying box (240 x 230 x 200 mm, cuboid, large enough for the animal to move freely). The marmoset was encouraged to voluntarily enter the carrying box, which had been connected to the cage. Once in, the animal was transported to a sound-attenuated test apparatus, which was located in a darkened room. The carrying box, with the subject, was then fitted into the internal frame of the apparatus (Figure 2.4). Light in the apparatus was provided by a 3W light bulb suspended from the ceiling. Three video cameras mounted on the inside walls of the apparatus recorded the behaviour of the subject (Figure 2.5). Cardiovascular data were collected by the telemetric receiver placed underneath the floor of the inner frame. Sound conditioning stimuli were generated in AdobeAudition software version 1.5 and played through a computer-controlled speaker (Biotronix, University of Cambridge, UK). An unconditioned aversive noise stimulus was generated by a siren controller box (Electronics Development Group, Engineering Department, University of Cambridge, UK) and played though a computer-controlled siren speaker (Biotronix, University of Cambridge, UK). The onset and offset of the light and sounds were controlled by a device control software, Whisker (ver. 2, Cambridge University Technical Service Ltd., UK, Cardinal & Aitken, 2010).

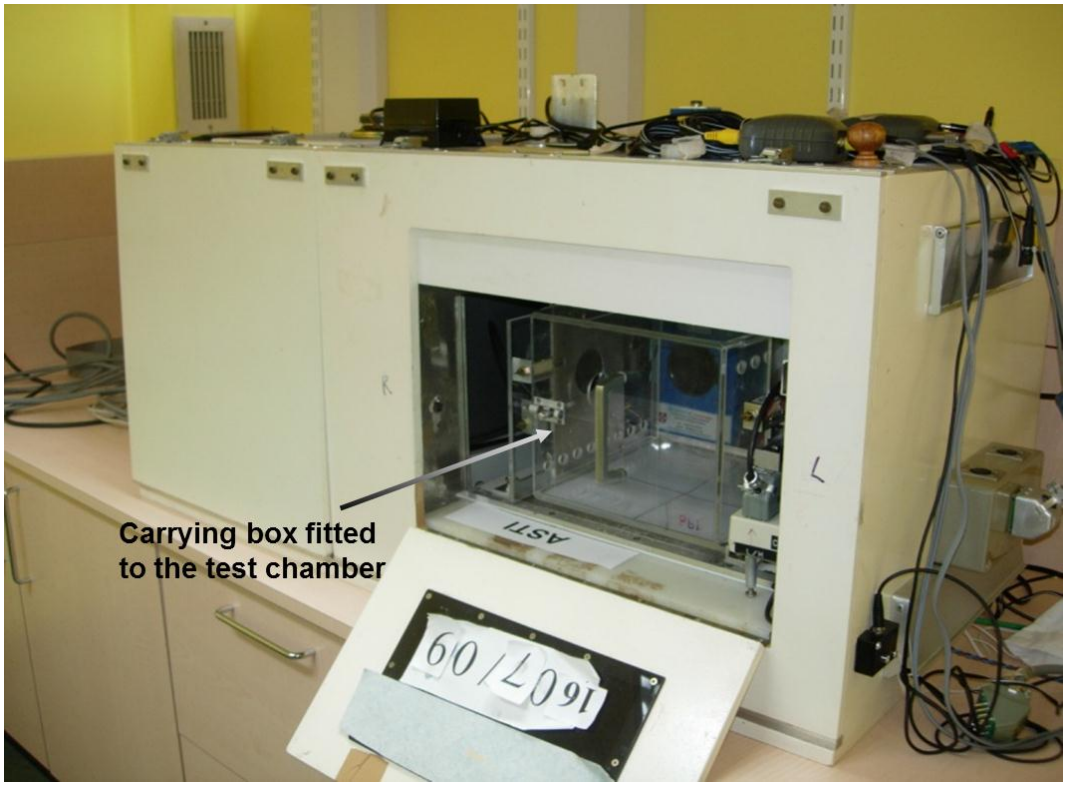



Figure 2.4 Photograph of test apparatus with carrying box placed in its internal frame.

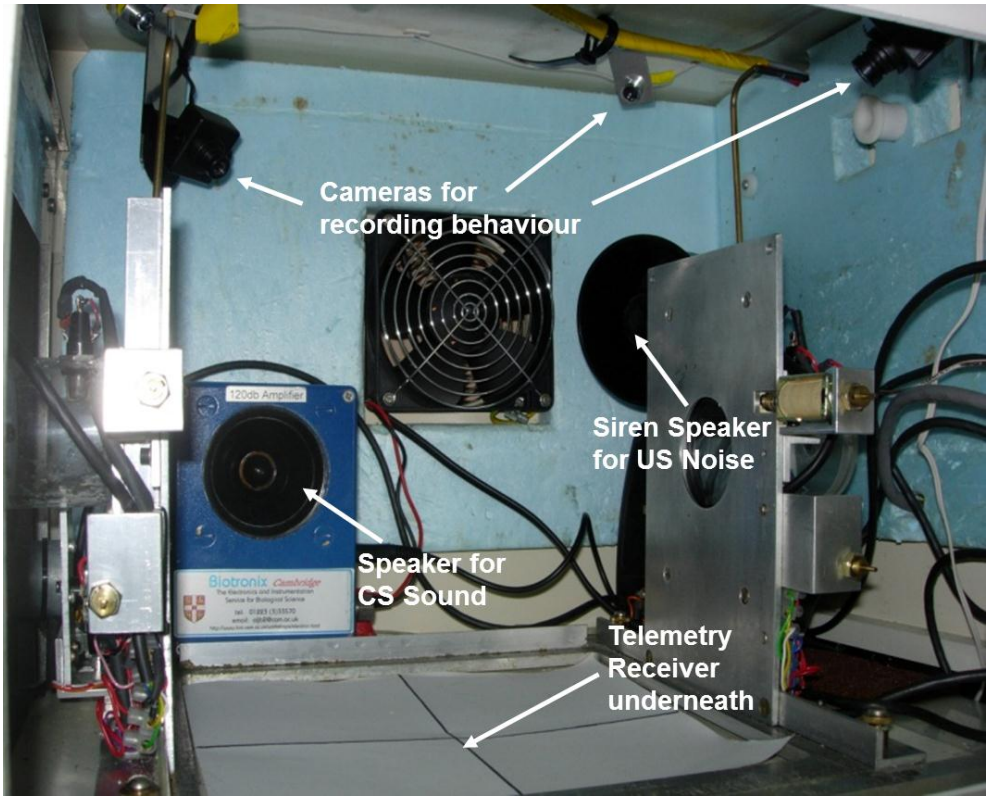



Figure 2.5 Photograph of the internal frame of the test apparatus with cameras, speakers and telemetry receiver indicated.

#### 2.2.1.4 Behavioural Procedures

***Habituation*** All animals received four to nine 10-min habituation sessions (mean: 4.7 SE: 0.2 sessions), in which the animal was placed in the test apparatus without any sound presentations. Once the subject had become accustomed to the apparatus as shown by a reduction in HR across sessions, they were moved to the next stage.

***Orienting*** Animals received two orienting sessions, in which two novel sounds, a 4-kHz tone and a clicker were presented. Each sound was played four times a session for a duration of 20 seconds, with a variable interval (VI) between presentations of 120-180 seconds. The VI was used to ensure that the onset of the novel sounds could not be predicted through temporal cueing. The aim of these orienting sessions was to monitor the behavioural and autonomic reactions of the animals towards the novel stimuli. The stimulus that elicited the smaller behavioural and autonomic reaction was chosen as the $CS^+$ (to be followed by the aversive loud noise), and the one that elicited the larger reaction became the $CS^-$ (to be followed by a neutral event), thus avoiding any stimulus preparedness (Agustín-Pavón et al., 2012). Out of 27 animals, 17 animals were assigned to the clicker and 10 animals were assigned to the tone as $CS^+$. After the two sessions of orienting, the animals were moved onto the conditioning.

***Discriminative Conditioning*** Animals were exposed to a Pavlovian conditioning paradigm (Figure 2.6) in which one of the sounds ($CS^+$) was associated with a burst of mildly aversive loud noise ($US^+$, 120 dB, 0.3-0.7 s) and the other ($CS^-$) with the brief offset of the house-light ($US^-$, 0.5-1 s). A session started with a variable interval (120-180 s) followed by 20-s presentations of the CSs which were immediately followed by the US. Four $CS^+/US^+$ pairs and four $CS^-/US^-$ pairs were presented pseudorandomly throughout a session with a 120-180-s inter-trial interval. Behavioural and cardiovascular measurements were taken during the CS periods and during the 20-s baseline (BL) periods prior to the onset of the CS.

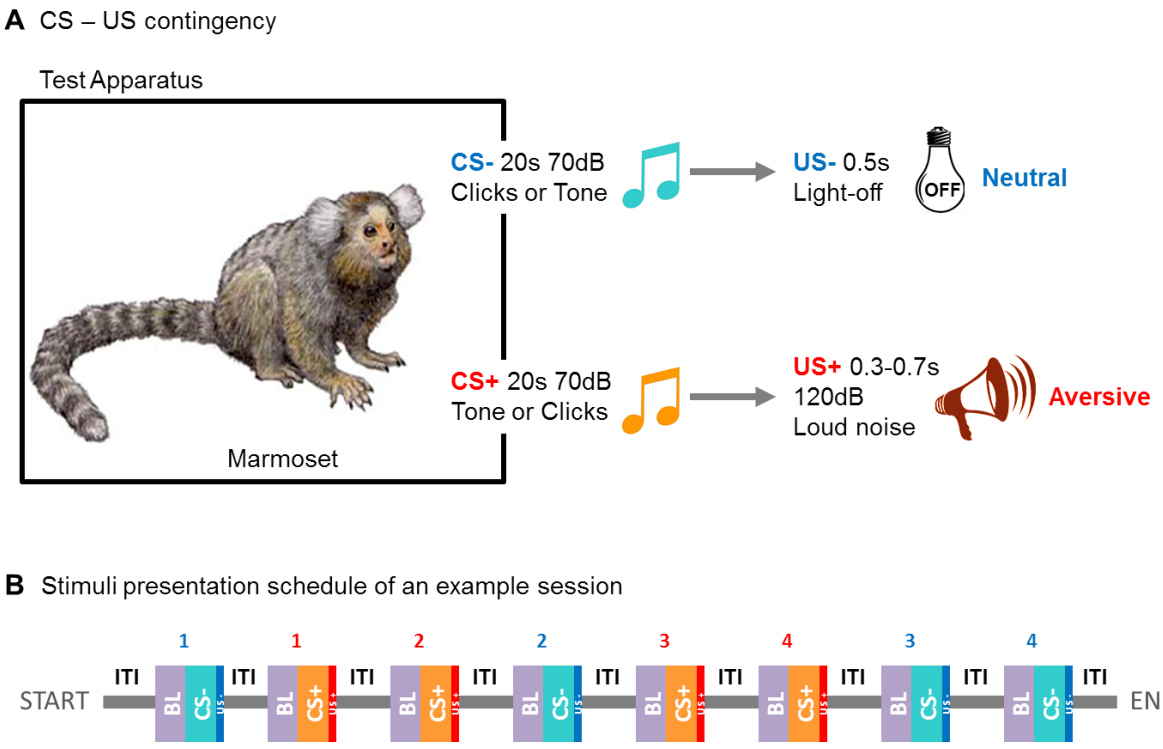



Figure 2.6 Schematic illustrations showing (A) contingency for the paired conditional and unconditional stimuli and (B) an example session with the stimuli pseudorandomly placed with variable ITI (120-180s). CS, conditional stimulus; US, unconditional stimulus; BL, baseline; ITI, inter trial interval.

### 2.2.1.5 Data Acquisition and Analysis

***Cardiovascular Measurements*** Blood pressure (systolic and diastolic) and heart rate information were collected by a receiver located at the base of the apparatus. The telemetric signals were then relayed to an analogue-digital signal converter via a calibrated pressure output adapter and the data was then collected at a sampling rate of 100Hz and recorded on a PC with a data acquisition software Spike2. Figure 2.7 depicts a typical Spike trace, where the upper level of the trace represents the systolic BP and the lower level of the trace represents the diastolic BP. Areas of darkness within the trace indicate periods of increased HR.

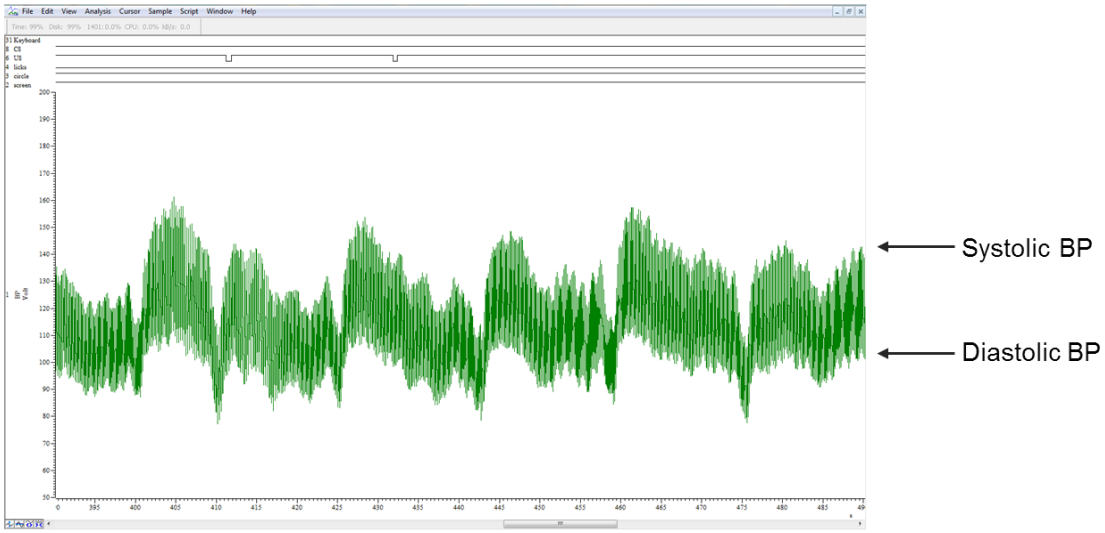



Figure 2.7 Snapshot of a computer screen showing a typical Spike trace depicting on-line blood pressure (BP) recording from the marmoset. Upper peak indicates the systolic component and lower peak indicates the diastolic component of BP.

Outliners were removed (abnormal blood pressure spikes typically above 400 mmHg or under 0 mmHg) and the remaining systolic and diastolic blood pressure events were extracted as local maxima (systolic BP) or minima (diastolic BP) during one heart beat cycle. Heat rate was calculated using the time interval between systolic BP events. Outliers or missing values were filled with cubic spline interpolation, though any disruptions in the trace longer than 0.4s were treated as missing values (Braesicke et al., 2005).

For the discriminative conditioning assessment, HR was analysed as the conditioned autonomic response since HR had the most consistent response during conditioning both within and across animals. BP had been shown previously to increase in some animals but not others (Mikheenko et al., 2010).

***Behavioural Measurements*** Behaviours during testing were digitally video-recorded through a video capture device (Xpert DVD Maker 2.0; KWorld, Taipei, Taiwan) and subsequently scored by an observer unaware of the experimental conditions. Figure 2.8 shows an example of an image used for scoring. The behaviours that developed to the $CS^+$ included vigilant scanning, defined as attentive visual search of the surroundings with movements of head and body, accompanied by tense posture (a forward extension of the body or head) or rearing (standing up on hind legs, with the upper body extended, as if to pounce). Such behaviours to threatening stimuli including real or simulated predators or

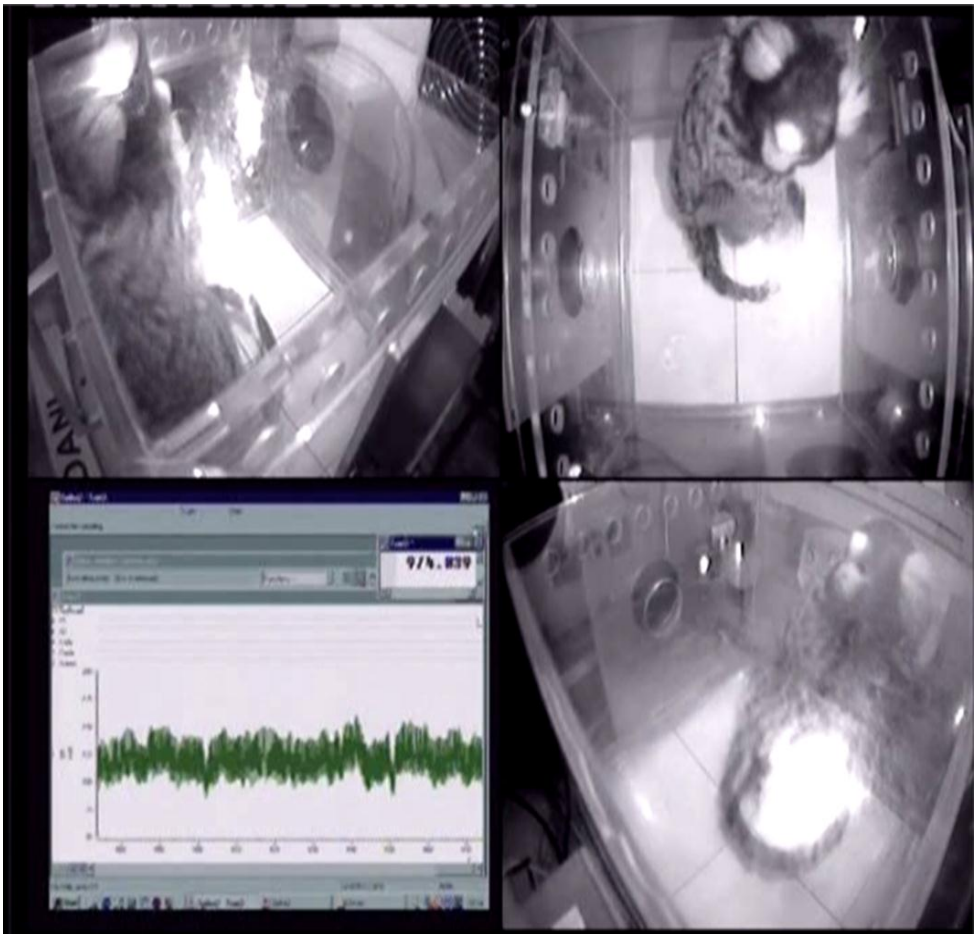


Figure 2.8 Snapshot image of the video recorded during the test showing the same animal from three different angles with the online cardiovascular trace. The video was used for behavioural scoring.

intruders have been described previously (Marilia Barros et al., 2004; Caine, 1998; Mikheenko et al., 2010; Stevenson & Poole, 1976). Occasionally, some animals displayed other, displacement-like activities specifically in the $CS^+$ period, such as gnawing the box or self-grooming. These behaviours were only included in the conditioned behavioural measures when shown consistently across sessions. The duration of the behaviour displayed during the BL and CS periods was scored using a program written in QuickBASIC 4.5.

***Conditioned Response Assessment Criterion*** For the assessment of conditioned response, the animal's unique response to the CS was obtained by subtracting the response during the BL period from the response during the CS period. The conditioned discrimination was assessed by comparing the animal's response to the $CS^+$ and its response to the $CS^-$.

The criterion for conditioned discriminative response for the animal was to show a statistically significant difference in both behavioural and autonomic responses between the CSs over the same three consecutive sessions within 30 sessions (Agustín-Pavón et al., 2012). Greater response to the $CS^+$ in comparison to the $CS^-$ was hypothesized. Each animal was given one session a day until the discriminative criterion was reached or until they had received 30 sessions, whichever occurred first.

***Statistical Analysis*** Statistical analyses were performed using statistic software SPSS (version 17.0). For the dependent variables, the assumption of normality was assessed via Kolmogorov-Sminov test and Shapiro-Wilk test; and, the homogeneity of variance and sphericity were checked by Leven's test and Mauchly's test respectively. When there was a violation of assumptions indicated by the significant values by those tests, necessary transformations were conducted and subsequent analyses were performed on the transformed data. The methods included Student *t*-test, mixed design analysis of variance (ANOVA) and logistic regression analysis.

## 2.2.2 Results

### 2.2.2.1 Individual Differences in the Acquisition of Aversive Discriminative Conditioning

***Discriminative Criterion Assessment*** Twenty of the 27 animals tested successfully acquired a discriminative conditioned behavioural and cardiovascular response; however, the remaining seven failed to display discriminative conditioning (for the discriminative conditioned criterion, refer to section 2.2.1.4 'Discriminative Conditioning'). One-sample Student's *t*-test assessed the difference score ($CS^+$ response minus $CS^-$ response) over a moving window of three consecutive sessions (e.g. 1-3, 2-4, 3-5…) against the null hypothesis which assumed no difference in the response between the CSs. Table 2.3 and 2.4 show the behavioural and autonomic results respectively (the absolute values from which the difference scores were derived are given in Table 2.5). Twenty animals developed a significantly greater response to the $CS^+$ than to the $CS^-$ satisfying the criterion; thus, they were labelled as the 'passed' group. Seven animals did not satisfy the criterion; thus, they were labelled as the 'failed' group. When considering the behaviour alone, two animals (animal 26, 27) from the 'failed' group displayed the opposite effect to those in the 'passed' group, i.e. a significant increase in the response to the $CS^-$, a safety signal, while the others did not show any significant difference in the responses between the $CS^+$ and the $CS^-$ (Figure 2.9 A-i). For the autonomic response, the animals in the 'passed' group showed an acceleration to the $CS^+$ and a deceleration to the $CS^-$; whilst seven of them showed both responses, six displayed mainly enhanced acceleration to the $CS^+$, seven showed predominantly the deceleration to the $CS^-$. Among the 'failed' group, one animal showed a decreased HR response to the $CS^+$, while the remaining six animals did not develop significantly different responses to the CSs (Figure 2.9 B-i).

Mean number of sessions to reach the discrimination criterion by the animals in the 'passed' group was 15.3 sessions (SE: 1.4) (all animals in the 'failed' group had 30 sessions). Typical learning curves of an animal from the 'passed' and the 'failed' groups are shown in Figure 2.10 A and B respectively.

***Group Comparison*** Direct comparison of the 'passed' and the 'failed' animals revealed that overall the 'passed' group developed a significant increase to the $CS^+$ and decrease to the $CS^-$ in both the behaviour and HR while the 'failed' group did not show differences in the responses to the CSs. A two-way ANOVA comparing the mean vigilant behaviour towards

the CSs in the three criterion sessions of the 'passed' group and the final three sessions (i.e. 28-30) of the 'failed' group revealed a significant group x CS interaction [$F(1, 25)=47.29$, $p<0.001$] (Figure 2.9 A-ii). Post hoc comparison of the groups revealed that the interaction was due to the 'passed' group showing a significantly greater response to the $CS^+$ than to the $CS^-$ [$F(1,25)=106.95$, $p<0.001$ ] and the 'failed' group showing no difference in the responses between the CSs [$F(1,25)=3.50$, $p=0.073$]. Post hoc comparison of the CSs revealed that the 'passed' group showed a significantly greater vigilant behaviour towards the $CS^+$ compared to the 'failed' group [$F(1,25)=5.17$, $p=0.032$], whereas the 'failed' group displayed a significantly greater response to the $CS^-$ compared to the 'passed' group [$F(1,25)=47.93$, $p<0.001$]. A similar pattern of results was seen for the HR response as revealed by a significant group x CS interaction [$F(1,25)=46.04$, $p<0.001$] (Figure 2.9 B-ii). Post hoc comparison of the groups revealed that the interaction was due to the 'passed' group showing a significantly greater HR acceleration to the $CS^+$ than to the $CS^-$ [$F(1,25)=115.72$, $p<0.001$]. No such difference was observed in the 'failed' group [$F(1,25)=2.31$, $p=0.141$]. Post hoc comparison of the CSs revealed that during the $CS^+$ the 'passed' group showed a significant HR elevation compared to the 'failed' group [$F(1,25)=8.78$, $p=0.007$], whilst to the $CS^-$ the 'passed' group showed a significantly lower HR than the 'failed' group [$F(1,25)=11.67$, $p=0.002$].

Table 2.3 Individual animal's mean behaviour score for $CS^+$, $CS^-$ and the difference between the CSs across three criterion sessions and accompanying *t*-test results. The animal numbers appearing in bold indicate those that were carried onto the subsequent experiments described in Chapter 3 and 4.

| Discrimination | Animal | CS+ (change from BL) | | | | CS- (change from BL) | | | | Difference (CS+ vs CS-) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SE | *t* | *p* | Mean | SE | *t* | *p* | Mean | SE | *t* | *P* |
| **Passed** | **1** | 8.54 | 1.31 | 6.51 | .000 | 1.73 | 1.86 | 0.93 | .372 | 6.80 | 2.28 | 2.99 | **.007** |
| | 2 | 7.98 | 1.02 | 7.80 | .000 | 1.25 | 0.43 | 2.90 | .014 | 6.74 | 1.11 | 6.06 | **.000** |
| | **3** | 7.13 | 1.21 | 5.88 | .000 | 3.34 | 1.07 | 3.12 | .010 | 3.80 | 1.62 | 2.35 | **.028** |
| | 4 | 6.53 | 1.18 | 5.52 | .000 | -0.44 | 1.49 | -0.29 | .774 | 6.97 | 1.92 | 3.62 | **.002** |
| | **5** | 6.30 | 1.34 | 4.70 | .001 | 1.67 | 0.72 | 2.33 | .040 | 4.63 | 1.52 | 3.04 | **.006** |
| | **6** | 6.06 | 0.96 | 6.28 | .000 | 1.61 | 1.62 | 0.99 | .341 | 4.44 | 1.89 | 2.36 | **.028** |
| | **7** | 6.05 | 1.08 | 5.62 | .000 | 0.63 | 0.83 | 0.76 | .462 | 5.41 | 1.36 | 3.98 | **.001** |
| | 8 | 5.69 | 1.25 | 4.54 | .001 | 1.02 | 1.13 | 0.90 | .386 | 4.67 | 1.69 | 2.77 | **.011** |
| | 9 | 5.36 | 0.73 | 7.34 | .000 | -0.51 | 0.63 | -0.80 | .439 | 5.87 | 0.96 | 6.08 | **.000** |
| | 10 | 4.96 | 1.41 | 3.51 | .005 | -0.23 | 0.88 | -0.26 | .799 | 5.19 | 1.67 | 3.11 | **.006** |
| | 11 | 4.94 | 1.86 | 2.66 | .022 | -2.32 | 1.04 | -2.23 | .047 | 7.26 | 2.13 | 3.41 | **.003** |
| | 12 | 4.82 | 1.27 | 3.80 | .003 | 0.33 | 0.30 | 1.09 | .298 | 4.49 | 1.30 | 3.44 | **.005** |
| | 13 | 4.61 | 0.94 | 4.90 | .000 | 1.02 | 0.66 | 1.54 | .151 | 3.59 | 1.15 | 3.11 | **.005** |
| | 14 | 4.41 | 1.29 | 3.42 | .006 | 0.02 | 0.82 | 0.03 | .978 | 4.38 | 1.53 | 2.87 | **.010** |
| | **15** | 3.44 | 0.49 | 7.03 | .000 | -0.45 | 0.66 | -0.69 | .505 | 3.89 | 0.82 | 4.76 | **.000** |
| | 16 | 3.20 | 0.65 | 4.94 | .000 | 0.35 | 0.55 | 0.64 | .535 | 2.85 | 0.85 | 3.35 | **.003** |
| | 17 | 3.09 | 0.80 | 3.86 | .003 | 0.52 | 0.90 | 0.59 | .570 | 2.57 | 1.20 | 2.14 | **.044** |
| | 18 | 2.62 | 0.74 | 3.56 | .004 | 0.35 | 0.35 | 0.99 | .344 | 2.27 | 0.82 | 2.78 | **.011** |
| | **19** | 2.53 | 0.53 | 4.74 | .001 | 0.39 | 0.51 | 0.77 | .460 | 2.14 | 0.74 | 2.91 | **.008** |
| | 20 | 1.13 | 0.19 | 6.03 | .000 | 0.29 | 0.13 | 2.25 | .046 | 0.84 | 0.23 | 3.67 | **.001** |
| **Failed** | **21** | 7.22 | 1.08 | 6.69 | .000 | 4.82 | 1.66 | 2.90 | .015 | 2.40 | 1.98 | 1.21 | **.240** |
| | **22** | 4.91 | 1.13 | 4.34 | .001 | 4.86 | 1.36 | 3.57 | .004 | 0.05 | 1.77 | 0.03 | **.977** |
| | **23** | 3.08 | 2.10 | 1.47 | .171 | 5.85 | 1.19 | 4.93 | .000 | -2.77 | 2.41 | -1.15 | **.264** |
| | **24** | 2.72 | 1.24 | 2.20 | .050 | 4.01 | 1.27 | 3.16 | .009 | -1.29 | 1.77 | -0.73 | **.475** |
| | **25** | 1.90 | 0.98 | 1.93 | .079 | 3.38 | 1.15 | 2.93 | .014 | -1.48 | 1.52 | -0.97 | **.341** |
| | **26** | 0.31 | 0.35 | 0.87 | .403 | 1.53 | 0.35 | 4.36 | .001 | -1.22 | 0.50 | -2.45 | **.023** |
| | 27 | 0.17 | 0.40 | 0.42 | .682 | 5.37 | 0.90 | 5.98 | .000 | -5.21 | 0.98 | -5.30 | **.000** |

Note: *df*=22, *p* values of the CS difference used for 'Passed' 'Failed' grouping appear in bold.

Table 2.4 Individual animal's mean HR for $CS^+$, $CS^-$ and the difference between the CSs across three criterion sessions and accompanying *t*-test results. The animal numbers appearing in bold indicate those that were carried onto the subsequent experiments described in Chapter 3 and 4.

| Discrimination | Animal | CS+ (change from BL) | | | | CS- (change from BL) | | | | Difference (CS+ vs CS-) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SE | *t* | *p* | Mean | SE | *t* | *p* | Mean | SE | *t* | *P* |
| **Passed** | 4 | 33.77 | 12.57 | 2.69 | .021 | -21.66 | 17.63 | -1.23 | .245 | 55.44 | 21.65 | 2.56 | **.018** |
| | 14 | 30.84 | 7.98 | 3.87 | .003 | 0.02 | 7.83 | 0.00 | .998 | 30.82 | 11.18 | 2.76 | **.012** |
| | 12 | 27.48 | 5.64 | 4.87 | .000 | 1.16 | 4.28 | 0.27 | .792 | 26.32 | 7.08 | 3.72 | **.001** |
| | **3** | 26.40 | 7.91 | 3.34 | .007 | -8.52 | 6.42 | -1.33 | .212 | 34.92 | 10.19 | 3.43 | **.002** |
| | 2 | 25.76 | 6.72 | 3.83 | .003 | -3.12 | 5.82 | -0.54 | .603 | 28.88 | 8.89 | 3.25 | **.004** |
| | 17 | 24.43 | 5.33 | 4.59 | .001 | 3.38 | 6.82 | 0.50 | .630 | 21.05 | 8.66 | 2.43 | **.024** |
| | 18 | 23.26 | 7.30 | 3.19 | .009 | -6.44 | 9.35 | -0.69 | .505 | 29.70 | 11.86 | 2.50 | **.020** |
| | 8 | 18.23 | 4.99 | 3.66 | .004 | -4.34 | 5.16 | -0.84 | .418 | 22.57 | 7.17 | 3.15 | **.005** |
| | 16 | 16.68 | 8.02 | 2.08 | .062 | -11.94 | 7.08 | -1.69 | .120 | 28.62 | 10.78 | 2.65 | **.015** |
| | 9 | 13.95 | 2.93 | 4.77 | .001 | 4.76 | 2.56 | 1.86 | .089 | 9.19 | 3.89 | 2.37 | **.027** |
| | **7** | 13.94 | 8.44 | 1.65 | .127 | -30.81 | 6.78 | -4.54 | .001 | 44.75 | 10.83 | 4.13 | **.000** |
| | **5** | 10.45 | 2.80 | 3.73 | .003 | -1.42 | 2.05 | -0.69 | .505 | 11.87 | 3.47 | 3.42 | **.002** |
| | **1** | 7.36 | 4.83 | 1.52 | .156 | -6.28 | 4.15 | -1.51 | .158 | 13.64 | 6.43 | 2.12 | **.046** |
| | **6** | 6.88 | 4.19 | 1.64 | .129 | -7.81 | 4.13 | -1.89 | .085 | 14.70 | 5.88 | 2.50 | **.020** |
| | 13 | 5.82 | 6.00 | 0.97 | .353 | -31.25 | 8.02 | -3.90 | .002 | 37.06 | 10.01 | 3.70 | **.001** |
| | 11 | 4.16 | 9.27 | 0.45 | .662 | -34.57 | 14.02 | -2.47 | .031 | 38.74 | 16.81 | 2.31 | **.031** |
| | **19** | 3.12 | 7.72 | 0.40 | .694 | -16.98 | 3.39 | -5.01 | .000 | 20.10 | 8.43 | 2.38 | **.031** |
| | **15** | 1.81 | 4.04 | 0.45 | .663 | -25.98 | 8.41 | -3.09 | .010 | 27.78 | 9.33 | 2.98 | **.009** |
| | 20 | -1.39 | 7.11 | -0.20 | .849 | -26.78 | 6.96 | -3.85 | .003 | 25.39 | 9.94 | 2.55 | **.018** |
| | 10 | -8.72 | 5.33 | -1.64 | .130 | -37.02 | 5.07 | -7.31 | .000 | 28.31 | 7.35 | 3.85 | **.001** |
| **Failed** | **21** | 12.89 | 4.64 | 2.78 | .018 | 16.01 | 5.24 | 3.06 | .011 | -3.12 | 7.05 | -0.44 | **.663** |
| | **22** | 5.70 | 5.11 | 1.11 | .289 | -6.22 | 6.19 | -1.01 | .336 | 11.92 | 8.02 | 1.49 | **.152** |
| | **26** | 4.82 | 7.30 | 0.66 | .522 | 14.05 | 3.83 | 3.67 | .004 | -9.22 | 8.47 | -1.09 | **.288** |
| | **25** | -1.60 | 7.89 | -0.20 | .843 | 4.08 | 4.46 | 0.91 | .380 | -5.68 | 9.06 | -0.63 | **.537** |
| | **24** | -2.47 | 6.23 | -0.40 | .700 | -3.76 | 8.09 | -0.46 | .651 | 1.29 | 10.21 | 0.13 | **.901** |
| | **23** | -4.34 | 9.24 | -0.47 | .648 | 13.28 | 4.41 | 3.01 | .012 | -17.62 | 10.55 | -1.67 | **.110** |
| | 27 | -20.87 | 3.41 | -6.13 | .000 | 2.66 | 6.25 | 0.43 | .679 | -23.53 | 7.11 | -3.31 | **.004** |

Note: *df*=22, *p* values of the CS difference used for 'Passed' 'Failed' grouping appear in bold.

Table 2.5 Individual animal's actual values of the vigilant behaviour scores and HR during BL and CS periods, from which the difference scores were derived, mean across three criterion sessions. The animal numbers appearing in bold indicate those that were carried onto the subsequent experiments described in Chapter 3 and 4.

| Discrimination | Animal | Vigilant behaviour (s) | | | | HR (bpm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | BL+ | CS+ | BL- | CS- | BL+ | CS+ | BL- | CS- |
| Passed | **1** | 0.63 | 9.17 | 3.43 | 5.17 | 218.69 | 226.05 | 221.11 | 214.83 |
| | 2 | 0.74 | 8.72 | 0.68 | 1.93 | 248.06 | 273.82 | 256.98 | 253.87 |
| | **3** | 1.70 | 8.83 | 3.26 | 6.60 | 221.13 | 247.54 | 230.71 | 222.20 |
| | 4 | 1.13 | 7.66 | 2.43 | 1.99 | 351.41 | 385.18 | 317.07 | 295.41 |
| | **5** | 0.68 | 6.98 | 0.93 | 2.61 | 220.90 | 231.35 | 218.33 | 216.91 |
| | **6** | 7.79 | 13.84 | 7.76 | 9.37 | 252.47 | 259.36 | 247.92 | 240.11 |
| | **7** | 2.07 | 8.12 | 1.70 | 2.33 | 302.84 | 316.77 | 300.49 | 269.68 |
| | 8 | 1.44 | 7.13 | 2.02 | 3.04 | 295.06 | 313.29 | 302.59 | 298.26 |
| | 9 | 1.46 | 6.82 | 2.21 | 1.70 | 191.15 | 205.11 | 186.32 | 191.08 |
| | 10 | 1.97 | 6.93 | 5.18 | 4.95 | 371.19 | 362.47 | 376.29 | 339.27 |
| | 11 | 5.64 | 10.59 | 3.49 | 1.17 | 343.49 | 347.65 | 327.67 | 293.09 |
| | 12 | 0.07 | 4.89 | 0.18 | 0.51 | 381.15 | 408.63 | 387.03 | 388.18 |
| | 13 | 2.02 | 6.63 | 1.40 | 2.42 | 281.99 | 287.81 | 287.05 | 255.81 |
| | 14 | 0.77 | 5.18 | 1.54 | 1.56 | 228.32 | 259.16 | 230.53 | 230.54 |
| | **15** | 2.59 | 6.03 | 2.60 | 2.15 | 321.40 | 323.20 | 321.47 | 295.49 |
| | 16 | 0.17 | 3.37 | 0.80 | 1.15 | 289.68 | 306.36 | 310.80 | 298.86 |
| | 17 | 3.42 | 6.51 | 3.06 | 3.59 | 311.96 | 336.39 | 303.81 | 307.19 |
| | 18 | 0.50 | 3.12 | 0.66 | 1.01 | 265.30 | 288.55 | 271.16 | 264.71 |
| | **19** | 1.25 | 3.77 | 0.90 | 1.29 | 365.11 | 368.23 | 361.70 | 344.72 |
| | 20 | 0.06 | 1.20 | 0.10 | 0.39 | 341.05 | 339.66 | 363.26 | 336.48 |
| Failed | **21** | 0.62 | 7.83 | 1.41 | 6.23 | 227.66 | 240.55 | 219.67 | 235.68 |
| | **22** | 6.33 | 11.24 | 4.25 | 9.10 | 182.09 | 187.79 | 173.83 | 167.60 |
| | **23** | 2.90 | 5.98 | 2.45 | 8.30 | 228.49 | 224.16 | 211.48 | 224.76 |
| | **24** | 1.43 | 4.15 | 2.53 | 6.54 | 257.80 | 255.34 | 250.22 | 246.46 |
| | **25** | 1.42 | 3.32 | 4.05 | 7.44 | 218.56 | 216.96 | 225.35 | 229.43 |
| | **26** | 0.07 | 0.38 | 0.10 | 1.63 | 210.70 | 215.53 | 206.49 | 220.54 |
| | 27 | 0.95 | 1.12 | 0.05 | 5.43 | 285.88 | 265.01 | 297.15 | 299.80 |

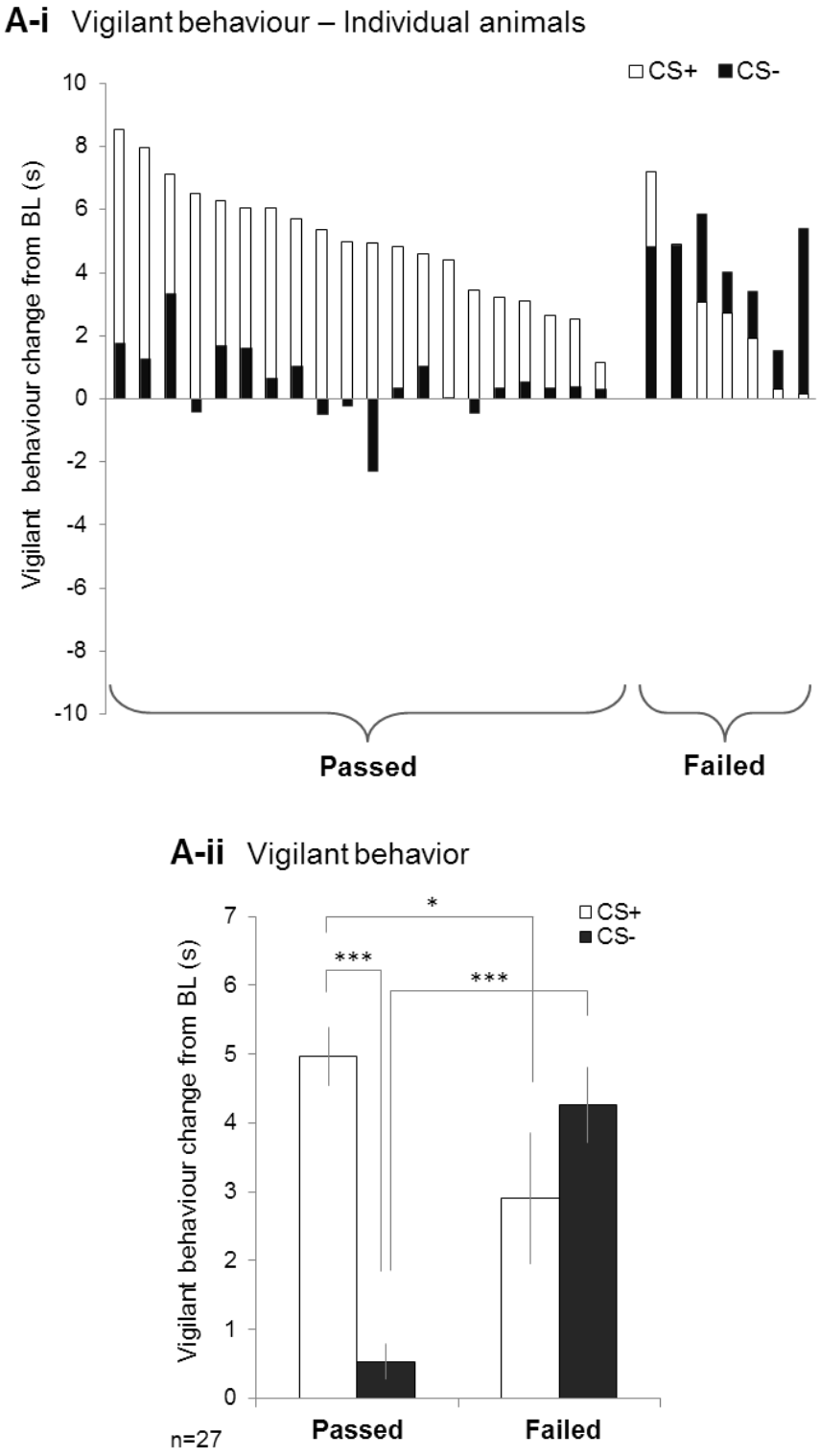



Figure 2.9 Individual variation (A-i) and group difference (A-ii) of the mean behavioural response to the $CS^+$ (open bar) and $CS^-$ (closed bar), compared to BL, in the three discrimination criterion sessions for the 'passed' group and sessions 28-30 for the 'failed' group

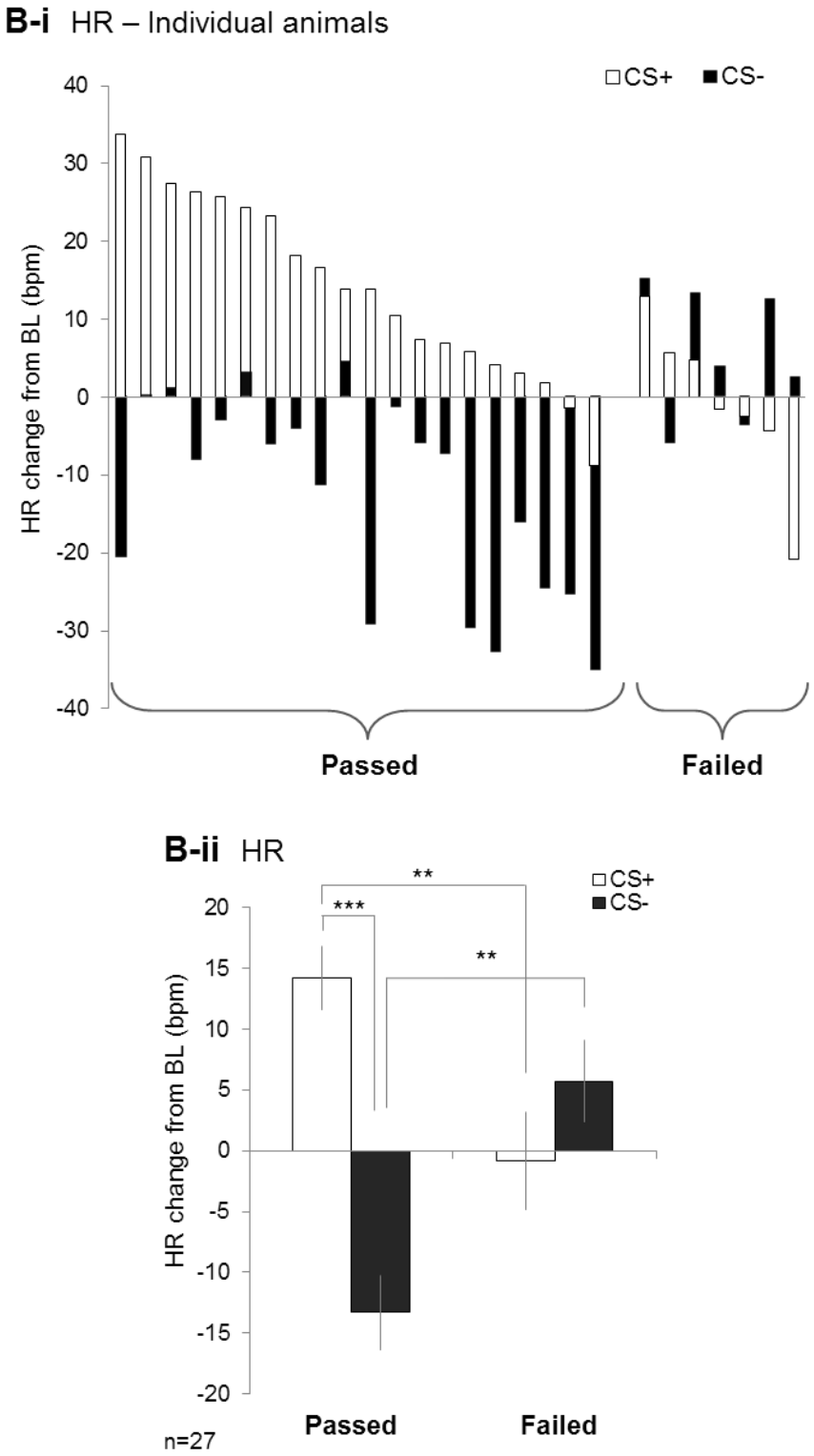



Figure 2.9 (continued) Individual variation (B-i) and group difference (B-ii) of the mean HR response to the $CS^+$ (open bar) and $CS^-$ (closed bar), compared to BL, in the three discrimination criterion sessions for the 'passed' group and sessions 28-30 for the 'failed' group. Error bars show the standard errors for each group. *$p$<.05, **$p$<.01, ***$p$<.001

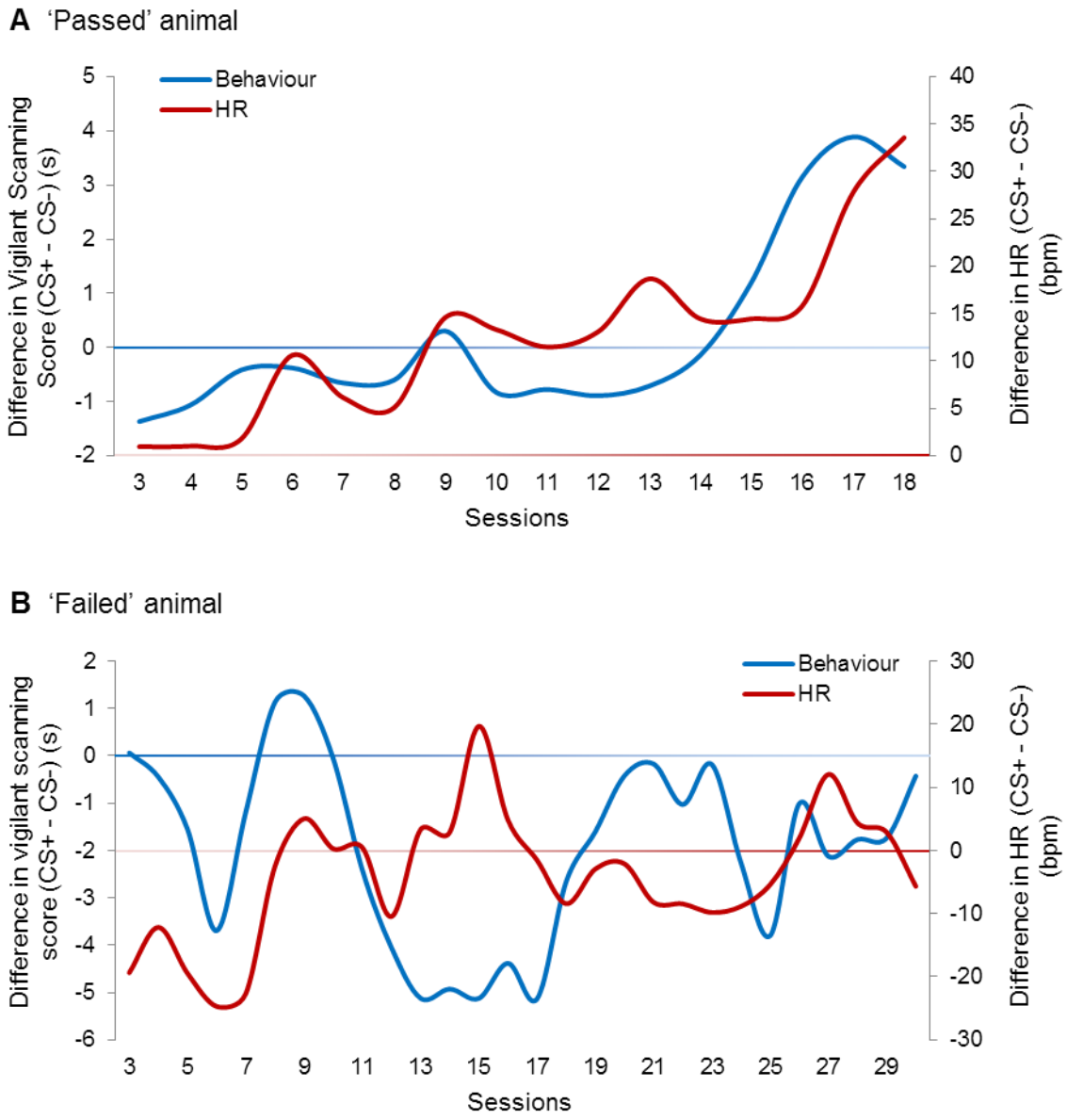



Figure 2.10 Learning curve (running mean of three consecutive sessions) for the vigilant behaviour (blue line) and HR (red line) of a typical (A) 'passed' animal and (B) 'failed' animal. HR, heart rate; CS, conditional stimulus.

#### 2.2.2.2 Measures in Early Sessions as Predictors of the Discriminative Outcome

To investigate whether individual differences in the responses to the cues in the early sessions may predict animal's eventual success or failure to display discriminative conditioning, a number of measures were investigated including behavioural and cardiovascular responses to the CS as well as at baseline and how HR and BP during the

baseline changed across sessions. The baseline measure was included in the analysis because previous studies of anxiety reported that anxious subjects not only display arousal responses to a safety cue but also display enhanced arousal during the baseline periods (Blechert, Michael, Grossman, Lajtman, & Wilhelm, 2007).

***Behavioural & Autonomic Response Analysis*** The behavioural and cardiovascular measures were compared across the first three sessions; a period before any animal showed discriminative conditioning. For the behaviour, the animals in the 'failed' group displayed significantly greater responses to both CSs compared to those that passed (Figure 2.11 A). A two-way factorial ANOVA of the vigilant scanning behaviour to the CSs across the first three sessions revealed a significant main effect of the group [$F(1,25)=6.71$, $p=0.016$] but no group x CS interaction [$F(1,25)=4.15$, $p=0.052$].

For the autonomic response, the animals in the 'failed' group showed a significantly enhanced response to the $CS^-$, i.e. safety signal, whereas the animals in the 'passed' group did not show such a response nor show a difference in the responses between the CSs (Figure 2.11 B). A two-way factorial ANOVA of the mean HR responses to the CSs revealed a significant group x CS interaction [$F(1,25)=11.57$, $p=0.002$]. Post hoc comparison of the groups revealed that the 'failed' group developed a significantly elevated HR response to the $CS^-$, than that to the $CS^+$ [$F(1,25)=8.96$, $p=0.006$] whereas the 'passed' group did not differ in their responses to the CSs [$F(1,25)=2.62$, $p=0.118$]. This is also supported by post hoc comparison of the CSs revealing a significant group difference in the response to the $CS^-$ [$F(1,25)=7.75$, $p=0.010$] but not to the $CS^+$ [$F(1,25)=0.25$, $p=0.619$].

Such enhanced vigilant and HR responses to the CSs by the 'failed' group were not seen in the orienting sessions prior to the start of conditioning. A two-way factorial ANOVA comparing the responses to the CSs (in the orienting, the USs were not introduced, thus strictly speaking these were CS-to-be sounds) between the groups across the two orienting sessions returned no significant main effect of the group [behaviour: $F(1,25)=3.26$, $p=0.083$; HR: $F(1,25)=0.62$, $p=0.438$] nor significant group x CS interaction [behaviour: $F(1,25)=1.99$, $p=0.170$; HR: $F(1,25)=0.38$, $p=0.543$].

***Baseline Response Analysis****.* Comparison of both baseline HR and systolic BP between the 'passed' and 'failed' groups revealed that there was a gradual decline in these measures across the early conditioning sessions (session 1-9) in the 'failed' group but not in the 'passed' group (Figure 2.11 C). A repeated-measures ANOVA across sessions 1-3, 4-6 and

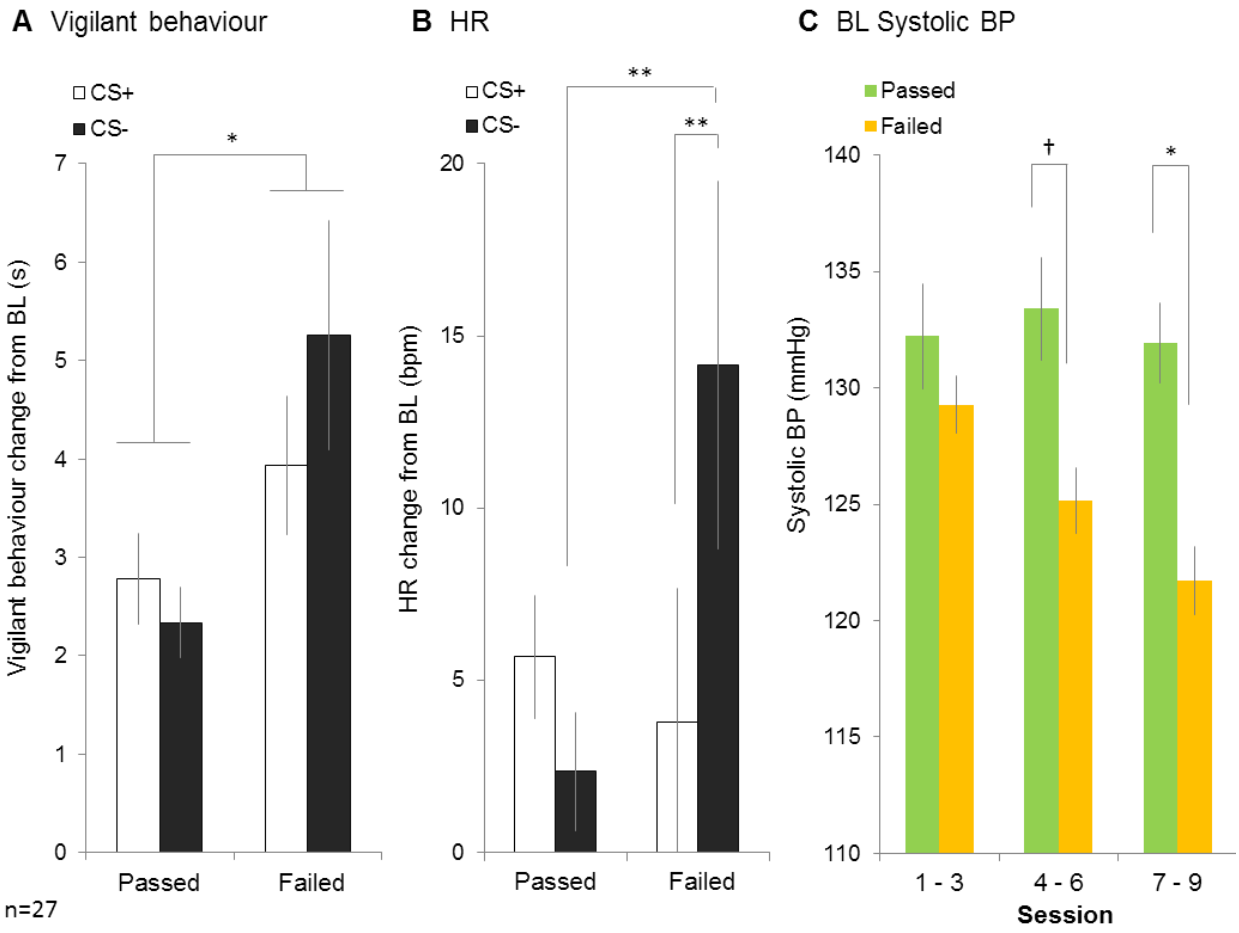



Figure 2.11 (A, B) Mean behavioral and HR responses to the $CS^+$ (open bar) and $CS^-$, (closed bar) compared to BL, in the first three sessions of animals in the 'passed' and 'failed' groups. (C) Mean BL Systolic BP across sessions 1-3, 4-6 and 7-9 in the 'passed' (green bar) and 'failed' (yellow bar) groups. Error bars show the standard deviation for each group. † $p$<.1, *$p$<.05, **$p$<.01

7-9 revealed a significant group x session interaction in BP [$F$(2,48)=4.49, $p$=0.016] but only a trend in HR [$F$(2,48)=2.87, $p$=0.067]. Post hoc comparison of the sessions revealed that the gradual decline of the systolic BP shown by the 'failed' group reached a trend during session 4-6 [$F$(1,24)=4.22, $p$=0.051] and a significant suppression in comparison to the 'passed' group's BP during session 7-9 [$F$(1,24)=11.59, $p$=0.002]. No difference in the vigilant behaviour was observed during the baseline period between the groups.

***Prediction of Discriminative Outcome*** In order to assess how reliably the responses in the early sessions predicted the eventual success or failure of the aversive discriminative conditioning, a binary logistic regression analysis was performed with the measures that showed significant contribution to the group differences in early sessions as predictor

Table 2.6 Regression coefficients, Wald statistics and Odds ratios from significant model predicting passing or failing the discrimination.

| Retained predictor | B | Standard Error | Wald's $\chi^2$ | *df* | *p* | eB (odds ratio) |
|---|---|---|---|---|---|---|
| Constant | 67.79 | 35.78 | 3.59 | 1 | .058 | |
| CS Vigilant Behavior Sessions 1-3 | 0.91 | 0.50 | 3.28 | 1 | .070 | 2.49 |
| BL BP Sessions 7-9 | -0.57 | 0.29 | 3.87 | 1 | .049 | 0.56 |

Note: $R^2$= .54 (Cox & Snell), .79 (Nagelkerke). Model $\chi^2(2)=20.86$, $p<.001$.

variables. Regression analysis assesses the relationships between variables and generates a model with a linear equation that best describes the data; thus, the analysis is used to predict values of the dependent variables from one or more independent variables (Field, 2009). Binary logistic regression was employed since our dependent variable was a categorical variable (i.e. 'passed' or 'failed') and independent variables were continuous. To obtain a reliable model with our relatively small sample size (n=27), four variables were selected as potential predictors based on their significance levels in the univariate tests described above. These were 1) the mean behavioural response to the $CS^+/CS^-$ in sessions 1-3, 2) the HR response to the $CS^-$ in sessions 1-3, 3) the baseline HR in sessions 7-9, and 4) BP in sessions 7-9. The forward likelihood method was used since there was no a priori hypothesis (Field, 2009). In the forward method, the analysis selects and inputs a predictor that has the highest correlation with the outcome to a model. If this predictor significantly improves the ability of the model to predict the outcome, then it is retained and the next predictor gets selected, otherwise the predictor is removed from the model. The model is constantly reassessed until the final model is reached with the predictors that have most significant contribution in accounting the variance in the data. Our analysis revealed a final significant model [$\chi^2(2)=20.86$, $p<0.001$] with two predictor variables retained: the mean vigilant behavioural response to the CSs in sessions 1-3 and the baseline BP in sessions 7-9 (Table 2.6). Out of the two variables, the baseline BP in sessions 7-9 showed the largest contribution to the model ($p$=0.049). The fact that its regression coefficient B was negative indicated that as the baseline BP decreased by one unit, the odds of passing the discrimination decreased from 1.0 to 0.56. Thus, the lower the baseline BP in sessions 7-9, the more likely the animal was to fail the discrimination. On the other hand, the positive coefficients for the CS vigilant behaviour in sessions 1-3 ($p$=0.070) suggested that as the vigilant behaviour score increased by one unit, the odds of failing the discrimination increased from 1.0 to 2.49.

## 2.3 Appetitive Discrimination Paradigm

### 2.3.1 Methods and Materials

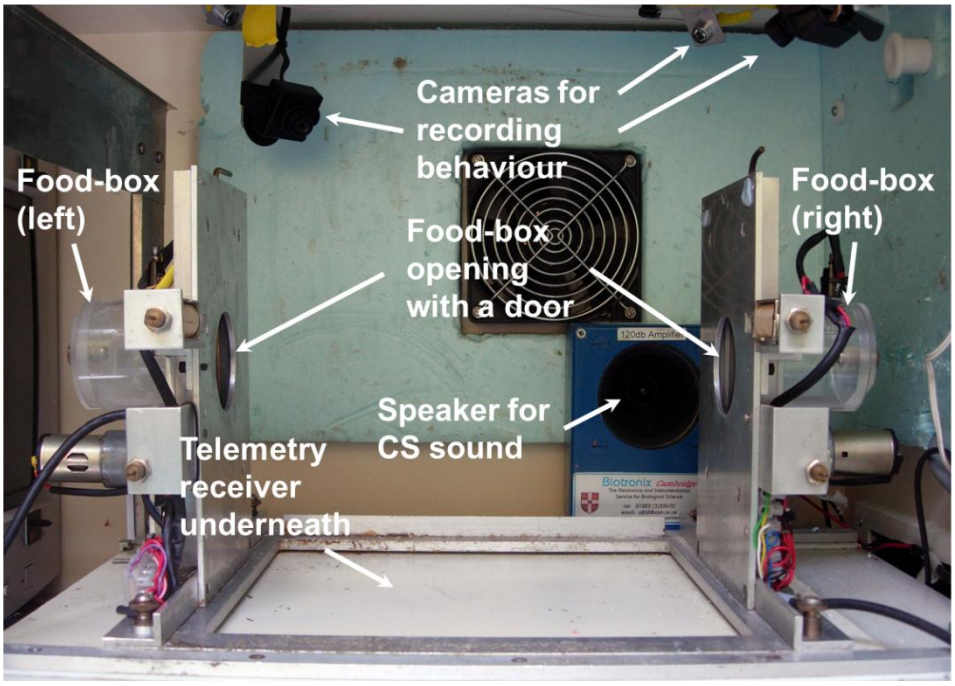
#### 2.3.1.1 Subjects

Six (3 females and 3 males) of the seven marmosets that were unable to show discriminatory conditioning in the aversive discrimination task ('failed' group) were tested on the appetitive discrimination task (the seventh animal died of an unexpected cause) (Table 2.1). The housing and feeding conditions were identical to the mild aversive Pavlovian discrimination paradigm procedure (section 2.2.1.1). All procedures were conducted in accordance with the project and personal licenses under the UK animals (Scientific Procedures) Act of 1986.

#### 2.3.1.2 Appetitive Discrimination Test Apparatus

The same test apparatus that had been used in the aversive discrimination paradigm was used (refer to section 2.2.1.3). In addition to the devices used for the aversive discrimination paradigm, two electrically controlled food-box units were attached to the left and right walls of the internal frame of the apparatus (Figure 2.12 A). The food-box was cylindrical (internal diameter 52mm and length 51mm). The inside of the food-box could be illuminated by a 28V, 0.04W encased light bulb. The clear Perspex carrying box (240 x 230 x 200 mm, cubioid) that was used to transport the marmoset also had two circular windows (diameter 30mm) on the opposite side. Thus, when the carrying box was fitted to the internal frame of the apparatus, the positions of the windows were aligned with the food-box openings allowing the animal access to the content of the food-box (Figure 2.12 B). However, the access was restricted by a black and opaque Perspex door attached to the food-box which could be opened remotely to allow access. The auditory stimuli were produced by the same system as in the aversive discrimination paradigm. Three cameras mounted on the inside walls recorded behaviour. Cardiovascular data were also collected by the telemetric receiver placed underneath the apparatus floor. The food-box door, light and sounds were controlled by a device control software, Whisker (Cardinal & Aitken, 2010).

**A** Test apparatus for the appetitive discrimination paradigm




**B** Carry box fitted to the test apparatus

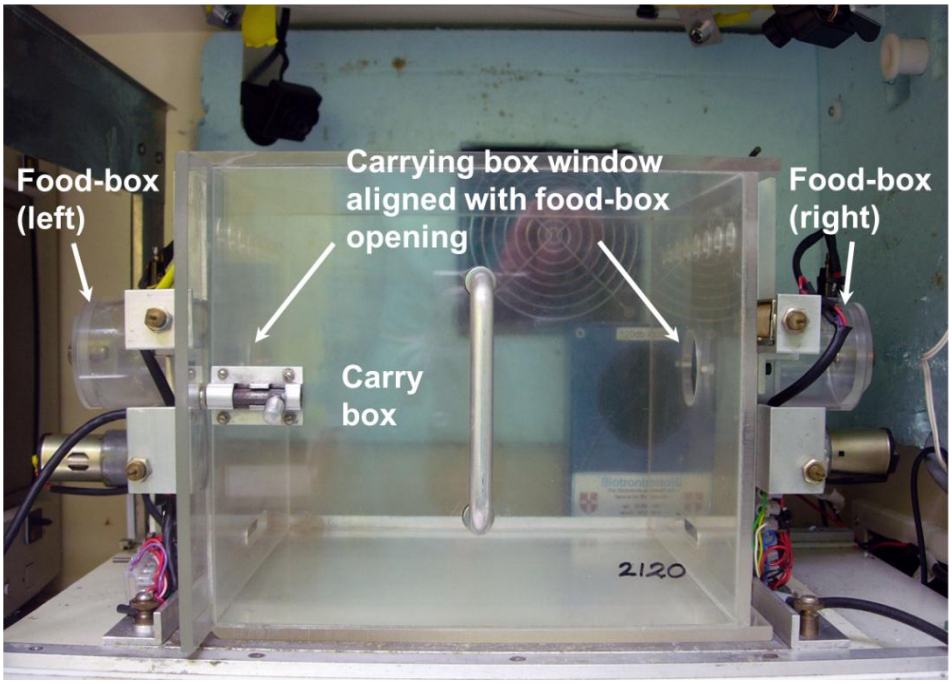



Figure 2.12 (A) Test apparatus setting with food-box for appetitive conditioning which was identical to the setting for aversive conditioning. (B) Carry box fitted within the internal frame of the test apparatus. The box's windows were aligned with the food-box opening.

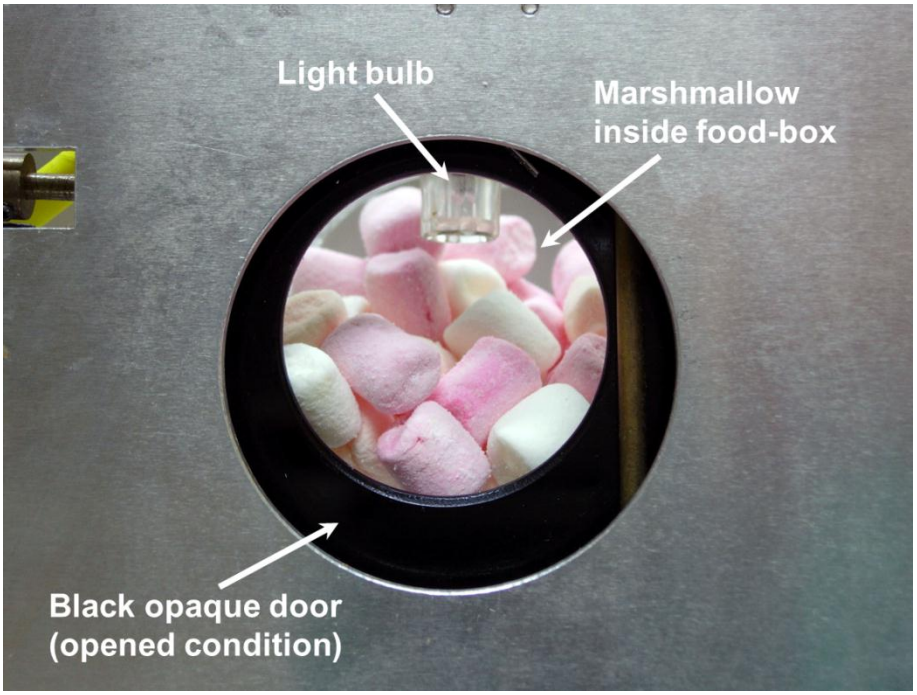



Figure 2.13 View to the food-box content from the inside of the carrying box.

#### 2.3.1.3 Behavioural Procedure

***Habituation*** Prior to conditioning, all marmosets were habituated to the sight and sound of the door of the food-box opening. During these habituation sessions, high incentive food (several pieces of marshmallow) was presented in either the left or right food-box (Figure 2.13). After the carry box with the animal inside was placed into the test apparatus, the door of the food-box was opened. When the animal stopped showing a startle response (i.e. rearing and jumping) to the opening of the door and started eating the food within 30 seconds of its opening, they were advanced to the conditioning session. The number of habituation sessions ranged from 4 to 12 (mean: 7.3 SE: 1.2 sessions) depending on the animal's performance.

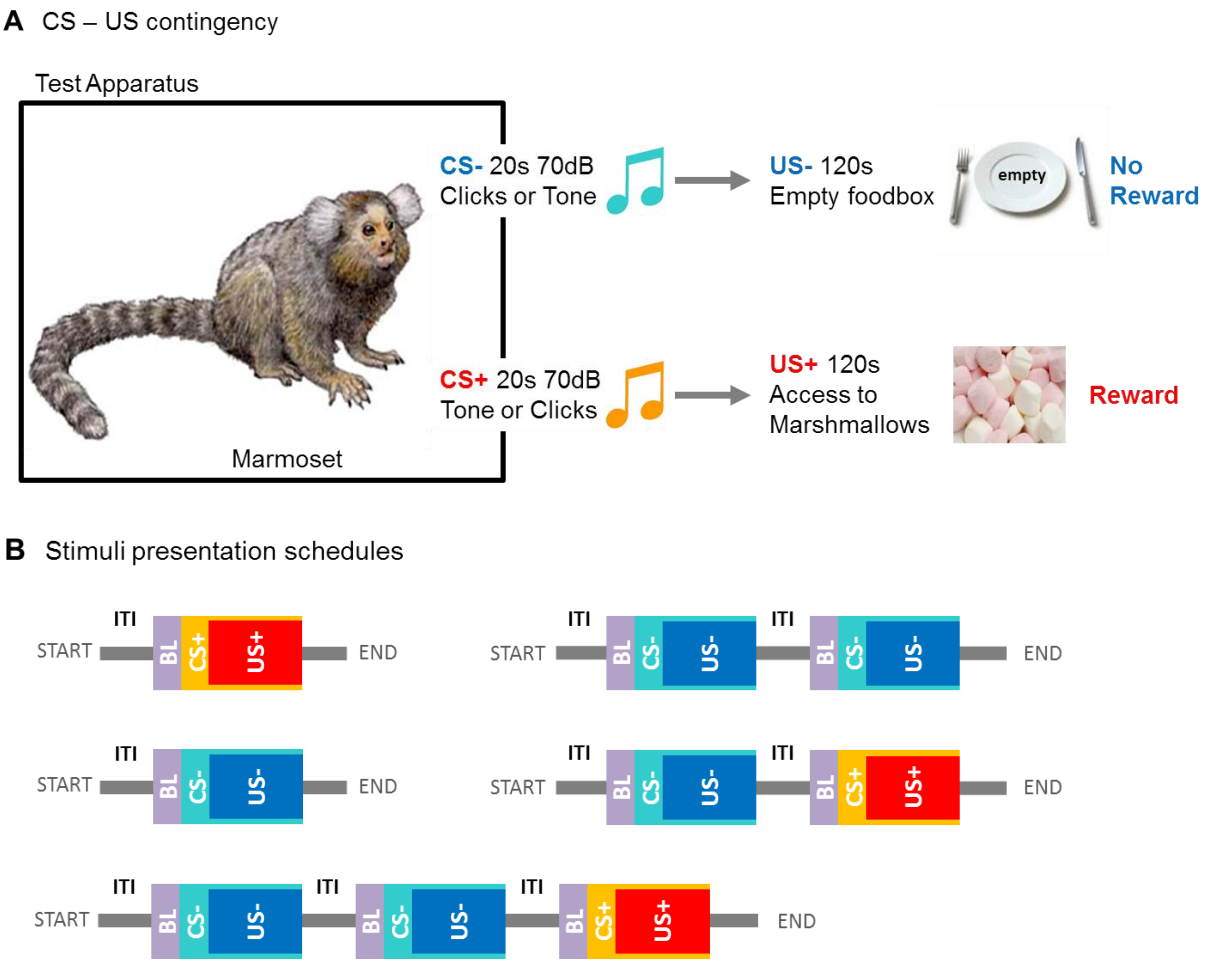



Figure 2.14 Schematic illustrations showing (A) paired conditioned and unconditioned stimuli for the appetitive discrimination paradigm. (B) five possible $CS^-/US^-$ and $CS^+/US^+$ combinations for a conditioning session with variable ITI (70-110s). CS, conditional stimulus; US, unconditional stimulus; BL, baseline; ITI, inter trial interval.

***Discriminative Conditioning*** During the appetitive Pavlovian conditioning procedure, the marmosets were exposed to the same sounds, tone and clicker, that were used as the CS in the aversive discrimination task. For each animal, the sound that was used as $CS^+$ stayed as $CS^+$ and the $CS^-$ stayed as $CS^-$. However, instead of the aversive noise and house-light-off, the sounds were associated with food reward (marshmallow, net weight: ~7.0g) ($US^+$) or no reward (US-) (Figure 2.14 A). The food reward was placed in the right food-box for the $US^+$ and the left food-box was kept empty for the $US^-$. A trial consisted of a 20-s CS period during which one of the sounds was played. At the end of this period, one or the other side of the food-boxes would open according to a predetermined randomized schedule, accompanied by the house-light offset, the onset of the food-box light and presentation of either the empty food-box (US-) or the high-incentive food reward ($US^+$). The auditory CS continued to be

played for the entire 120-s duration of the US period. In multiple-trial sessions, the offset of the US periods was indicated by termination of the CS, closure of the black opaque food-box door and onset of the house light. If a trial was the last in a session, all lights were turned off at the end of the US period indicating session termination. The intervals between trials were pseudorandomly varied between 70-110 seconds. The number of trials in a session varied from one to three trials. No more than one $CS^+/US^+$ trial was given in a session and the $CS^+/US^+$ trial was given always as the final trial. Thus, a session could consist of a single $CS^-/US^-$ or $CS^+/US^+$ trial, two $CS^-/US^-$ trials, or a combination of one or two $CS^+/US^-$ trials and one $CS^+/US^+$ trial (Figure 2.14 B). Marshmallows were chosen as the food reward since marmosets invariably favour them over other types of food (Caldwell, Watson, & Morris, 2009). Behavioural and cardiovascular measurements were taken both during the CS periods and during the 20-s BL periods prior to the onset of the CS.

#### 2.3.1.4 Data Acquisition and Analysis

***Cardiovascular Measurements*** Collection of BP and HR data were conducted with the same system and the procedure described in section 2.2.1.5. In comparison to HR, systolic BP has been shown to be a more reliable cardiovascular measure of emotional arousal in an appetitive paradigm (Braesicke et al., 2005; Reekie et al., 2008; Shabel & Janak, 2009). Therefore, systolic BP was analysed as the conditioned autonomic response.

***Behavioural Measurements*** Behaviours were recorded using the same system as in the aversive Pavlovian discrimination paradigm (section 2.2.1.4) and subsequently scored manually. Marmosets display head-jerking (a rapid side-to-side turn/flick of head) as an orienting response to auditory appetitive CSs (Holland, 1977; M.-S. Man et al., 2011; Reekie et al., 2008). Head jerks were rarely observed outside the $CS^+$ periods. The number of head jerks displayed during the BL and CS periods was counted. The total amount of food reward consumed was also recorded.

***Conditioned Response Assessment Criterion*** For the assessment of conditioned response, the animal's unique response to the CS was obtained by taking the difference between the 20-s baseline (BL) response and the 20-s CS response. The criterion for learning the appetitive discrimination was the same as that used previously (M.-S. Man et al., 2011), i.e. significantly ($p<.05$) greater $CS^+$ responses compared to $CS^-$ responses for both behavioural and autonomic measures over a block of sessions that included six consecutive $CS^+$ presentations (e.g. $1^{st}$-$6^{th}$ $CS^+$'s, $2^{nd}$-$7^{th}CS^+$'s, $3^{rd}$-$8^{th}CS^+$'s…) and intervening $CS^-$ trials

(ranged between 6-14, mean:7.9, SE: 0.3). Each animal was given one session a day until the discrimination criterion was reached.

***Statistical Analysis*** Statistical analyses were performed using statistic software SPSS (version 17.0). For the dependent variable, the assumption of normality was assessed via Kolmogorov-Sminov test and Shapiro-Wilk test. Student *t*-test was used for the conditioning criterion assessment. Paired *t*-test was used to compare for subsequent analyses.

## 2.3.2 Results

### 2.3.2.1 Performance of the 'Failed' Group in Appetitive Discriminative Conditioning

Despite the fact that these animals were unable to discriminate the $CS^+$ from the $CS^-$ in the aversive discrimination paradigm ('failed' group), all of them successfully developed conditioned discriminative responses to the exact same CSs when they were paired with an appetitive US (for the discriminative conditioned criterion, refer to section 2.3.1.4 'Data acquisition and analysis'). They displayed significantly greater numbers of head jerks to the $CS^+$, the sound that was currently paired with food reward but that had previously been paired with the aversive loud noise. Similarly, they displayed a significantly elevated systolic BP to the $CS^+$ in comparison to their response to the $CS^-$. This analytical procedure was in line with the one used for aversive discriminative conditioning (section 2.2.1.5). A one-sample Student's *t*-test of the difference score (mean $CS^+$ response - mean $CS^-$ response) for both the behavioural and the BP responses over a moving block of sessions that contained six consecutive $CS^+$ trials and intervening $CS^-$ trials revealed that all 6 animals achieved criterion within 6 to 24 $CS^+$'s presentations (Table 2.7). Table 2.7 also shows the final level of performance of both behavioural ('Number of Head Jerks') and autonomic ('Systolic BP') measures. A comparison of the difference scores ($CS^+$ - $CS^-$) across the group of animals (Figure 2.15 A, B) using a paired sample *t*-test confirmed the successful discrimination with a significant main effect of CS, for the number of head jerks [$t(5)=3.74$ $p=0.014$] and for the BP [$t(5)=3.33$ $p=0.021$].

The mean number of $CS^+$ to reach criterion was 14.3 (SE: 2.9) which was within the normal range for animals achieving the discriminative conditioning in previous studies using the same paradigm (M=16.6, SE=2.6, n=23) (Reekie et al., 2008, unpublished data). A typical learning curve from one of the animals is shown in Figure 2.16. The pattern was similar to the learning curve observed for the 'passed' animal in the aversive discrimination paradigm (Figure 2.10 A).

Table 2.7 Individual animal's difference score between the CSs across six $CS^+$ and corresponding $CS^-$ criterion trials for behavioural and autonomic measures and accompanying *t*-test results. Also, the number of $CS^+$'s to reach criterion is shown.

| Animal | Number of Head Jerks | | | Systolic BP (mmHg) | | | Number of CS+'s to reach criterion |
|---|---|---|---|---|---|---|---|
| | Difference (CS+ vs CS-) | | | Difference (CS+ vs CS-) | | | |
| | Mean | *t* | *p* | Mean | *t* | *p* | |
| 1 | 0.42 | 2.24 | **.039** | 2.25 | 2.17 | **.048** | 24 |
| 2 | 1.83 | 2.45 | **.026** | 2.91 | 2.34 | **.031** | 19 |
| 3 | 1.50 | 2.31 | **.035** | 3.33 | 2.34 | **.035** | 11 |
| 4 | 1.61 | 2.31 | **.036** | 2.85 | 3.07 | **.011** | 12 |
| 5 | 2.93 | 3.29 | **.009** | 10.51 | 2.74 | **.021** | 6 |
| 6 | 0.41 | 2.26 | **.039** | 3.51 | 3.95 | **.002** | 14 |

*Note*: *p* values of the CS difference used for the discriminative criterion appear in bold.

The amount of food reward consumed during each of six $CS^+/US^+$ criterion trials was recorded for each animal. A one-sample *t*-test assessing the weight of the food consumed across the criterion trials against the null hypothesis (no food consumption) revealed a significant main effect for all the animals (Table 2.8). This substantial food consumption following the $CS^+$ presentation verified the appetitive property of the $US^+$ and supported the use of the orienting behaviour seen during the $CS^+$ period as an appetitive response in expectation of the food delivery.

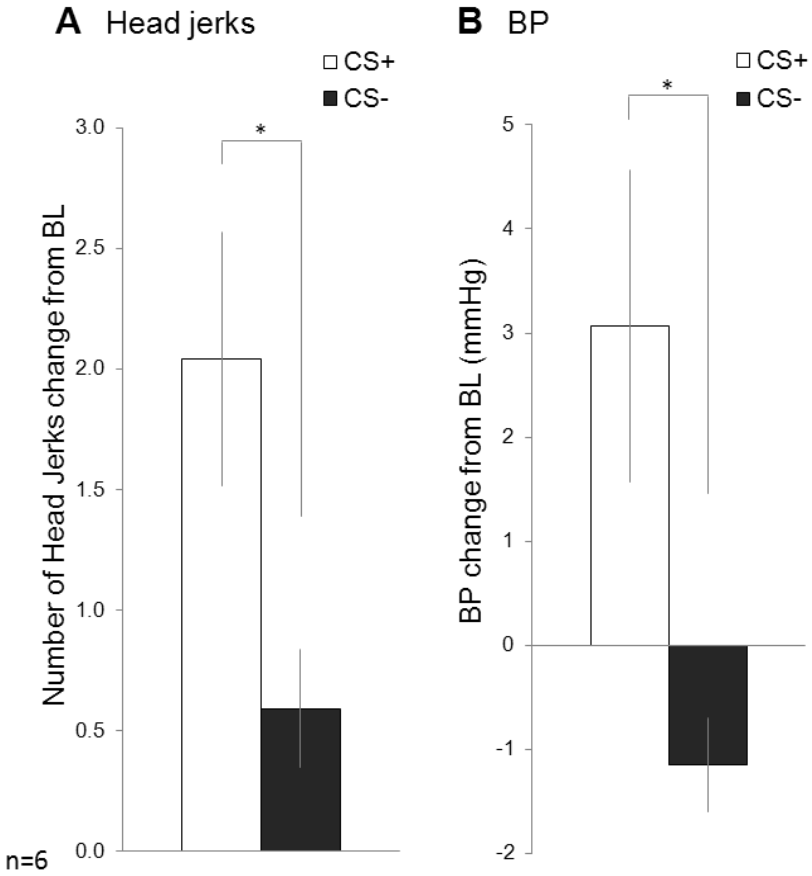



Figure 2.15 Mean (A) behavioural and (B) BP responses to the $CS^+$ and $CS^-$, compared to BL, in the appetitive discrimination criterion sessions of the 'failed group' (animals that were unable to discriminate the aversive CSs). BP, blood pressure; CS, conditional stimulus. Error bars show the standard error for each CS. *$p$<.05

Table 2.8 Mean weight of food consumed over the criterion sessions for individual animal and accompanying *t*-test results.

| | Amount of Food Consumed (g) | | | |
|---|---|---|---|---|
| Animal | Mean | SE | *t* | *p* |
| 1 | 0.9 | 0.32 | 2.58 | **.049** |
| 2 | 3.3 | 0.16 | 19.37 | **.000** |
| 3 | 2.6 | 0.14 | 21.90 | **.000** |
| 4 | 4.0 | 0.14 | 32.81 | **.000** |
| 5 | 3.5 | 0.11 | 33.67 | **.000** |
| 6 | 3.6 | 0.24 | 14.43 | **.000** |

*Note*: *df*=5. *p* values appear in bold.

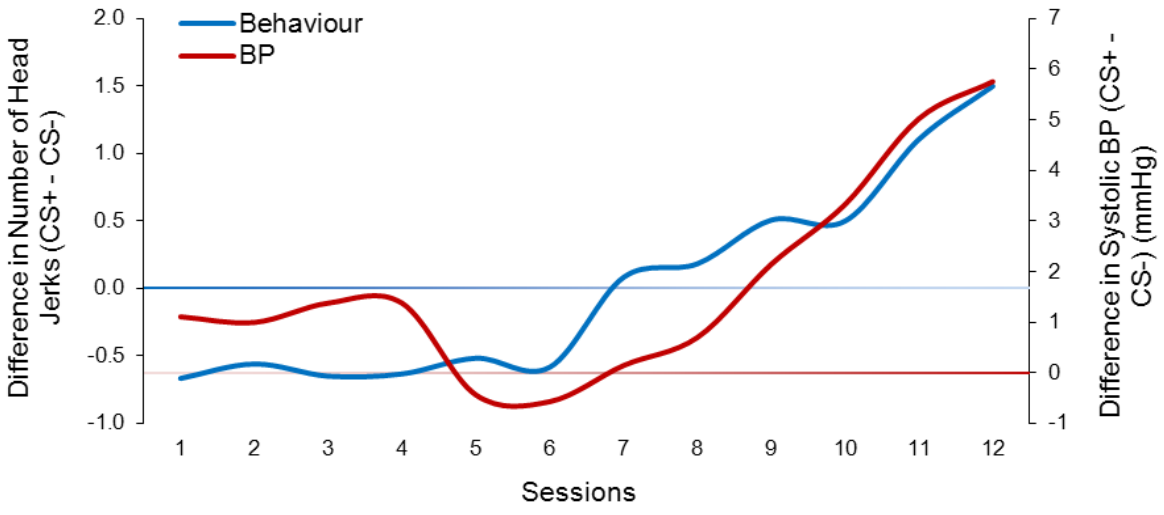



Figure 2.16 Learning curve (running mean of consecutive sessions containing six $CS^{+}$s) for the head jerk (blue line) and BP (red line) of a typical animal. BP, blood pressure; CS, conditional stimulus.

## 2.4 Discussion

A normal cohort of common marmosets was tested on a newly developed aversive discriminative conditioning paradigm. Previous studies on pathological and high trait anxiety using the same paradigm have provided evidence for two alternative hypotheses. The first 'fear generalisation' hypothesis states that individuals with high trait anxiety should show over-generalization of fear, displaying undifferentiated anxiety responses to both CS's (Grillon & Morgan, 1999; Lissek & Grillon, 2010; Mauchnik et al., 2010). In contrast, the hyper fear discrimination hypothesis states that high trait anxiety enhances conditionability, leading to a larger difference in the responses between $CS^+$ and $CS^-$ (Michael et al., 2007; Orr et al., 2000; Peri et al., 2000).

The main results of the current study revealed that out of the 27 animals tested, 20 animals (74%) successfully developed differential responses between the $CS^+$ and $CS^-$ ('passed' group), whilst the remaining seven animals (26%) persistently showed indiscriminative responses to both CS's ('failed' group). This failure in the discrimination is not due to a general learning impairment or auditory perception deficit, because, when tested on an appetitive discriminative conditioning task, in which the same auditory CS's that were paired with aversive noise in the aversive paradigm, were paired with food reward, the animals that had failed the aversive discrimination all successfully developed differential responses in the appetitive paradigm. Thus, a more likely explanation for their failure on the aversive discrimination paradigm is that they were more anxious than those marmosets that displayed successful discrimination. However, what evidence is there for such an account?

Two hypotheses have been proposed to explain how trait anxiety may interfere with CS processing, leading to fear generalisation. The fear inhibition hypothesis postulates that high anxiety interferes with the learning of a 'safety cue'. Thus, an anxious individual fails to inhibit a fear response in the presence of a safety signal (Lissek et al., 2005). In support of this, Lissek and colleagues (Lissek et al., 2009) demonstrated that the discrimination deficit displayed by individuals with PD was attributed to an enhanced anxiety response (fear potentiated startle) to the safety cue rather than aberrant reactivity to the danger cue. The PD patients, expressed as high expectancies of a dangerous outcome in the presence of the safety cue as they did for the danger cue, in contrast to healthy controls that displayed far greater expectancies in the presence of the danger cue. Jovanovic and colleagues (Jovanovic, Kazama, Bachevalier, & Davis, 2012) also suggested that fear generalization occurs due to the lack of safety signal learning. If the association between the $CS^-$ and the

safety outcome is learnt, when both $CS^+$ and $CS^-$ are simultaneously presented post conditioning, the transfer of the learnt safety signal to the compound presentation occurs, resulting in an attenuation of the anxiety response. Whilst this was observed in healthy subjects, PTSD patients displayed the same level of an anxiety response (fear potentiated response) across both conditions, indicating their failure to learn about the safety signal and to transfer that learning across contexts.

In contrast, the contextual fear hypothesis postulates that during aversive conditioning, the initial presentation of the shock (US) is unpredictable, and fear generalizes to the surrounding context. Following several CS-US pairings, cue-specific learning develops and fear to the context is inhibited. Thus, during fear conditioning, subjects learn to identify the CS's as the danger/safety signal. However, enhanced anxiety interferes with this process and leads to higher conditionability to the context rather than to the cues. Grillon (Grillon, 2002a) reported that the individuals with higher trait anxiety not only failed to display differential anxiety responses (fear potentiated startle) between the CS's but also showed marked contextual conditioning (measured during the intertrial interval) and avoidance of the experimental context (higher rate of not returning to the second test session), in comparison to those who were able to discriminate. Baas and colleagues (J M P Baas et al., 2008) investigated the contextual fear hypothesis further by using two different contexts in the cue discriminative conditioning paradigm. Half of the subjects discriminated both the cues ($CS^+/CS^-$) and contexts ($CXT^+/CXT^-$). However, the other half only discriminated the contexts and failed to discriminate the CS's. Those who showed only context discriminative conditioning scored higher in trait anxiety scale than those who discriminated both cue and context. In an animal study (Duvarci et al., 2009), the rats that showed poor fear discrimination of the CS's, when returned to the same context without any CS-US presentation, displayed greater freezing to the context than those that were able to discriminate. The poor discriminators also exhibited a high anxiety-like trait in the EPM.

How do the results from the present study compare with these two hypotheses? In regard to the response to the cues, the results revealed that whilst animals in the 'passed' group displayed an enhanced response to the $CS^+$ and a suppressed response to the $CS^-$, the animals in the 'failed' group displayed a similar magnitude of *enhanced* responses to both CS's. This indicates that their lack of differential responsivity was not due to a reduced response to the $CS^+$ but to an increased response to what is considered a safety signal, the $CS^-$, suggesting an impaired fear inhibition mechanism. This was particularly noticeable with respect to the vigilant behaviour. During early sessions when the discriminative response

was not apparent even among the animals that eventually 'passed' the discrimination, all animals showed similar levels of vigilant scanning (and HR acceleration) to both CS's although the 'failed' group displayed generally elevated responses. Therefore, as the animals experienced repeated CS-US presentations, those in the 'passed' group developed the discriminative response, whilst the animals in the 'failed' group did not learn the $CS^-$ - safety association and continued to show the enhanced response to both CS's. This observation is in line with the fear inhibition hypothesis. As Grillon (Grillon & Ameli, 2001) suggests, high anxiety is not associated with increased levels of fear. It is the response to safety signals that are impaired in high anxious subjects, suggesting impairment in the inhibition of fear.

In contrast to the responses during the cue presentations, an animal's responses during baseline can indicate their responsivity to the context. Grillon (Grillon, 2002b) postulates that during fear conditioning, subjects are kept at a sustained level of apprehensive anticipation of an aversive event. Consequently, the baseline is not neutral and the response to the CS's is riding on an already elevated baseline level reflecting fear to the overall context. The analysis of vigilant scanning and cardiovascular reactivity during the baseline revealed a significant difference between the 'passed' and 'failed' groups in their cardiovascular activity. Specifically, this difference was due to the 'failed' group developing a suppressed BP response across repeated conditioning sessions (a weaker effect was also seen for HR).

High anxiety has been associated with a greater risk of coronary heart disease (Booth-Kewley & Friedman, 1987; Rozanski, Blumenthal, & Kaplan, 1999) and anxiety disorders are frequently associated with altered autonomic cardiovascular function (Berntson, Sarter, & Cacioppo, 1998). Although many reports support a view that pathological anxiety is associated with exaggerated autonomic reactivity (tachycardia and hypertension), which is especially evident in phasic responses to specific stimuli or cues, any association with basal cardiac state is less clear (Berntson et al., 1998). Phobic subjects are reported to show a normal resting HR but an exaggerated tachycardia to fear-relevant stimuli (McNeil, Vrana, Melamed, Cuthbert, & Lang, 1993). Although PTSD patients are reported to exhibit elevated sympathetic and attenuated parasympathetic activations at baseline (Blechert et al., 2007), another study showed no elevated baseline HR or BP but exaggerated autonomic reactivity to trauma-relevant stimuli (McFall, Murburg, Ko, & Veith, 1990). Studies with PD and generalized anxiety disorder (GAD) and obsessive compulsive disorder (OCD) patients also show mixed results, including a reduction in baseline autonomic responses (Berntson et al., 1998; Hoehn-saric & Hipsley, 1995; Hoehn-saric, 1989; Massana et al., 2001). In animal studies, aversive context conditioning in rats and mice has been associated with

hypertension and tachycardia accompanied with defensive behaviours (Carrive, 2000; Dielenberg, Carrive, & McGregor, 2001; Stiedl, Tovote, Ogren, & Meyer, 2004). Baseline HR in fear conditioning, however, was reported not to differ between high and normal trait anxiety mice (Gaburro et al., 2011). On the other hand, fear generalization in rabbits has been associated with bradycardia (Schreurs, Smith-Bell, & Burhans, 2011).

With such mixed reports, the association between trait anxiety and baseline cardiovascular activity is clearly not straightforward. It is even proposed that anxiety disorders, such as specific phobia, in which anxiety is associated with a particular stimulus, give rise to exaggerated autonomic reactivity, whilst more global and diffuse anxiety states, such as GAD, are associated with more blunted reactivity (Berntson et al., 1998). The current findings suggest that the suppressed baseline BP in the fear discriminative conditioning is possibly an anxiety-related response in the common marmoset to a repeated stressor, i.e. loud noise. In fact, the regression analysis revealed that, together with the vigilant behavioural response to the CS's during early sessions, the baseline BP turned out to be a reliable predictor of whether the animals 'passed' or 'failed' the discrimination (detailed discussion below). Since the baseline response is an index of context conditioning, the suppressed BP displayed by the animals in the 'failed' group may suggest their enhanced conditioning to the context.

Thus, to summarise, the current results provide evidence for both the fear inhibition and contextual fear hypotheses. It could be argued that these two hypotheses are not contradictive, but describe the same mechanism from different viewpoints. The failure to learn the safety signal makes the aversive US unpredictable, which leads to the animals conditioning to the context. This is reflected in the indiscriminate responses to both cues and context.

**Biomarkers predicting eventual discriminative failure**

In addition to the above, the analysis also revealed possible signs of anxiety displayed by those in the 'failed' group during early sessions of the experiment. Having found the two groups at the end of the experiment, various measures during early sessions were analysed for any indications of the animal's eventual fate, 'passing' or 'failing' the discrimination. Interestingly, several measures, both behavioural and autonomic, differentiated the animals even at the early stage. As well as the development of suppressed BP and HR mentioned in the previous paragraphs, the animals in the 'failed' group showed generally heightened vigilant behaviours to the CS's and accelerated HR to the $CS^-$, the safety signal, in comparison to those in the 'passed' group. These differences were not observed during the

orienting sessions, in which only the CS's were presented without the aversive US, although the behaviour showed a trend for such an effect. Thus, as soon as the animals started experiencing the stressful event, those that would eventually fail the discrimination developed those enhanced reactions. A binary logistic regression analysis revealed two measures, the heightened vigilant behaviour to the CS's and the suppressed baseline BP, out of the four, as significant predictors of the failure of the discrimination.

A few studies reported subjects' responses during early learning stage in the fear discriminative conditioning paradigm. Grillon (Grillon & Ameli, 2001) reported that during initial blocks of the discriminative conditioning, both high and low anxious subjects displayed increased startle response to the cues. But, the reactivity to the safety signal was higher among those in high anxious group. In contrast, Lissek (Lissek et al., 2009) reported no difference in startle response to the cues between PD patients and the controls during initial blocks of the discriminative conditioning. Although the literatures are few and not conclusive, the obtained results may suggest an association between the enhanced negatively biased sensitivity under a stressful condition and high trait anxiety. Human subjects with high trait anxiety have been reported to show a tendency to negatively interpret emotionally ambiguous stimuli (Chan & Lovibond, 1996; Dunsmoor et al., 2011; Mathews, Richards, & Eysenck, 1989; Richards et al., 2002). During early sessions of the discriminative conditioning, the animals have experienced only a few CS-US pairings, thus the stimuli association has not been apparent yet. The cues would appear semantically ambiguous to the subjects. Having been placed under emotionally stressful condition, those that are high in trait anxiety (i.e. the animals in the 'failed' group) might have interpreted the CS's negatively, showing heightened attentional response to the CS's. This negatively biased attention might eventually have caused the animal the failure to learn the safety signal. The same animals also developed the suppressed BP during early sessions which persisted until the end of the experiment. As mentioned above, this may indicate the animals' enhanced conditionability to the context. The fact that together these two measures were predictive of the discriminative outcome suggests that they may act as biomarkers of underlying trait anxiety in the common marmoset.

**Neural underpinnings**

Differences in both cue and contextual processing between those animals that 'passed' and those that 'failed' might reflect the involvement of different neural pathways. LeDoux (J. E. LeDoux, 1995) proposed that regardless of the types of fear conditioning paradigm, simple, discriminative or contextual, the amygdala is activated. The amygdala receives sensory

information, processes it and produces physiological and behavioural responses that are similar across these different paradigms. However, the pathway by which sensory information reaches the amygdala differs, depending on the types of conditioning. Simple conditioning, in which a single CS is paired with an aversive US, mainly involves the direct thalamic pathway to the amygdala. This thalamo-amygdala pathway is short and fast, and just sufficient for the rapid triggering of an emotional response by simple stimulus features. On the other hand, in discriminative conditioning, cortical processing is required before the information reaches the amygdala. This thalamo-cortico-amygdala pathway is longer and slower, but capable of more accurate processing of perceptually complex stimulus objects. Contextual conditioning, on the other hand, is dependent on the hippocampus as well as the amygdala. The hippocampus is a crucial structure in the representation of background/contextual stimuli. This hippocampal-amygdala pathway allows the animals to distinguish between those situations in which it is appropriate to defend oneself against a stimulus from situations in which it is not necessary (J. E. LeDoux, 1995). The bed nucleus of stria terminalis (BNST), by virtue of its connection with the hippocampus, is also involved in contextual conditioning (Sullivan et al., 2004), especially in the situation of an unpredictable aversive US (Michael Davis et al., 2010).

As the thalamo-cortico-amygdala pathway is proposed for the neural network specifically involved in aversive discriminative conditioning (J. E. LeDoux, 1995), the next question is which cortical area is responsible for the neural computation that gives rise to the discrimination of the safety from danger cues. Apart from sensory cortical areas that are required for accurate processing of sensory information (Armony, Servan-Schreiber, Romanski, Cohen, & LeDoux, 1997; Chen & Barnes, 2011; Dunsmoor et al., 2011), several regions in the prefrontal cortex have been reported for their roles in discriminative learning. These include the infralimbic / prelimbic prefrontal cortex (Zelinski, Hong, Tyndall, Halsall, & McDonald, 2010), ventromedial prefrontal cortex (vmPFC) (Courtin & Herry, 2011), ventralateral prefrontal cortex (vlPFC) (Agustín-Pavón et al., 2012) and orbitofrontal cortex (OFC) (Agustín-Pavón et al., 2012; Zelinski et al., 2010). Although the prefrontal cortex in general is involved in various executive higher order functions including cognitive flexibility, emotional regulation, evaluation of contingencies between different stimuli and response inhibition (Bishop, 2007; R. J. Davidson, 2002; Ghashghaei & Barbas, 2002; Milad & Rauch, 2007; G J Quirk & Gehlert, 2003; Sotres-Bayon & Quirk, 2010), the specific role of its sub-region in fear and anxiety has not been clearly identified. This issue is further discussed in Chapter 4, in which the experimental results of the OFC- and lPFC-dependent cognitive flexibility tests on the animals from the ‘passed’ and ‘failed’ groups were reported.

In summary, the results of this chapter demonstrate that by using an aversive discriminative conditioning paradigm, a normal cohort of common marmosets can be categorized into those that 'passed' (ones able to discriminate the $CS^+$ from $CS^-$: 74%) and those that 'failed' (unable to discriminate: 26%). The subsequent testing of the 'failed' group on the appetitive discriminative conditioning paradigm using the same CS as that in the aversive discrimination paradigm, confirmed that the 'failure' of the discrimination was not due to the impairment of a general learning ability or auditory perception. Based on the previous findings, it is suggested that the discrimination failure is a detrimental effect of trait anxiety on cognition, known as fear generalization. Individuals with high trait anxiety tend to over-generalize environmental cues under stressful condition. In addition, the analysis of behavioural and autonomic measures during early stages of conditioning revealed two predictors of the final discriminative performance, namely, vigilant scanning to the CS's and baseline BP. These measures are interpreted as potential indicators of trait anxiety in the common marmoset.

The successful development of the aversive discriminative conditioning model in the common marmoset provides substantial benefit for the future research in neurobiology of anxiety and fear. The discriminative conditioning paradigm bears several advantages over classical anxiety tests that measure animal's unconditioned response in a fearful environment. 1) the observed variables are specific to anxiety and fear, and less confounded by task irrelevant measures such as exploratory trait, 2) the binary categorical outcome explicates the detrimental effect of trait anxiety, providing a clear border between low and high anxiety. In fact, many of the previous studies on pathological / high trait anxiety conducted the aversive discriminative conditioning experiment following the measurement of unconditioned anxiety responses, proving the power of the discriminative paradigm in the identification of high / low anxious individuals. Moving in the opposite direction, the animals from the 'passed' and 'failed' groups identified in this study will now be tested on two classic tests previously used to identify trait anxiety in monkeys, namely, the human intruder test and the rubber snake test. If the hypothesis is correct, and the animals in the 'failed' group are more anxious, then they are also expected to show greater anxiety-related unconditioned responses to the potential threat in the human intruder and rubber snake tests in comparison to those from the 'passed' group. These results are reported and discussed in Chapter 3.

# Chapter 3

## Is failure to show discriminative fear conditioning a marker of high trait anxiety in marmosets?

## Abstract

The previous chapter identified a cohort of marmosets that displayed an over-generalisation of fear responses on a Pavlovian fear discrimination task, a potential marker of high trait anxiety (Lissek et al., 2010). To assess more directly the evidence that these animals were high anxious, their performances were studied on two conventional tests of anxiety commonly used in primates, an unfamiliar human intruder and a snake stimulus. In the case of the former, it has been reported that anxiety responses were reduced following treatment with diazepam in both rhesus monkeys (Kalin & Shelton, 1989) and marmosets (Carey, Costall, Domeney, Jones, & Naylor, 1992),

In order to first characterise the behavioural repertoire displayed by marmoset monkeys in response to a human intruder and rubber snake, a large cohort of neurobiologically intact animals, including the animals that went through the aversive discriminative conditioning task, were tested, and their behavioural responses were analysed by principal component analysis (PCA). The underlying psychological dimensions extracted by PCA were then used to compare the 'passed' and 'failed' groups.

The results revealed a significant aversive effect of both a human intruder and a rubber snake on marmoset behavioural patterns. In both paradigms, PCA revealed two psychological components, which were subsequently labelled 'emotionality' and 'coping strategy'. The comparison of the groups from the aversive discriminative conditioning task revealed a significant difference in the 'emotionality' component of the rubber snake test. The animals that showed over-generalization of fear exhibited enhanced emotional reactivity toward the snake, supporting the findings in the previous chapter that fear generalization is a marker of high trait anxiety. In addition, the 'emotionality' scores were inversely correlated with one of the two predictors of the over-generalization of fear, the baseline blood pressure. Multiple regression analysis predicting the 'emotionality' scores produced a significant model retaining not only the baseline blood pressure but also the cue-specific vigilance behaviour, supporting these two measures as possible biomarkers of enhanced trait anxiety in marmosets. No group difference in the human intruder test and a significant, but weak correlation between the two paradigms, together imply that the two emotionally relevant stimuli may differ somewhat and be processed through different neural pathways.

## 3.1 Introduction

The previous chapter introduced the newly developed aversive discriminative conditioning paradigm for the common marmoset model of trait anxiety. The paradigm was designed to detect a detrimental effect of trait anxiety known as 'fear generalization' (Dunsmoor et al., 2011). Based on their discriminative conditioning performance, a normal cohort of marmoset monkeys were categorized as either animals that were able to discriminate the CS's (the 'passed' group) or ones that persistently displayed undifferentiated responses to the CS's (the 'failed' group). Since the 'failed' group were able to learn an appetitive discrimination relatively well, it was unlikely that the 'failed' group were generally poor learners. Thus, it was hypothesized that the animals in the 'failed' group were more anxious than those in the 'passed' group and this was supported by their enhanced HR and behavioural responses to the CS- early on in learning. However, to test this hypothesis more explicitly the behaviour of these same animals was measured on more conventional tests of anxiety in non-human primates, namely the human intruder and rubber snake tests.

This is the first attempt in non-human primates to link impaired discriminative conditioning (fear generalization) with anxiety. However, it has been demonstrated that rats that showed a poor ability to discriminate between $CS^+$ and $CS^-$ show a high anxiety-like trait in the EPM (Duvarci et al., 2009) and a similar relationship has been reported in humans (J M P Baas et al., 2008; Grillon, 2002a). Thus, it is expected that animals that were unable to show discriminative conditioning (the 'failed' group) would display greater anxiety-related responses in comparison to those animals that did display discriminative conditioning (the 'passed' group) on both the human intruder and rubber snake tests.

In contrast to the aversive discriminative conditioning paradigm which measures animal's conditioned response to cues or contexts, the human intruder test and rubber snake test measure animal's unconditioned responses to threatening or stressful stimuli (Rodgers et al., 1997). The human intruder test, also known as the human threat/confrontation paradigm, has been one of the most fruitful and widely adopted methods to test fear/anxiety in non-human primates, including rhesus monkeys and common marmosets (for review, see Barros & Tomaz 2002). In a typical human intruder paradigm, an experimenter, who is previously unknown to the subject animal, enters the test room and maintains eye contact with the animal for a few minutes while the animal's responses are recorded. The modified versions include extra conditions in which, first, the subject is removed from the home cage and left

alone in the test room (Kalin & Shelton, 1989) and then second, a human intruder enters but doesn't make eye contact (Izquierdo & Murray, 2004; Kalin, Shelton, Davidson, & Kelley, 2001; Meunier, Bachevalier, Murray, Málková, & Mishkin, 1999). Laboratory animals receive human handling on a daily basis. These include not only favourable treatment such as feeding but also occasional aversive treatment such as health check and vaccination. Therefore, the mere presence of a human can be stressful (Cagni, Gonçalves, Ziller, Emile, & Barros, 2009). In addition, the direct eye contact is seen as a highly threatening gesture, especially in macaque monkeys, inducing fear/anxiety (Machado & Bachevalier, 2008), which is why there is an additional condition in which the intruder stands in front of the cage but looks sideways instead. On the other hand, direct eye gaze is not so threatening to marmoset, therefore in the marmoset version of the task, there is no comparison between direct and indirect gaze conditions (Cagni et al., 2009). The current marmoset human intruder test protocol was designed following the paradigm reported in Carey et al. (Carey et al., 1992) in which upon entering the test room, the intruder stood approximately 40 cm from the cage front and made eye contact with the target animal of the pair throughout the 2-min test period. A variety of anxiety-related behavioural responses are induced by the presence of the intruder and these have been shown to be sensitive to both anxiogenics and anxiolytics (Carey et al., 1992; Costall, Domeney, Farre, & Kelly, 1992)

The snake test has also been a widely used paradigm for the anxiety/fear research both in humans and non-human primates (Öhman & Mineka, 2001). The paradigm typically involves the presentation of an alive or fake snake, or an image of a snake to the target animal, while the animal's responses are recorded (Marilia Barros, Boere, et al., 2002; Deloache & Lobue, 2009; Kalin et al., 2001; Levine, Atha, & Wiener, 1993; Meunier et al., 1999; Vogt, Coe, & Levine, 1981). Modified version of snake paradigm includes a setting in which a snake stimulus is placed between a rhesus monkey and food reward. The subject's latency to reach for the reward as well as unconditioned responses are measured (Nelson, Shelton, & Kalin, 2003). Although the debate as to whether the fear of snakes in primates is an innate trait or a learnt response is still ongoing (S Mineka & Cook, 1993; Öhman & Mineka, 2001), the evidence from the studies of human infants (Deloache & Lobue, 2009), the comparisons between laboratory-born and feral-born rhesus monkeys (Izquierdo, Suda, & Murray, 2005; Levine et al., 1993; Nelson et al., 2003) and the evolutionary considerations (Isbell, 2006; Shibasaki & Kawai, 2009) suggest that there can be a natural tendency to associate a snake with fear. Marked individual differences along a continuum of mild to extreme fear responses have been reported in rhesus monkeys in response to alive or rubber snakes (Nelson et al., 2003) indicating the sensitivity of this stimulus to individual differences in trait anxiety

spectrum. The current marmoset rubber snake test protocol was designed by mainly following the paradigms reported in Barros et al.(Marilia Barros, Boere, et al., 2002) and Cagni et al. (Cagni, Sampaio, Ribeiro, & Barros, 2011) in which the observation period was divided into three intervals: baseline, stimulus exposure and post-exposure periods. Unlike other marmoset snake paradigms (Marilia Barros, Boere, et al., 2002; Cagni et al., 2011; Clara, Tommasi, & Rogers, 2008; N Cross & Rogers, 2006), in the current test, a rubber snake was presented inside the test cage, providing the robust effect of the aversive stimulus.

**Validation of an animal model for the study of anxiety**

In order to validate an animal model for the investigation of human anxiety, three proposed criteria can be applied (Belzung & Griebel, 2001; Bourin, Petit-Demoulière, Dhonnchadha, & Hascöet, 2007).

- Face validity, where the model is phenotypically similar and implies that the anxiety/fear-related response observed in the animal model should be identical to the behavioural and physiological responses observed in humans.
- Predictive validity implies that the animal model should be sensitive to clinically effective pharmacological agents. Therefore, anxiolytic and anxiogenic compounds should elicit opposite effects on the observed variables, while agents that have no effect in the clinic should have no effect in these tests.
- Construct validity relates to the similarity between the theoretical rationale underlying the animal model and the human behaviour. This requires that the etiology or causal attributes of the anxiety behaviour and the physiological factors may be similar in animals and humans. One approach to this criterion is to investigate and compare the neural basis of anxiety responses elicited by the same or similar threatening stimuli across the species (Maximino, Brito, & Gouveia Jr., 2010).

**Face Validity of the Human Intruder and Snake Tests**

The human intruder test and snake test, together with the anxiety paradigms in rodent such as the EPM, open field arena, light/dark box and holeboard test, are categorized as ethologically based animal models of fear and anxiety, that simulate the natural conditions under which such emotional states are elicited (Marilia Barros & Tomaz, 2002). Ethologically elicited fear/anxiety has been suggested to provide a wider range of defensive behaviours. Some observed behaviours are species specific, for instance whilst freezing is seen in

rhesus monkeys (Kalin et al., 2001; Kalin & Shelton, 2003; Meunier et al., 1999), it is rarely observed in the marmosets (Marilia Barros, Boere, et al., 2002; Stevenson & Poole, 1976). Nonetheless, compared to the behavioural repertoire of rodents, non-human primate species not only share many emotional responses such as facial expressions, postures and vocalizations between each other but also these behaviours bear similarity to human ethogram (Marilia Barros & Tomaz, 2002; F. A. King, Yarbrough, Anderson, Gordon, & Gould, 1988). Importantly, upon encountering a potential threat, the species-specific behaviours should reflect survival strategies driven by underlying emotional states (i.e. fear and anxiety) of the organism. These survival strategies are common across species and include avoidance, escape, behavioural inhibition, alarm/mobbing vocalization and hypervigilance (Belzung & Griebel, 2001). For instance, whilst a rhesus monkey and human may run from a threat, a marmoset often jumps away from it. Regardless of the difference in these observed behaviours, they function to escape from the potential danger. Moreover, these survival responses, when overtly or inappropriately expressed, are suggested to be analogous to the behavioural disturbances observed in human anxiety disorders (Prut & Belzung, 2003; Rodgers et al., 1997). These commonalities across species and the analogy to human anxiety provide the face validity for the human intruder and snake paradigm as the animal models of human anxiety.

**Predictive Validity of the Human Intruder and Snake Tests**

Predictive validity requires that clinical drugs that are effective in humans exert a similar effect in animal models. The human intruder test has been widely used to examine the effects of both anxiolytic and anxiogenic agents. Benzodiazepine agents such as diazepam and chlordiazeproxide are GABA receptor agonists and have been traditionally used as anxiolytic drugs to reduce tension and anxiety (Blanchard, Griebel, Rodgers, & Blanchard, 1998). Kalin and Shelton (Kalin & Shelton, 1989) showed that the treatment with diazepam on young rhesus monkeys reduced their anxiety/fear related response (freezing and barking) to a human intruder. Carey et al (Carey et al., 1992) reported that when treated with diazepam, marmosets reduced anxiety-related postures (tail posture, scent marking, slit stare and arched pilo) and increased the time spent at the cage front, indicating significant anxiolytic effect of the drug. On the other hand, while some anxiogenic agents (FG7142, yohimbine, caffeine and pentylenetetrazole) did not show any effect, the administration of amphetamine induced stereotyped behaviours that may indicate hypervigilance. Walsh and colleagues (Walsh, Stratton, Harvey, Beresford, & Hagan, 1995) also reported significant anxiolytic effects of diazepam and chlordiazeproxide on the marmoset human intruder test.

The effects of anxiolytic agents targeting the serotonin (5-HT) system have been also tested on the human intruder paradigm. Acute administration of a specific serotonin transporter inhibitor (SSRI) and 5-$HT_{1A/B}$ autoreceptor antagonist significantly attenuated anxiety-related postures in marmosets without any sedative effect (Starr et al., 2007) and a 5-$HT_{1A}$ receptor ligand was also shown to be effective in reducing anxiety-related behaviours in marmosets (Costall et al., 1992).

Although there has been no study reporting the effects of anxiolytic or anxiogenic agents on the snake test, similar paradigms using other predator models such as a taxidermized cat have been shown to be effective in detecting the effects of these compounds (Marilia Barros et al., 2003, 2007; Cagni et al., 2009).

**Construct Validity of the Human Intruder and Snake Tests**

Construct validity refers to whether observable phenomena in the model actually reflect the underlying psychological concept that the model is designed to measure. In other words, it asks whether the measures in the human intruder and snake tests actually reflect the animal's anxiety/fear state. One of the approaches to this question would be to show that the neurobiological mechanisms that are associated with the responses measured in the model are the same or similar to the ones related to human anxiety/fear (Maximino et al., 2010). Anxiety disorders can be seen as disorders of the defense mechanism. Since appropriate control of a defense response is crucial to the organism's survival, the neural systems involved in the defense response are strongly conserved in evolution (Rodgers et al., 1997). Therefore, in the situation of fear/anxiety, the neural systems activated should be comparable between animals and humans (J. E. LeDoux, 1995).

Neuroimaging studies in humans have reported positive associations between the magnitude of the amygdala reactivity to threatening stimuli and inter-individual variation in indices of trait (Dunsmoor et al., 2011; Etkin et al., 2004; Haas, Omura, Constable, & Canli, 2007; Most, Chun, Johnson, & Kiehl, 2006; Ray et al., 2005) and state (Bishop, Duncan, & Lawrence, 2004) anxiety. Reports that patients with bilateral damage to the amygdala were unable to recognize the facial expression of fear also point out that the amygdala is a key structure in the processing of anxiety/fear (Adolphs, Tranel, Damasio, & Damasio, 1995; Broks et al., 1998; Calder, 1996). A number of studies using the human intruder and snake paradigms investigated the involvement of the amygdala in the emotional processing and expression of fear/anxiety. Amygdala lesions in rhesus monkeys have been reported to reduce fear and

aggression towards snakes, however no effect of the lesion was found on the response to a human intruder (Amaral, 2002; Kalin et al., 2001; Meunier et al., 1999; Meunier & Bachevalier, 2002). Similar results were found with an exctitoxic lesion restricted to the central nucleus of the amygdala (Kalin et al., 2004).

Other brain structures such as the hippocampus, and various prefrontal areas have also been implicated in human anxiety/fear (Bishop, 2007; Lisa M Shin & Liberzon, 2010). Machado and Bachevalier (Machado & Bachevalier, 2008) reported that the lesions to the hippocampus and orbitofrontal cortex (OFC) caused alterations in the defensive behaviours toward a human threat. The OFC lesioned rhesus monkeys also showed the attenuation of snake fear as well as the reduction in the threat-induced freezing to a human intruder (A. S. Fox et al., 2010; Kalin et al., 2007). Another study (Izquierdo et al., 2005) reported similarly attenuated emotional response to a model snake by the bilateral OFC lesion, however the effect of the lesion on a human intruder was milder.

Together, these findings indicate that the same neural systems, which are implicated in human anxiety/fear, are also associated with the process and expression of observable emotional responses measured in the human intruder test and snake test. This implies a causal relationship between the behavioural outcomes measured in those paradigms and the underlying psychological constructs, i.e. anxiety and fear, elicited by the threatening stimuli, supporting the construct validity for the human intruder and snake tests as animal models of human anxiety.

**Aim of the Experiment**

Overall, the findings from previous studies employing the human intruder and snake paradigms strongly support the view that these paradigms fulfill the three validation criteria (face, predictive and construct) for animal models of human anxiety and fear. Thus, the present study employs these well-validated and established tests of anxiety as reliable tools to detect individual differences in anxiety in those marmosets that either failed or passed the discrimination task. Since these tests had not previously been employed in my PhD laboratory, I characterised the performance of a large cohort of marmosets on these tests. A pilot study determined the range of behaviours that characterized the marmosets' responsivity to a human intruder and a rubber snake. These included the anxiety-related behavioural and locomotor measures described previously (Marilia Barros, Boere, et al., 2002; Marilia Barros & Tomaz, 2002; Cagni et al., 2009, 2011; Carey et al., 1992). The

current paradigm also recorded and scored various vocalizations that were made in the presence of the intruder and snake that had not been described in earlier psychopharmacological studies using these tests (Barnes et al., 1990; Costall et al., 1992; Starr et al., 2007; Walsh et al., 1995). However, they have been described in the ethological literature (Bezerra & Souto, 2008) and one of them, the tsik call, has been associated with an increase in cortisol levels after exposure to a threatening stimulus (Nicola Cross & Rogers, 2004).

## 3.2 Human Intruder Test

### 3.2.1 Methods and Materials

#### 3.2.1.1 Subjects

In total, 63 adult common marmosets (28 females and 35 males, aged 1.6 to 4.1 years, average age 2.5 years) were tested on the human intruder test paradigm. These included 13 animals (described in section 3.5) out of the 27 animals that were tested in the aversive discrimination paradigm (section 2.2). All other animals were experimentally naïve (Table 2.1). All animals were housed and fed in the same condition as described in the aversive discrimination paradigm (section 2.2.2.1). All procedures were conducted in accordance with the project and personal licenses under the UK animals (Scientific Procedures) Act of 1986.

#### 3.2.1.2 Test Apparatus

***Home Cage*** Testing took place in an animal's home cage (280 cm high x 120 cm wide x 98 cm deep) (Figure 3.1 'Home Cage'). The back wall was made of stainless steel. The sides and bottom consisted of trespa and plastic panels respectively while the roof was covered with a clear plastic dome. The cage front consisted of stainless mesh. Inside, the cage was equipped with ropes, wooden perches and other objects for the purpose of environmental enrichment. Just before the start of testing, a target animal was isolated from its partner into the right upper quadrant of the home cage (94 cm high x 60 cm wide x 98 cm deep) by opaque separator panels (Figure 3.1 'Test Quadrant'). All the objects were removed from the quadrant except for a nestbox (19 cm high x 25 cm wide x 19 cm deep, 67 cm from the floor and 57 cm from the front mesh) that was hung on the upper right corner; upper and lower perches (both 60 cm in length, 68 cm and 20 cm from the floor respectively, and both 35 cm from the front mesh); back shelf (50 cm wide x 17 cm deep, 66 cm from the floor and 68 cm from the front mesh); middle shelf (50 cm wide x 17 cm deep, 40 cm from the floor and 35 cm from the front mesh); front shelf (41 cm wide x 13 cm deep, 30 cm from the floor, attached to the front mesh) and a rope (hang from one side to another). The partner was isolated into a left lower quadrant; therefore, the pair did not have visual contact during testing. In order to avoid any aversive contact with the experimenter, the animal was encouraged to enter the quadrant voluntarily.

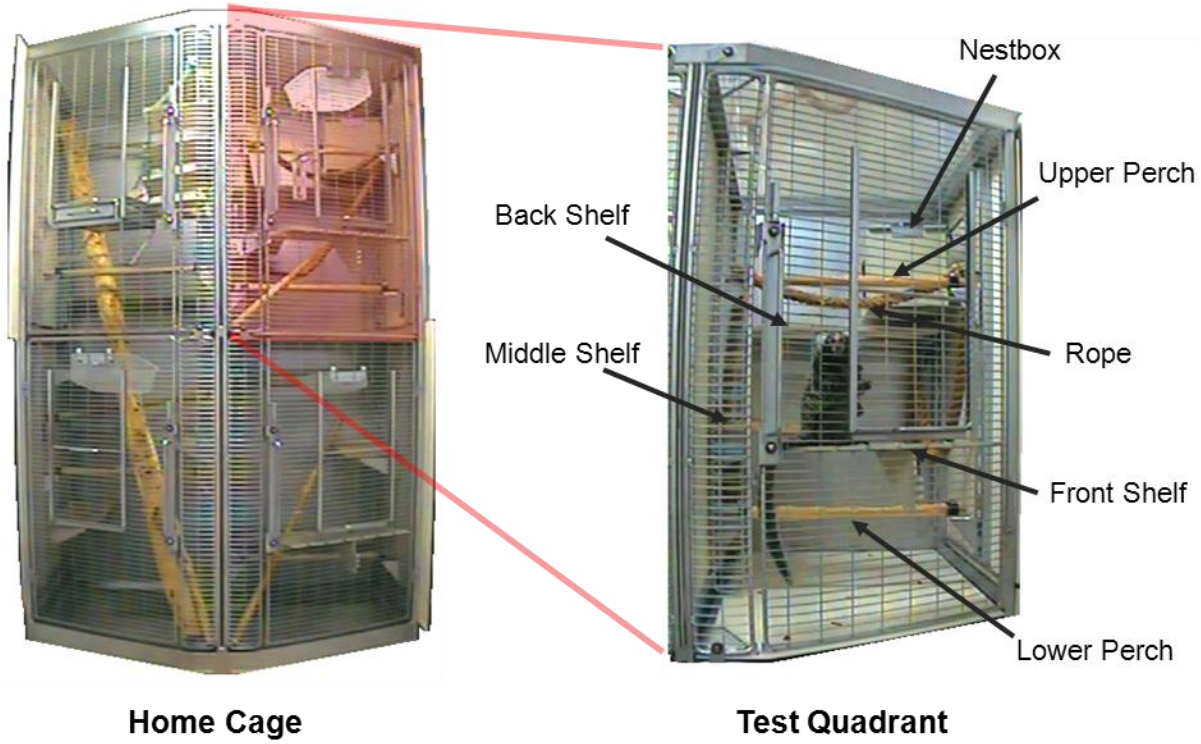



Figure 3.1 A photo of the home cage containing environmental enrichment objects, and a photo of the upper right test-quadrant separated from other quadrants by white plastic panels. The objects kept in during the testing are indicated by arrows.

#### 3.2.1.3 Behavioural Procedure

***Habituation*** To habituate the animal to the presence of the camera equipment, a habituation session was conducted the day before the testing day. The session took place between 12:00 and 13:00 on a weekday. First, the animal was separated into the quadrant, then a video camera (Genie CCTV, C5351/12) mounted on a tripod and a shotgun microphone (Pulse, NPM702) were positioned in front of the front mesh (120 cm and 15 cm from the mesh, respectively). The camera and microphone were connected to a digital recorder (Pinnacle, Video Transfer) placed in a hallway enabling the experimenter to remotely record animal's behaviour. The recording lasted for 20 min during which no one entered the room.

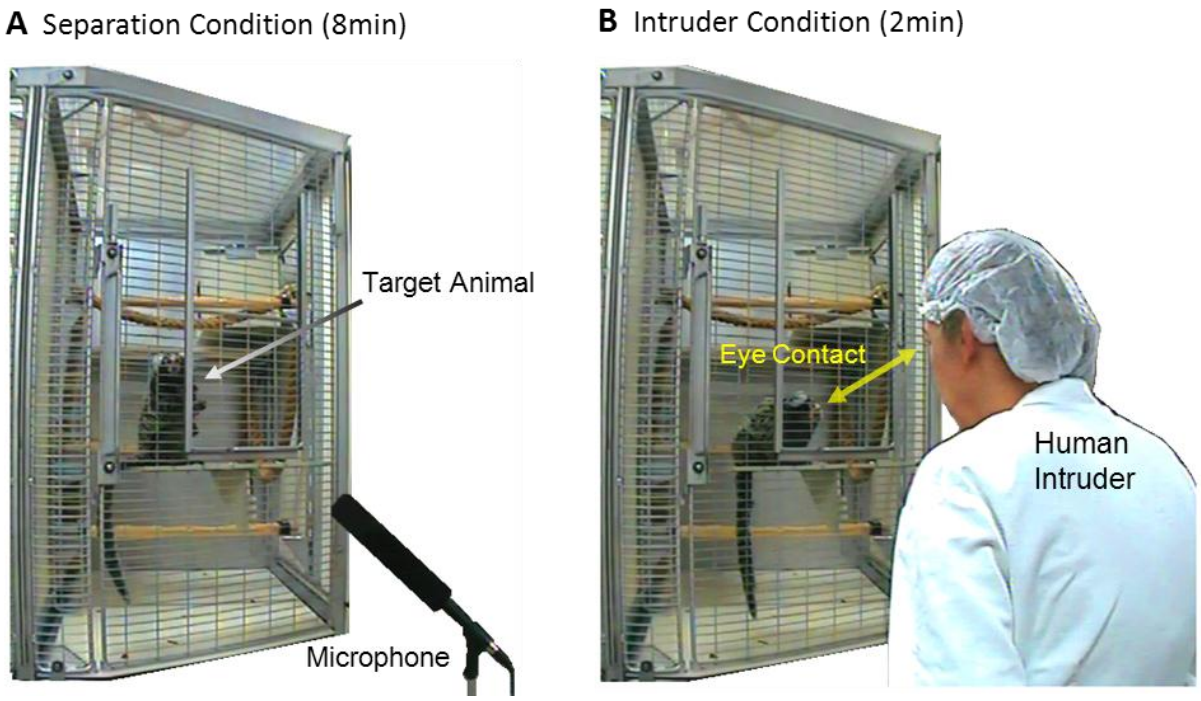



Figure 3.2 (A) A photo of the 8-min separation condition and (B) a photo of the 8-min intruder condition, during which the human intruder kept eye contact with the target animal.

***Human Intruder*** On the next day, the animal was exposed to the human intruder. A person whom the animal had never seen before was selected as the intruder. The 10-min test consisted of two conditions: 'separation' condition (Figure 3.2 A) and 'intruder' condition (Figure 3.2 B), and took place between 12:00 and 13:00 on a weekday. After the target animal was separated into the test quadrant, the camera and microphone were positioned in front of the cage as in the habituation session. After 8 min of recording without the presence of a human (separation condition), the intruder entered the room and stood 40 cm away from the cage front. Throughout the 2-min test period, the intruder maintained eye contact, whenever possible, with the target animal (intruder condition). The intruder was wearing a white lab coat and hair net. Previous studies using a similar paradigm reported that the time spent at the cage front was sensitive to anxiolytic drugs (Carey et al., 1992), therefore the animal's locations in the quadrant as well as behaviours directed toward the intruder and any calls made during the session were video-recorded for post-test scoring purpose. Up to four animals were tested in one day. If both paired partners were scheduled to be tested, they were tested on different days. All the animals received the same treatment.

### 3.2.1.4 Data Acquisition and Analysis

***Behavioural Measurements*** Marmosets exhibit various behavioural responses when encountering a potential threatening stimulus (Marilia Barros et al., 2004; N Cross & Rogers, 2006). To assess the animal's behavioural responses toward the human intruder, the following parameters were scored by an observer using a quantitative analysis program JWatcher, Ver. 1.0 (Blumstain, Daniel, Evans, & Blumstein, 2011).

1. Average distance in the test quadrant. The proportion of the time spent by the animal at a specific location zone in the quadrant was scored. For the scoring purpose, the quadrant was divided into three zones on the horizontal plane (Figure 3.3 A) and into five zones on the vertical plane (Figure 3.3 B). The horizontal zones included the cage front (middle point: 6.5 cm), middle (35.3 cm), back of the cage (71.0 cm) and inside the nestbox (after scoring, it was found that animals rarely went inside the nestbox, thus for statistical analysis the inside nestbox was incorporated with the back). The vertical zones included floor (9 cm), low (24 cm), middle (48 cm), high (71 cm) and on top of the nestbox (85 cm). Top of the nestbox was the furthest point away from the intruder. The middle points were obtained by measuring the distance from the cage front (horizontal plane) or the floor (vertical plane) to the midline of each zone. How much time the animal spent in each zone during the test session was scored separately for the horizontal and vertical planes. This produced up to eight location measures for each animal.

   In order to obtain a single numeric figure that represented an average location of the animal in the quadrant over the test duration, the following formula was applied. First, the middle points from the horizontal and vertical zones were used to calculate the hypotenuse for each zone in the three dimensional representation of the quadrant (Table 3.1). Then, the proportion of time spent in the horizontal and vertical zones were combined to produce the proportion of time spent in each box of the matrix. Finally, the proportion of time was multiplied by the hypotenuse for each box and they were summed, consequently producing a single numeric figure. This number represents each animal's average distance from the floor front (location nearest to the intruder) over the duration of the test session.

2. Locomotion. The duration of time the animal spent in translational movement during the test session was scored. Subsequently, its proportion over the 2-min session was

calculated and used as a measurement of locomotion. Translational movement was defined as the relocation of the body from one location to another.

3. Postures. Number of specific postures exhibited by the animal during the test session was scored. Observed postures included:
    - *Head and body bobbing* – The animal stares at the intruder and rapidly moves its head and upper body side to side. This behaviour is sometimes accompanied by an emission of warning calls.
    - *Jump toward the front* – The animal jumps from back to the front of the cage. A jump is defined as a leap with four paws in air momentarily. Jumps have been shown to be anxiogenic sensitive (Carey et al., 1992).

    These behaviours were shown to be anxiety/stress related (Stevenson & Poole, 1976; Carey et al., 1992; Baross et al., 2004). Other reported postures such as tail posture (display of anogenital area accompanied by tail lift), scent marking, slit stare (staring an object with eyelids half closed), piloerection and self-grooming/scratching were included in the listed behaviours for scoring; however, subsequently it was found that these postures were rarely observed during the test session thus not included in the analysis.

4. Vocalizations. Number of specific calls was counted. Marmosets live in a family group and use a variety of vocalization to communicate between members (Stevenson & Poole, 1976; Bezerra & Souto, 2006). Types of calls observed included:
    - *Egg call* – A very short call with a few harmonics, uttered singly or in series (≤ 3 call units). Frequency range: 9.8±1.7 ~ 1.7±0.8 kHz.
    - *Tsik call* – A short and loud 'tsik' sound, uttered either singly or in series. Frequency range: 14.6±0.6 ~ 2.7±0.4 kHz, most prominent part falls between 14 - 6 kHz.
    - *Tsik-egg call* – Tsik call produced in combination with egg call. Frequency range: 18.0 ~ 2.0 kHz (tsik) + 10.0 ~ 1.0 kHz (egg).
    - *Tse call* – A short call similar to tsik without low frequency 'k' sound. Frequency range: 16.9±0.7 ~ 11.8±0.7 kHz, no frequency observed below 8 kHz.
    - *Tse-egg call* – Tse call followed by egg call. Frequency range: 18.0 ~ 12.0 kHz (tse) + 10.0 ~ 1.0 kHz (egg).

    It has been reported that marmosets use tsik calls as a mobbing and warning call when there is a potential predator in the environment (Marilia Barros et al., 2004; Bezerra & Souto, 2008; Clara et al., 2008; N Cross & Rogers, 2006; Stevenson &

Poole, 1976). Egg calls are also associated with vigilance behaviour in the presence of a threat (Bezerra & Souto, 2008). Tse call has been observed under the situation involving intragroup aggression as well as during vigilant behaviour (Bezerra & Souto, 2008).

Although other calls such as the phee call (a long and loud high-pitched call) and ng/ock call (short low frequency sound from throat) were counted, the occurrence was so rare that they were not included in the analysis.

For scoring purposes, recorded sound information was extracted from the video files using an audio editor program Audacity (ver. 1.3.13, http://audacity.sourceforge.net/) and analysed via Syrinx-PC sound-analysis software (Burt, 2006) which produced frequency sonogram (Figure 3.4). To ensure objectivity, an observer visually matched the frequency and pattern of recorded calls with their prescribed description while listening to the sound.

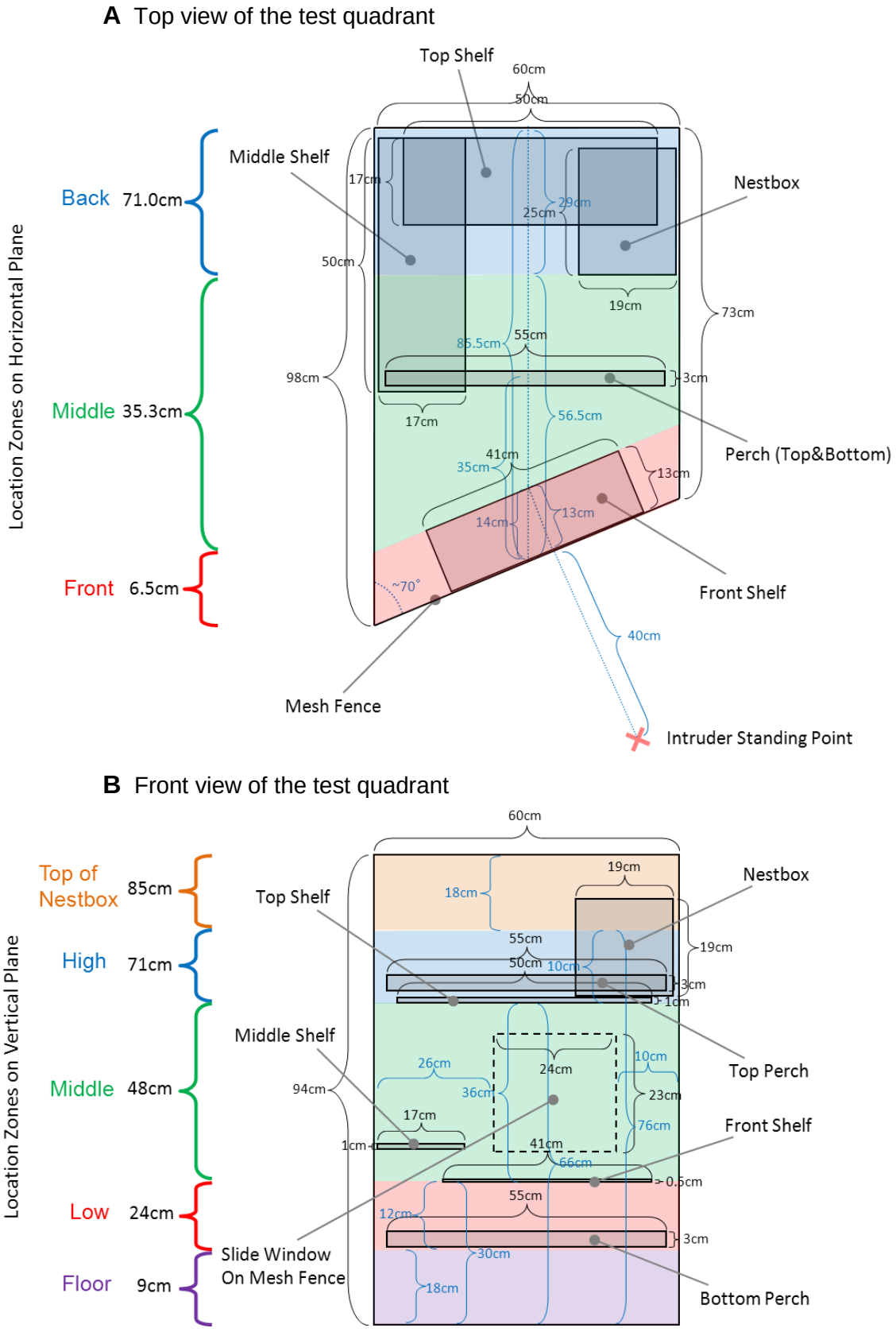



Figure 3.3 (A) Top view and (B) front view of the test quadrant with the dimensions of all the objects contained. Location zones and their middles points are also indicated.

Table 3.1 Matrix with the hypotenuses for each location zone and the middle points from which the hypotenuses were calculated.

| | | | Horizontal Zone and Middle Point (cm) | | |
|---|---|---|---|---|---|
| | **Hypotenuse (cm) from Front & Floor** | | Back | Middle | Front |
| | | | 71.0 | 35.3 | 6.5 |
| Vertical Zone and Middle Point (cm) | Top of Nestbox | 85.0 | **110.75** | **91.92** | **85.25** |
| | High | 71.0 | **100.41** | **79.16** | **71.30** |
| | Middle | 48.0 | **85.70** | **59.41** | **48.44** |
| | Low | 24.0 | **74.95** | **42.44** | **24.86** |
| | Floor | 9.0 | **71.57** | **36.14** | **11.10** |

***Statistical Analysis*** Statistical analyses were performed using a statistic software SPSS (version 19.0). The animals' performances between the separation condition and the intruder condition were compared by using a Student's *t*-test (for parametric data) and a related-samples Wilcoxon signed rank test (for non-parametric data). Within the intruder condition, the behavioural paradigm produced multiple variables, so in order to elucidate underlying psychological dimensions driving these variables, principal component analysis (PCA) was performed. Adequacy of sample size was checked by Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy, of which the test score of larger than 0.5 was ensured (Field, 2009, p.647). For PCA, both zero correlation and extreme multicollinearity are problematic; the former was tested by Bartlett's test, whose significance was ensured; the latter was assessed by Pearson's correlation, whose *r* values were noted to be under 0.8. Since the paradigm was designed to test animal's psychological construct, of which observed measures are not completely independent from each other (Field, 2009, p.644), oblique rotation (direct oblimin) was used to calculate the loadings of the variables on each principal component. Component scores for individual animals were then calculated by using the Anderson-Rubin method (Field, 2009, p.635). Pearson's correlation was used to examine the relationships between the multiple variables. The assumption of normality was checked by Kolmogorov-Sminov test and Shapiro-Wilk test. The homogeneity of variance was tested by Leven's test. The data satisfied all assumptions, otherwise noted.

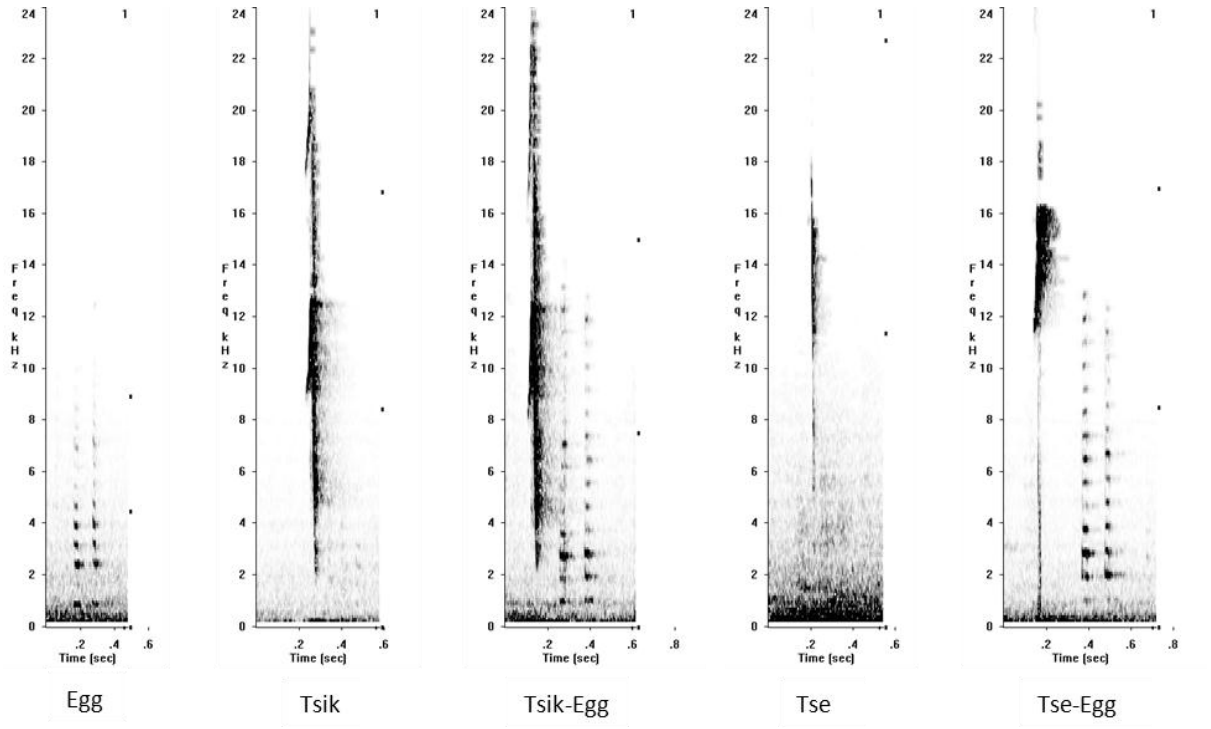



Figure 3.4 Examples of the five types of call recorded, with their typical bandwidth and pattern of frequency graphically represented against time in sonogram.

## 3.2.2 Results

### 3.2.2.1 Effect of Intruder Presence on an Animal's Behaviour

All animals' behaviour markedly changed in the presence of the human intruder. Some of the behaviours were elicited specifically during the intruder condition only, namely the emission of calls and the head-body bobbing. Thus, out of nine measures (four behavioural measures and five calls, section 3.2.1.3) only the average distance and locomotion could be compared across the separation and intruder conditions. It was found that in the presence of the intruder the animals stayed further back in the test quadrant (Figure 3.5 A) and were more active (Figure 3.5 B) than in the separation condition. Paired Student's *t*-test comparing the average distance between the separation condition and the intruder condition revealed that there was a significant difference between the conditions [$t(62)=-6.03$, $p<0.001$], with the animals keeping a significantly greater distance from the cage front in the intruder condition (mean: 79.98 cm, SE: 2.14) than in the separation condition (mean: 66.98 cm, SE: 2.31). Since the proportion of time spent in locomotion during the separation condition violated the assumption of normality [Shapiro-Wilk test: $W(63)=0.95$, $p=0.017$], a non-parametric test was used to compare the conditions. Related-samples Wilcoxon signed rank test revealed a significant difference between the conditions [$Z=5.82$, $p<0.001$], in that the animals spent greater proportion of time in locomotion during the intruder condition (mean: 7.65%, SE: 0.94) than during the separation condition (mean: 2.75%, SE: 0.25).

It is evident from Figure 3.6 in which the scores of all 63 animals tested are depicted, that there was marked individual variation in the behavioural responses to the human intruder. Most of the animals emitted egg calls (Figure 3.6 A) but far fewer emitted the other types of calls, namely, tsik, tsik-egg, tse and tse-egg (Figure 3.6 B, C, D). Those that did emit these other calls often emitted them in quite large numbers, which is why the distributions are positively skewed with a long tail. Head-body bobbing tended to be displayed, simultaneously, with egg calls. Together, these behaviours indicate a strong attentional, orienting response (Marilia Barros et al., 2004; Bezerra & Souto, 2008). The histogram depicting head-body bobbing shows a platykurtic distribution, indicating that the animals were evenly spread from the low to high displayers of this behaviour (Figure 3.6 G). The least common response was that of making jumps to the front, towards the human intruder (Figure 3.6 H). 46% of animals did not show this behaviour but those that did, tended to be the animals that approached the intruder and were more active in their presence, as evidenced by significant correlations with these measures (Table 3.2). For more discussion of this, see the following PCA results

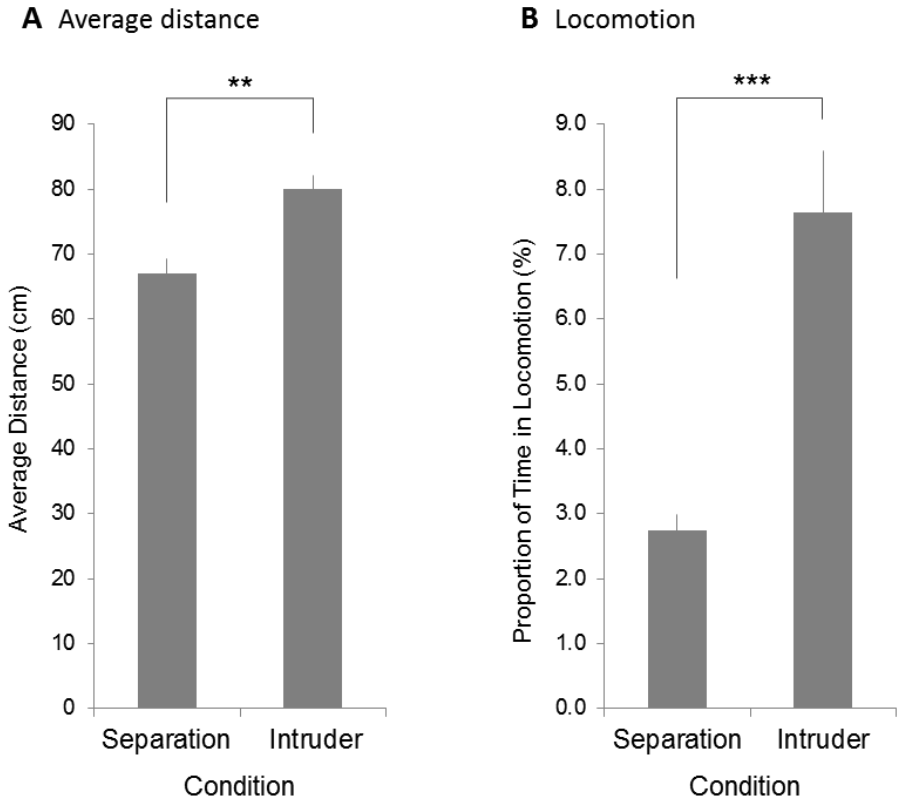



Figure 3.5 Comparison of (A) the average distance in the test quadrant and (B) the proportion of time spent in locomotion between the separation and intruder conditions. n=63. $p<.01$**, $p<.001$***.

(section 3.2.2.2). The majority of animals stayed significantly further back in the presence of the intruder as described above, with the distance scores being symmetrically distributed, centering around the mean of 80 cm from the intruder. Thus, average distance shows a fairly normal distribution (Figure 3.6 E). The positively skewed distribution of the locomotion measure with a long tail indicates that while about one third of the animals were relatively inactive, the more active animals showed a wide spread in their degree of mobility (Figure 3.6 F).

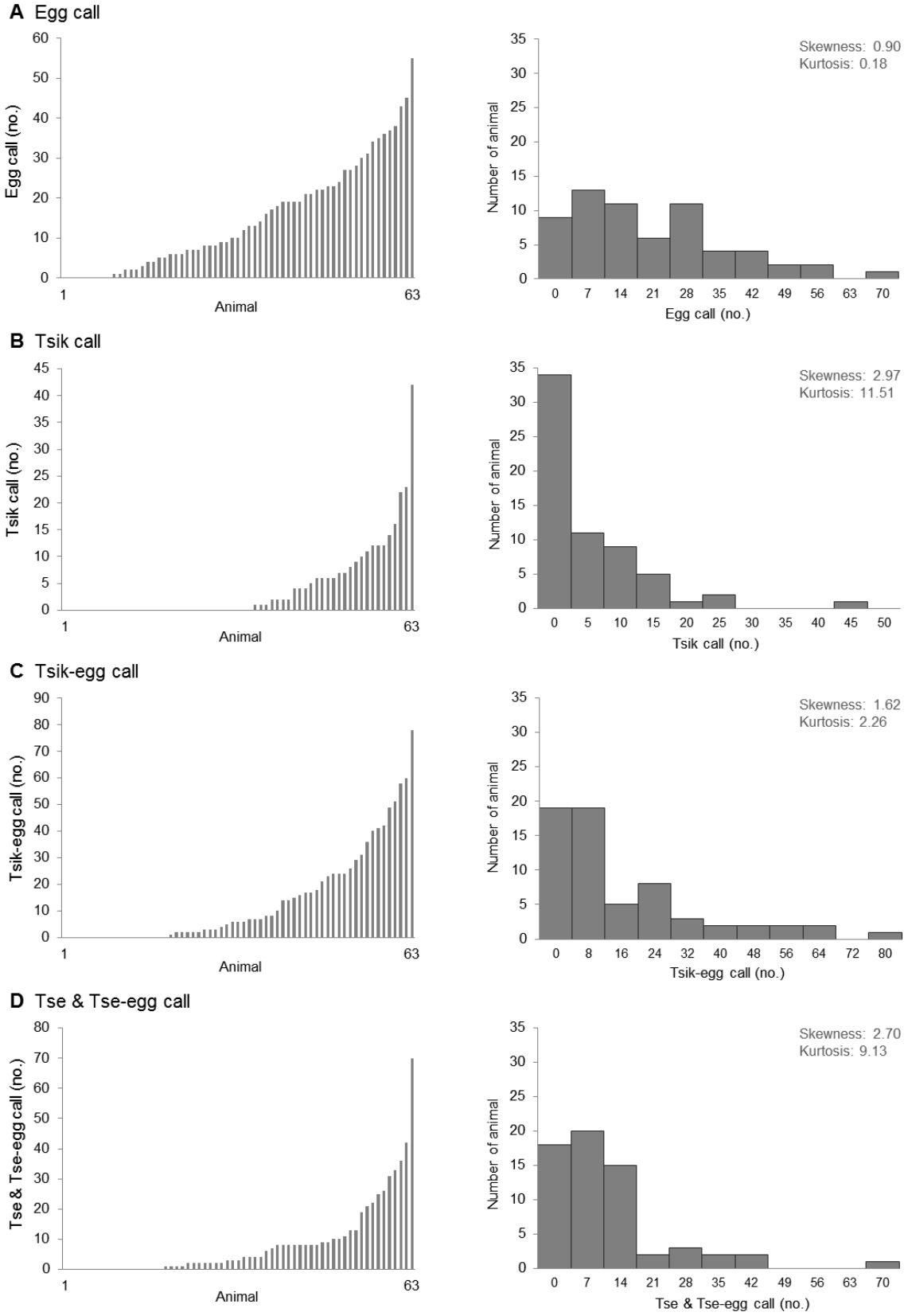



Figure 3.6 Behavioural measures during the intruder condition. Graphs on the left show the scores of individual animals and graphs on the right show the distribution histogram with corresponding skewness and kurtosis statistics. (A) The number of egg calls. (B) The number of tsik calls. (C) The number of tsik-egg calls. (D) The number of tse & tse-egg calls.

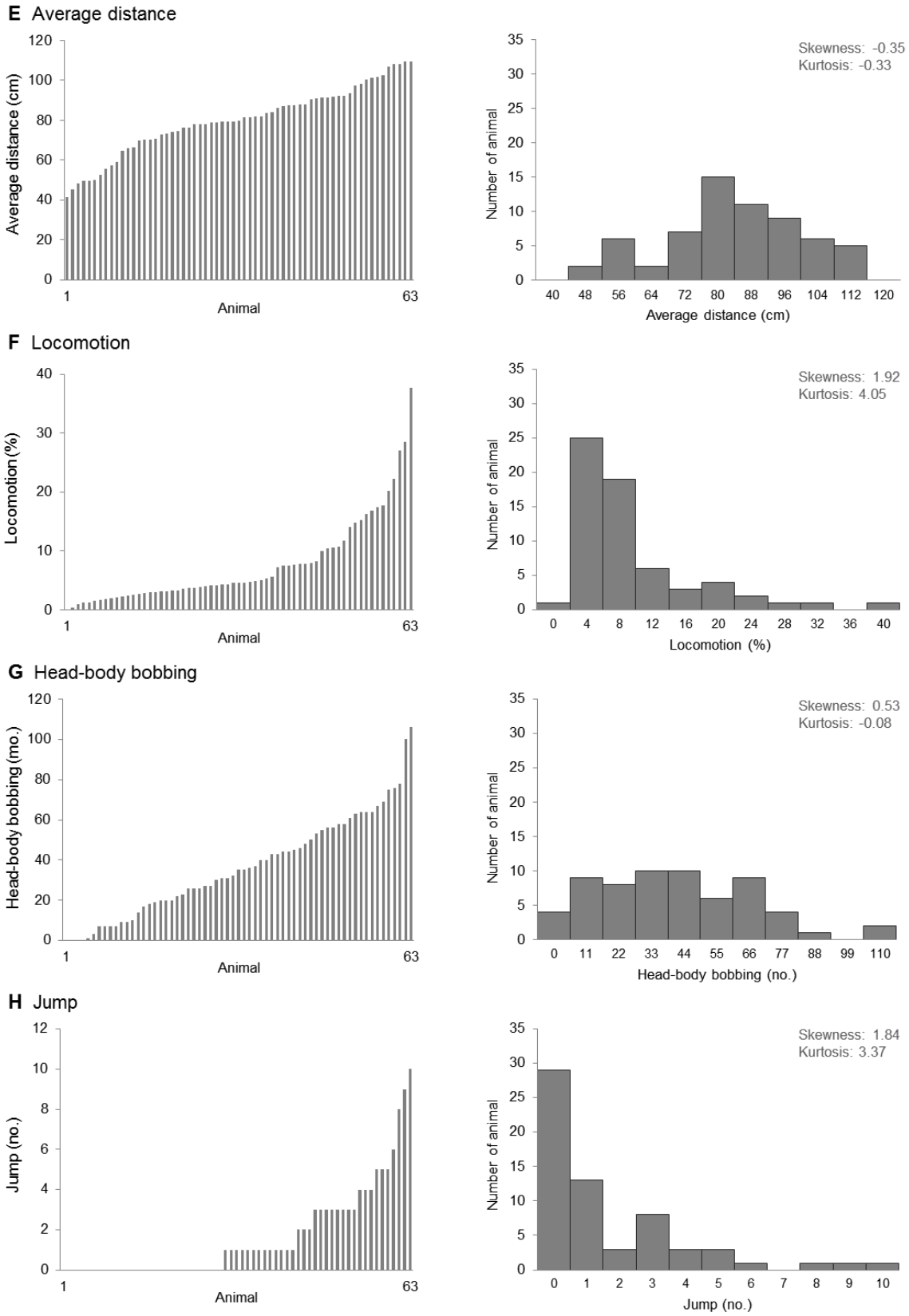



Figure 3.7 (continued) (E) The average distance from the intruder. (F) The proportion of time spent in locomotion. (G) The number of head-body bobbing. (H) The number of jumps toward the front. A perfect normal distribution has skewness and kurtosis values at zero.

Table 3.2 Pearson correlation matrix of the measures in the intruder condition.

| | Egg call | Tsik call | Tsik-egg call | Tse & Tse-egg call | Average distance | Locomotion | Head-body bobbing | Jump |
|---|---|---|---|---|---|---|---|---|
| **Egg call** | 1.000 | -.409*** | .016 | .177 | .441*** | -.417*** | .615*** | -.263* |
| **Tsik call** | | 1.000 | .187 | -.070 | -.228* | .186 | -.336** | .032 |
| **Tsik-egg call** | | | 1.000 | .110 | .272* | -.265* | .296** | -.280* |
| **Tse & Tse-egg call** | | | | 1.000 | .425*** | -.308** | .305** | -.277* |
| **Average distance** | | | | | 1.000 | -.726*** | .701*** | -.575*** |
| **Locomotion** | | | | | | 1.000 | -.563*** | .683*** |
| **Head-body bobbing** | | | | | | | 1.000 | -.429*** |
| **Jump** | | | | | | | | 1.000 |

*Note*: * $p<.05$, ** $p<.01$, *** $p<.001$

### 3.2.2.2 PCA Reveals Two Psychological Dimensions: 'Emotionality' and 'Coping Strategy'

It is clear from the results so far that there is a range of different behaviours displayed by marmosets on this test with varying degrees of relatedness. Thus, in order to elucidate the underlying psychological dimensions driving these variables the data were subject to PCA. Out of the four calls observed during the intruder condition, the tse call had the lowest counts (Table 3.3), and a highly significant correlation between tse and tse-egg calls indicating their similarity (Pearson's correlation $r$=.35, $p$=0.003). Therefore, these two calls were combined for subsequent analyses. In total, eight variables (egg call, tsik call, tsik-egg call, tse & tse-egg call, average distance, head-body bobbing, locomotion and jumps) were analysed with PCA. The sampling adequacy of the analysis was verified by KMO=.79 (Field, 2009). Bartlett's test of sphericity $X^2(28)=190.50$, $p<0.001$, indicated that correlations between items were sufficiently large for PCA (Table 3.2). An initial analysis was run to obtain eigenvalues for each component in the data. Two components had eigenvalues over Kaiser's criterion of 1 and in combination explained 62.35% of the variance. The scree plot also showed an inflexion point after the second component. Both results justified retaining components 1 and 2 in the final analysis.

Table 3.3 Means and standard deviations of nine measures recorded during the intruder condition.

| | Vocalization | | | | | Behaviour | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Egg call (no.) | Tsik call (no.) | Tsik-egg call (no.) | Tse call (no.) | Tse-egg call (no.) | Average Distance (cm) | Proportion of Time Spent in Locomotion (%) | Head-body Bobbing (no.) | Jump (no.) |
| Mean | 14.78 | 4.08 | 13.68 | 1.68 | 13.68 | 79.98 | 7.65 | 36.48 | 1.63 |
| Standard Error | 1.69 | 0.92 | 2.27 | 0.54 | 2.27 | 2.14 | 0.94 | 3.16 | 0.29 |

Table 3.4 and Figure 3.8 show the component loadings after rotation. The measures that loaded highly positively on component 1 included the average distance from the intruder and the number of head-body bobbing; tsik-egg and egg calls were loaded moderately; on the other hand, locomotion and the number of jumps to the front showed high negative loadings. Those animals with higher component 1 scores distanced themselves from the intruder, made frequent head-body bobbing, emitted a fair number of tsik-egg and egg calls, stayed relatively immobile and made few jumps to the cage front. According to the description of these behaviours in previous studies (refer to section 3.2.1.3), the pattern indicated high anxiety/emotionality. Thus, the component 1 was labelled 'emotionality'. The measure that loaded highly positively on the component 2 was the number of tsik calls; the tsik-egg call was loaded moderately; while the number of egg calls showed a fairly negative loading. Those animals with higher component 2 scores emitted a greater number of tisk calls but few egg calls. The tsik call is a mobbing call and has been associated with a proactive coping strategy against a potential threat (Agustín-Pavón et al., 2012). Based on this pattern of variable loadings, the component 2 was labelled 'coping strategy'. From the loadings, the component coefficients were derived via the Anderson-Rubin method (Table 3.5), which in turn provided component scores for individual animals (Figure 3.9). The coordinates of individual points on the two axes, 'emotionality' and 'coping strategy', represent the psychological construct of the animal facing the situation with a potential threat, a human intruder.

Table 3.4 Component loadings of the measures in the human intruder test. Eigenvalues for and proportion of variance accounted by each component. (n=63)

| | Rotated Component Loadings | |
|---|---|---|
| Measures | **Component 1 'Emotionality'** | **Component 2 'Coping Strategy'** |
| Average distance | **.856** | -.137 |
| Locomotion | **-.844** | .058 |
| Jump to front | **-.791** | -.159 |
| Head-body bobbing | **.742** | -.339 |
| Tsik-egg call | **.548** | **.541** |
| Tse & Tse-egg call | **.504** | .009 |
| Tsik call | -.068 | **.829** |
| Egg call | **.449** | **-.626** |
| Eigenvalues | 3.59 | 1.40 |
| % of variance | 44.85 | 17.50 |

*Note*: Component loadings over .40 appear in bold.

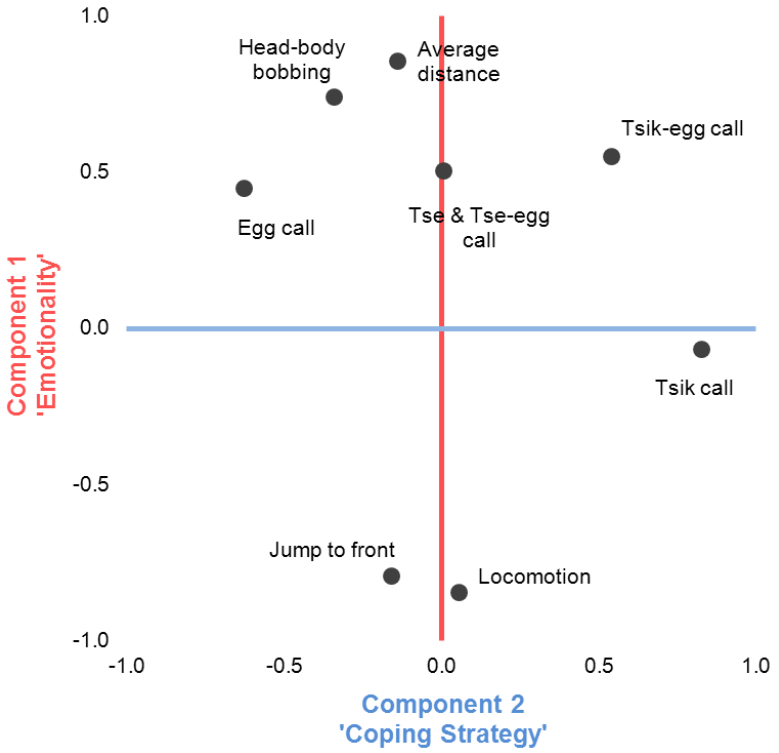



Figure 3.8 Component loadings of the measures in the human intruder test plotted in rotated space with the 'emotionality' and 'coping strategy' axes.

Table 3.5 Component score coefficients of the measures in the human intruder test, from which component scores for individual animals were calculated.

| | Component Score Coefficients | |
|---|---|---|
| Measures | **Component 1 'Emotionality'** | **Component 2 'Coping Strategy'** |
| Average distance | .250 | -.036 |
| Locomotion | -.251 | -.016 |
| Jump to front | -.249 | -.157 |
| Head-body bobbing | .202 | -.178 |
| Tsik-egg call | .201 | .396 |
| Tse & Tse-egg call | .153 | .039 |
| Tsik call | .033 | .547 |
| Egg call | .095 | -.387 |

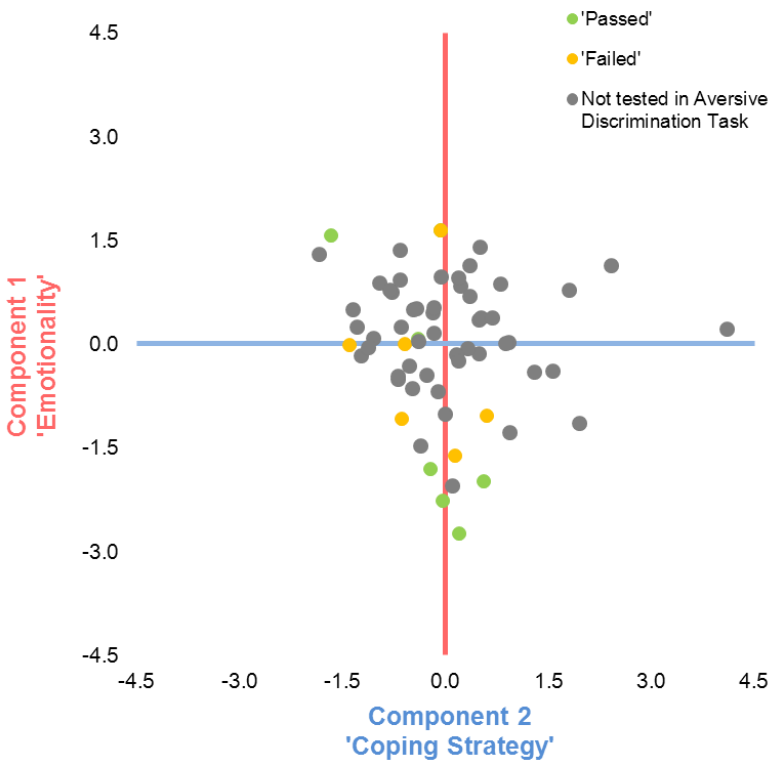



Figure 3.9 Component scores of individual animals plotted on the axes of 'emotionality' and 'coping strategy'. The animals in the 'passed' and 'failed' groups were indicated with green and yellow dots respectively (for the group comparison, refer to section 3.5). (total n=63)

## 3.3 Rubber Snake Test

### 3.3.1 Methods and Materials

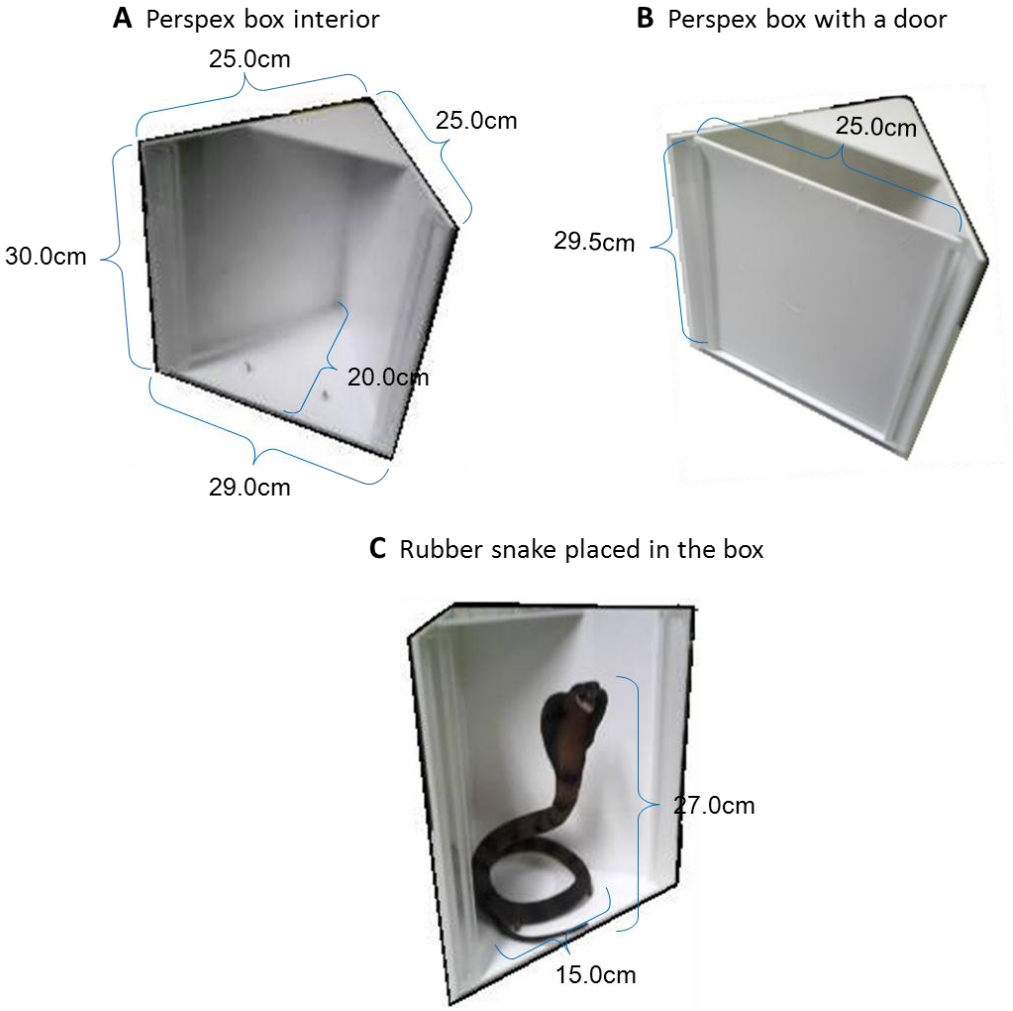
#### 3.3.1.1 Subjects

In total, 44 adult common marmosets (21 females and 23 males, aged 1.7 to 4.3 years, average age 2.9 years) were tested on the rubber snake test. These included 13 animals (described in section 3.5) out of the 27 animals that were tested in the aversive discrimination paradigm (section 2.2, Table 2.1). Prior to receiving the rubber snake test, all animals had been tested on the human intruder test (section 3.2). The interval between the two tests was at least three weeks. All subjects were housed and fed in the same conditions as described in the aversive discrimination paradigm (section 2.2.1). All procedures were conducted in accordance with the project and personal licenses under the UK animals (Scientific Procedures) Act of 1986.

#### 3.3.1.2 Test Apparatus

***Home Cage*** Testing took place in a subject's home cage (dimensions and materials are described in section 3.2.1.2). As in the human intruder paradigm (section 3.2.1.2), just before the start of testing, a target animal was isolated from its partner into the right upper quadrant of the home cage. The setup in the test quadrant was the same as described in the human intruder paradigm. The paired partner was isolated in the left half of the cage, preventing visual contact during testing. In order to avoid any aversive contact with the experimenter, the subject was encouraged to enter the quadrant voluntarily.

***Stimulus*** A model snake made of rubber was used as a stimulus. It resembled a cobra and was coiled, with its head raised (27 cm in height) and dark brownish in colour with black stripes (Figure 3.10 C). A triangular prism box made of opaque white Perspex (25.0cm x 25.0cm x 29.0cm triangle sides x 30cm high) (Figure 3.10 A) contained the rubber snake. By removing the sliding door at the front (Figure 3.10 B), the snake could be revealed to the animal. The animals had never seen the snake or the box before the experiment.




### 3.3.1.3 Behavioural Procedure

***Habituation*** To habituate the subject to the presence of the camera equipment and the snake box, a habituation session was conducted the day before the testing day. The session took place between 12:00 and 13:00 on a weekday. First, the target animal was separated into the test quadrant, then a video camera (Genie CCTV, C5351/12) mounted on a tripod and a shotgun microphone (Pulse, NPM702) were positioned in front of the cage (120 cm and 15 cm from the front, respectively) (Figure 3.11 A). To provide a view from the top, a small wireless camera (Swann, PPW-245) was placed on the clear plastic ceiling of the quadrant (Figure 3.11 B). The cameras and microphone were connected to a digital recorder (Pinnacle, Video Transfer) placed in a hallway enabling the experimenter to remotely record

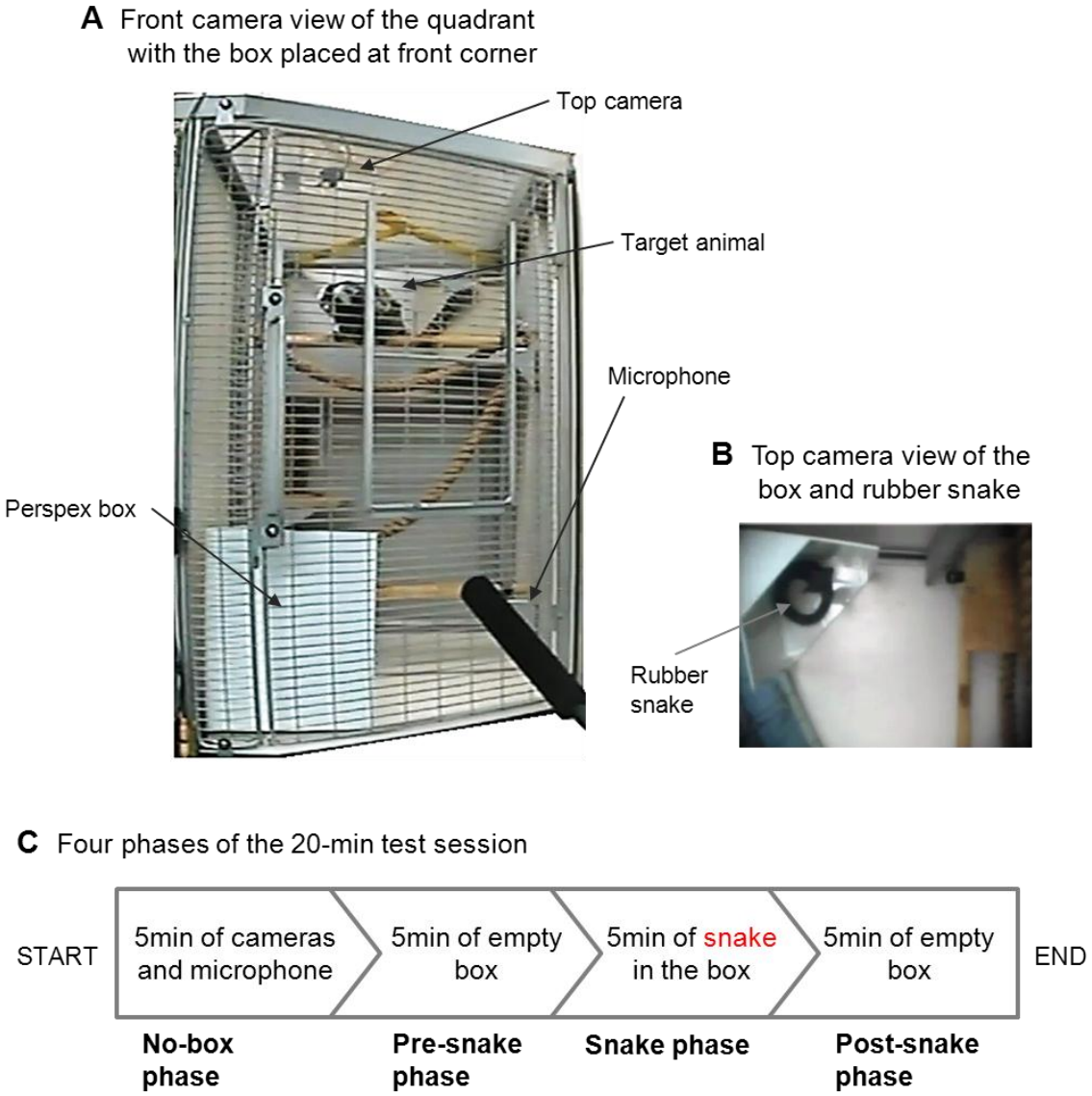



Figure 3.11 Photos of the test quadrant from (A) the front camera view and (B) the top camera view, and (C) the time line of the four phases of the 20-min test session.

animal's behaviour. In order to simulate the test procedure, the 20-min recording period was divided into four phases. After the initial five minutes with only the cameras and microphone, the experimenter entered the room with the empty box and placed the box into the front left corner of the test quadrant carefully avoiding any eye or physical contact with the animal. Subsequently, in two other occasions with a 5-min interval (i.e. 10$^{th}$ minute and 15$^{th}$ minute) the experimenter entered the room and replaced the box with another identical empty box until the 20-min session was completed.

***Rubber Snake Test*** Twenty four hours later, the subject was exposed to the rubber snake. The 20-min test consisted of four 5-min phases: 'no-box phase', 'pre-snake phase', 'snake phase' and 'post-snake phase' (Figure 3.11 C). The procedure was identical to the habituation session except that on the third of the 5-min phases, the box contained the rubber snake ('Snake phase'). Up to three subjects were tested in one day but only one subject per holding room. Each subject received the same treatment.

### 3.3.1.4 Data Acquisition and Analysis

***Behavioural Measurements*** Marmosets exhibit various behavioural responses when encountering a potentially threatening stimulus (Marilia Barros et al., 2004; N Cross & Rogers, 2006). To assess the subject's behavioural responses toward the model snake, the following behavioural parameters were scored by an observer using a quantitative analysis program JWatcher, Ver. 1.0 (Blumstain et al., 2011).

1. Average distance in the test quadrant. The proportion of the time spent by the animal at a specific location zone in the quadrant was scored. For scoring purposes, the quadrant was divided into seven zones based on their approximation to the rubber snake and the nestbox (the furthest structure from the rubber snake). In order to obtain the middle point with which the distance from the rubber snake was measured, multiple locations an animal could position itself within each zone were taken. The centre of these multiple location points was taken as the middle point. The seven zones and their distances from the rubber snake were 'contact snake box' (0.0 cm), 'proximity snake box' (23.0 cm), 'floor' (44.0 cm), 'middle' (50.5 cm), 'proximity nestbox' (84.0 cm), 'inside nestbox' (103.5 cm) and 'top of nestbox' (126.0 cm) (Figure 3.12). During the no-box phase when no box was present, the front left corner was regarded as the imaginary box location. How much time the animal spent in each zone over the 5-min phase was scored.

   In order to obtain a single numeric figure that represented an average location of the animal in the quadrant over the 5-min phase, first the proportion of time spent in each zone was multiplied by its mean distance from the snake; the products were then added across the zones. The resulting number represents each animal's average distance from the rubber snake over the duration of each 5-min phase.

2. Locomotion. Proportion of time an animal spent in translational movement over the 5-min phase was scored and used as the measurement of locomotion. The translational

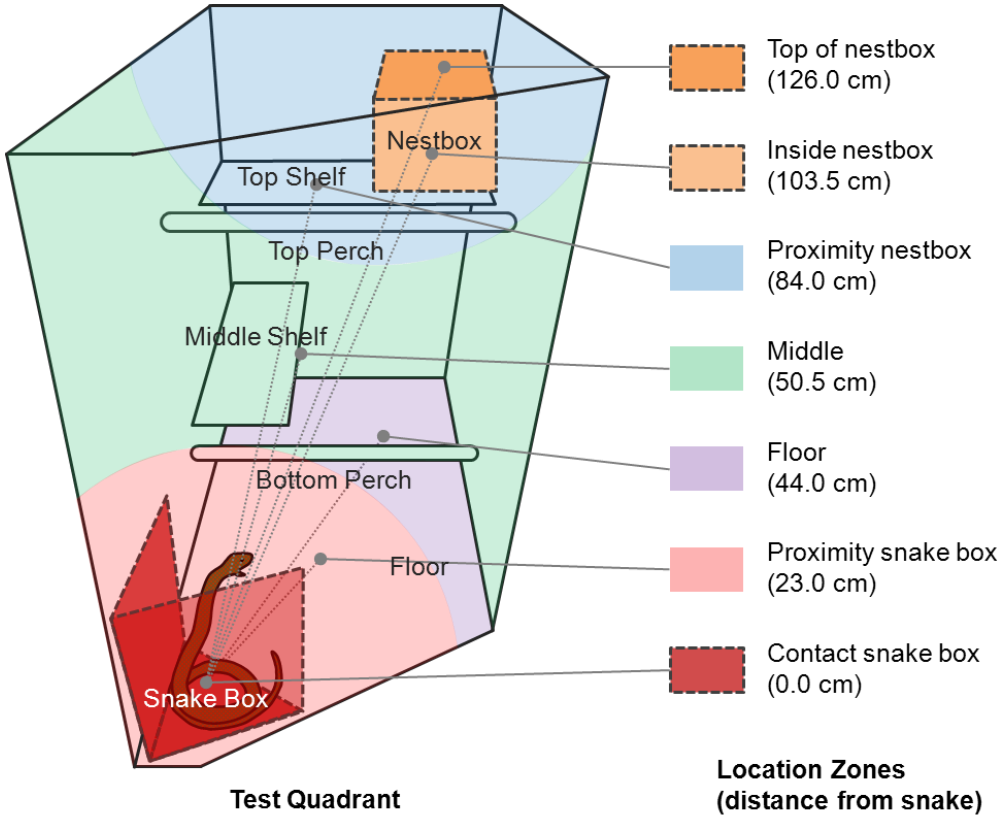



Figure 3.12 Schematic diagram of the test quadrant viewed from the upper front corner, with the location zones depicted with different colours and the mean distance of each zone from the rubber snake.

movement was registered when an animal altered its body position involving the displacement of all four limbs.

3. Stare. The proportion of time an animal spent staring at the model snake was recorded. Staring was defined as when an animal's eyes and head were oriented directly toward the rubber snake.

4. Stare-frequency. The number of occasions an animal stared at the model snake was also scored.

5. Head-cock. The number of occasions an animal cocked his head, defined as head movements from side to side while the animal's attention was directed towards the rubber snake was scored. This behaviour has been reported as an observational behaviour (Marilia Barros, Boere, et al., 2002). Other postures as described in section

3.2.1.3 were included in the scoring scheme; however, those postures were rarely observed during the test session and thus not included in the analysis.

6. Vocalizations. The number of specific calls was counted. Types of calls observed (frequency ranges of the calls were provided in section 3.2.1.3) included:
    - *Tsik call* – An alarm/mobbing call emitted in the presence of a potential predator (Bezerra & Souto, 2008; Cagni et al., 2011; Clara et al., 2008; N Cross & Rogers, 2006).
    - *Tsik-egg call* – A tsik call closely followed by an egg call (a short call with a few harmonics), associated with vigilance behaviour (Bezerra & Souto, 2008; Pistorio, Vintch, & Wang, 2006).

    In addition, all types of calls described in section 3.2.1.3 were included in the scoring scheme; however, apart from the above two calls, the other types of calls were either rarely, or not emitted, in response to the rubber snake (egg, tse and tse-egg all had a mean of fewer than three calls). Therefore, they were not included in the analysis.

    As in the human intruder test, to aid objective scoring, the sound information was extracted and analysed by using an audio editor program Audacity (ver. 1.3.13, http://audacity.sourceforge.net/) and a sound-analysis program Srynx-PC software (Burt, 2006).

All of above behavioural parameters were observed during the snake phase; thus, these were scored and used for subsequent analyses. However, in the absence of the rubber snake, no calls were observed and stare measure was irrelevant without the target to look at. Therefore, the average distance was used as a behavioural measure for the three other phases ('no-box', 'pre-snake' and 'post-snake') outside the snake phase.

***Statistical Analysis*** Statistical analyses were performed using a statistic software SPSS (version 19.0). A repeated-measures ANOVA was used to compare the animals' performances across the phases. Within the 'snake phase' analysis, PCA, with oblique rotation (direct oblimin), was performed to condense correlated measures into their principal components. Tests for the assumptions and detailed analyses were the same as described in section 3.2.1.4 Pearson's correlation was used to examine the relationships between the multiple variables. The assumption of normality was checked by Kolmogorov-Sminov test and Shapiro-Wilk test. The homogeneity of variance was tested by Leven's test. The data satisfied all assumptions, otherwise noted.

## 3.3.2 Results

### 3.3.2.1 Effect of Rubber Snake on an Animal's Behaviour

As in the human intruder test, the majority of behavioural measures were responses induced specifically by the experimental procedure, namely, in this case, the presence of the snake. The only behaviour that was measured across all phases of the test was the average distance from the snake box. It was found that while the animals were attracted to the empty box during the pre-snake phase, when the rubber snake was placed in the box, they kept a wide distance from the snake & box. After the rubber snake was removed, some animals went back to the empty box to investigate during the post-snake phase (Figure 3.13). A repeated-measures ANOVA comparing the average distance of all 44 animals across the four phases revealed a main effect of phase [$F(3, 129)=44.19$, $p<0.001$]. Post-hoc pairwise comparisons revealed a significant difference between the snake phase (mean: 72.3cm, SE: 2.53) and all the other phases: the no-box phase (mean: 64.4cm, SE: 3.10) [$F(1, 43)=4..42$, $p=0.041$], the pre-snake phase (mean: 34.2cm, SE: 3.56) [$F(1, 43)=102..58$, $p<0.001$], the post-snake phase (mean: 59.2cm, SE:2.93) [$F(1, 43)=19.36$, $p<0.001$], that is, the animals stayed further back in the quadrant in the presence of the rubber snake than in any other conditions. The pre-snake phase also significantly differed from any other phases: the no-box phase [$F(1, 43)=60.37$, $p<0.001$], the snake phase, the post-snake phase [$F(1, 43)=64.75$, $p<0.001$], that is, the animals stayed closer around the empty box before the exposure to the rubber snake than in any other conditions. No significant difference was found between the no-box phase and the post-snake phase [$F(1, 43)=2.38$, $p=0.130$].

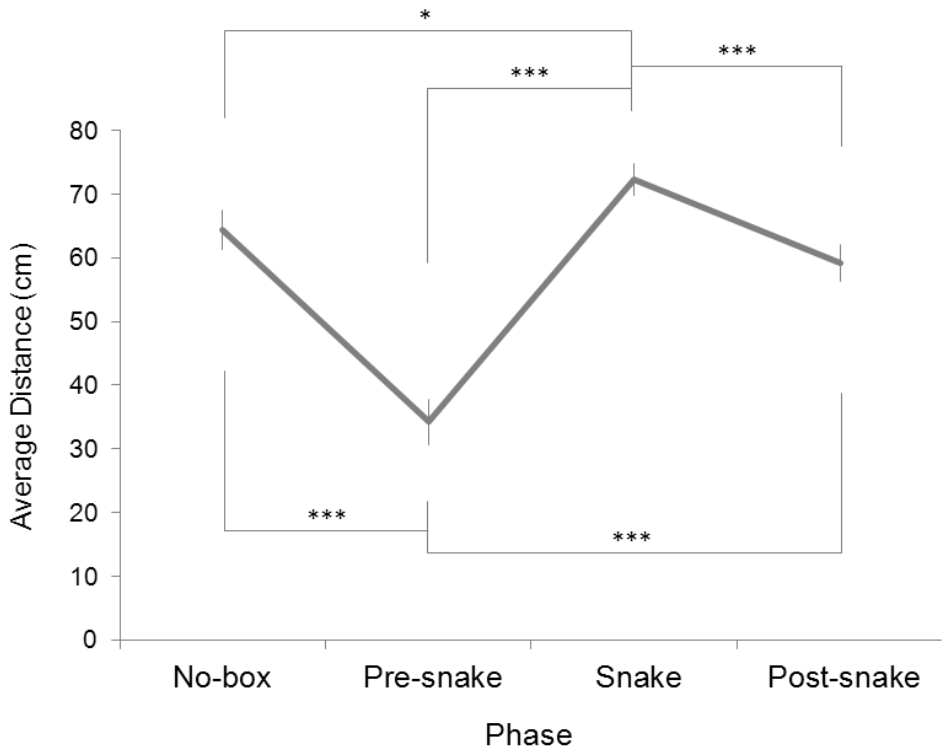



Figure 3.13 Comparison of the average distance across the four phases. *$p$<.05, ***$p$<.001Figure 3.2.1. Photos and dimensions of (A) Figure 3.10

It is evident from Figure 3.14 that there was marked individual variation in the behaviour of all 44 animals tested, similar to that seen in the human intruder test. However, it should be noted, that some of the behavioural responses were different to those seen in the presence of the human intruder. Egg calls were extremely rare in the presence of the snake. Instead, far more common was the emission of tsik-egg calls. Tsik calls were also more common and certainly given in greater numbers when they were emitted, compared to the human intruder test (Figure 3.14 A). The histogram depicting the number of tsik-egg calls shows a mildly positively skewed distribution, indicating that, although only a few animals made extremely large numbers of calls, the majority still made a fair number (Figure 3.14 B). Other behaviours exhibited by the marmosets in the presence of the snake included staring at the snake and making head-cocks. The frequency distributions depicting stare duration and stare frequency show symmetrical bell-shaped curves, with the largest cluster around the mean and few cases at the extremes (Figure 3.14 E, F). Interestingly, stare frequency, but not stare duration, was highly positively correlated with the number of tsik and tsik-egg calls (Table 3.6). This relationship is discussed further in the following PCA results (section 3.3.2.2). The distribution of the number of head-cocks is mildly platykurtic, with the majority of animals

falling in the range of 6-15 head-cocks (Figure 3.14 G). As described above, all animals increased their distance away from the snake box in the presence of the snake. The distribution shows a cluster around a mean of 72 cm with only a very few animals approaching close to the snake or staying in the furthest corner (Figure 3.14 C). Locomotion too showed a fairly normal distribution, centering around 5% (Figure 3.14 D).

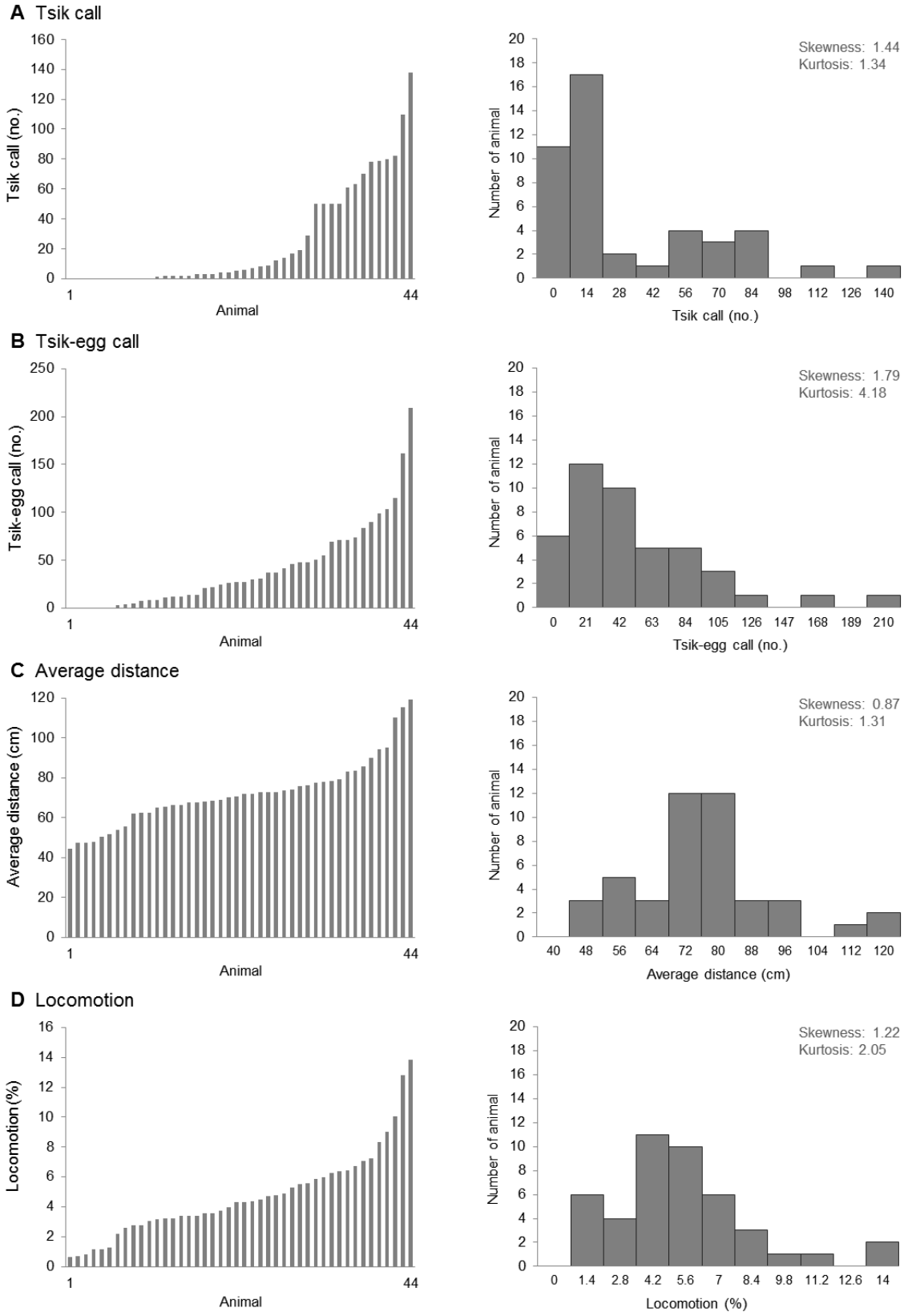



Figure 3.14 Behavioural measures during the snake phase. Graphs on the left show the scores of individual animals and graphs on the right show the distribution histogram with corresponding skewness and kurtosis statistics. (A) The number of tsik calls. (B) The number of tsik-egg calls. (C) The average distance from the rubber snake. (D) The proportion of time spent in locomotion.

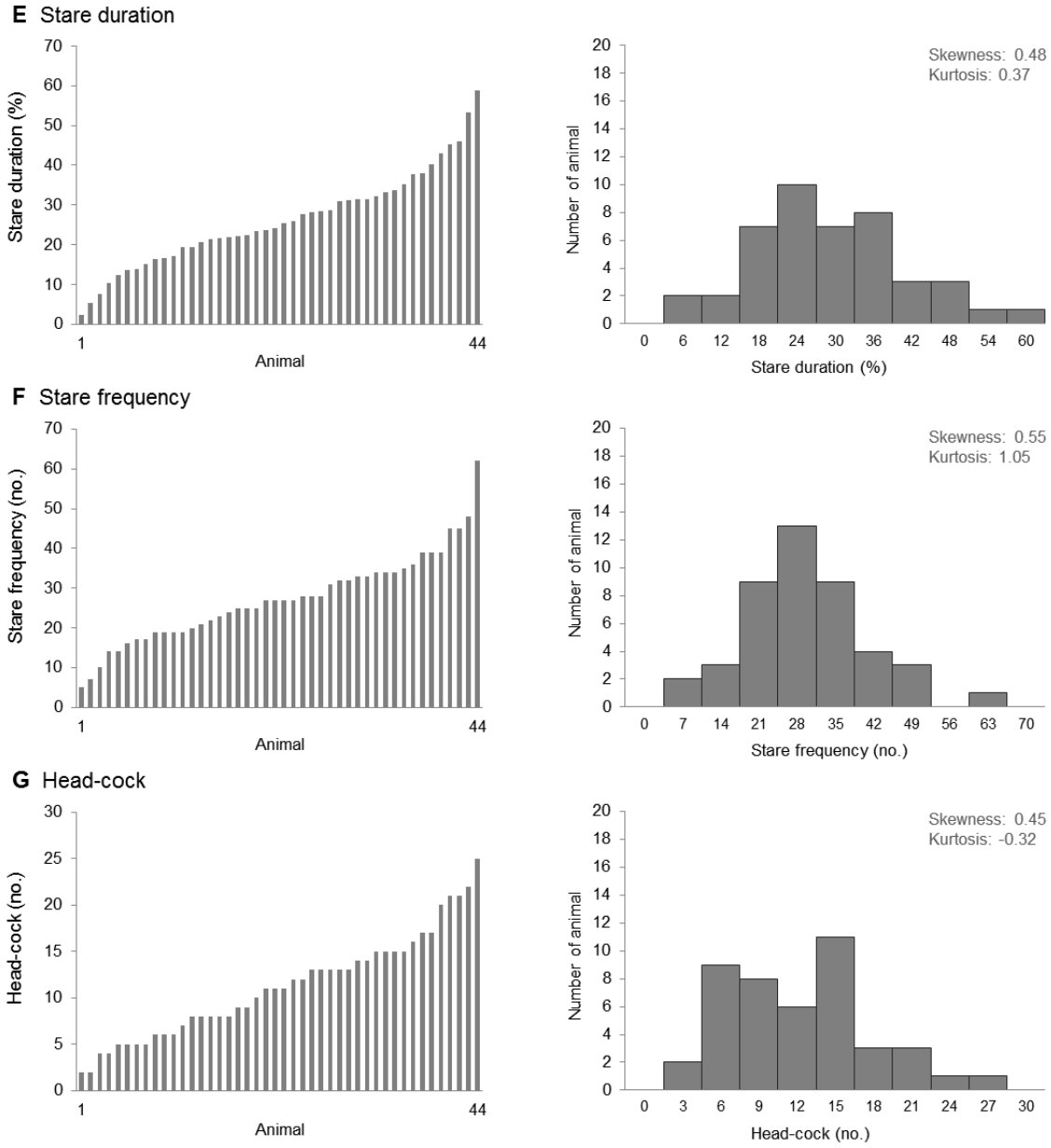



Figure 3.14 (continued) (E) The proportion of time spent staring at the rubber snake. (F) The number of occasions looking at the rubber snake. (G) The number of head-cocks. A perfect normal distribution has skewness and kurtosis values at zero.

Table 3.6 Pearson correlation matrix of the measures in the snake phase.

| | Tsik call | Tsik-egg call | Average distance | Locomotion | Stare duration | Stare frequency | Head-cock |
|---|---|---|---|---|---|---|---|
| **Tsik call** | 1.000 | .690*** | -.017 | .120 | .057 | .536*** | .258 |
| **Tsik-egg call** | | 1.000 | -.125 | .070 | .222 | .581*** | .167 |
| **Average distance** | | | 1.000 | -.619*** | -.685*** | -.248 | -.441** |
| **Locomotion** | | | | 1.000 | .370* | .304* | .213 |
| **Stare duration** | | | | | 1.000 | .380* | .597*** |
| **Stare frequency** | | | | | | 1.000 | .309* |
| **Head-cock** | | | | | | | 1.000 |

*Note*: * $p<.05$, ** $p<.01$, *** $p<.001$

### 3.3.2.2 PCA Reveals Two Psychological Dimensions: 'Emotionality' and 'Coping Strategy'

In order to identify possible psychological dimensions driving the observed latent variables, PCA was performed on the following seven measures; tsik call, tsik-egg call, average distance, locomotion, stare duration, stare frequency and head-cock (Table 3.7.). The sampling adequacy of the analysis was verified by KMO=0.64 (Field, 2009). Bartlett's test of sphericity $X^2(21)=125.00$, $p<0.001$, indicated that correlations between items were sufficiently large for PCA (Table 3.6). An initial analysis was run to obtain eigenvalues for each component in the data. Two components had eigenvalues over Kaiser's criterion of 1 and in combination accounted for 68.33% of the variance. The scree plot also showed an inflexion point after the second component. Both results justified retaining components 1 and 2 in the final analysis.

Table 3.8 and Figure 3.15 show the component loadings after rotation. The measure that loaded highly positively on component 1 was the average distance from the rubber snake while stare duration and locomotion loaded highly negatively. In addition, the number of head-cocks was moderately negatively loaded. Those animals with higher component 1 scores maintained a considerable distance from the rubber snake, avoided staring at the snake, and displayed reduced locomotion and head-cocks. According to the description of

Table 3.7 Means and standard deviations of nine measures recorded during the snake phase.

| | Vocalization | | Behaviour | | | | |
|---|---|---|---|---|---|---|---|
| | Tsik call (no.) | Tsik-egg call (no.) | Average Distance (cm) | Proportion of Time Spent in Locomotion (%) | Proportion of Time Spent in Staring at Snake (%) | Stare Frequency (no.) | Head-cock (no.) |
| Mean | 25.30 | 41.25 | 72.34 | 4.72 | 26.27 | 27.36 | 11.23 |
| Standard Error | 5.30 | 6.80 | 2.53 | 0.44 | 1.84 | 1.69 | 0.84 |

these behaviours in previous studies (refer to section 3.2.1.3 and 3.3.1.3), the pattern indicated high anxiety/emotionality. Moreover, two of the major variables loading on component 1 (i.e. average distance and locomotion) showed a very similar pattern to the same measures loading on the 'emotionality' component of the human intruder paradigm (Table 3.4). Therefore, the component 1 of the snake test was also labelled 'emotionality'. The measures that loaded highly positively on component 2 included the tsik call, tsik-egg call and stare frequency. Those animals with higher component 2 scores emitted greater numbers of tsik and tsik-egg calls and displayed a higher frequency of short latency 'looks' at the snake. It should be noted that the mobbing tsik call, which has been associated with a proactive coping style against a potential threat (Bezerra & Souto, 2008), was also highly positively loaded on the 'coping strategy' component of the human intruder paradigm (Table 3.4). In addition, a significant positive correlation between tsik call and stare frequency (Table 3.6) suggested that short latency 'looks' or repeated inspection of the snake is part of the proactive coping strategy. Therefore, the component 2 was labelled 'coping strategy'. From the loadings, the component coefficients were derived using the Anderson-Rubin method (Table 3.8), which in turn provided component scores for individual animals (Figure 3.15). The co-ordinates of individual points on the two axes, 'emotionality' and 'coping strategy', represent the psychological construct of the animal facing the situation with a simulated predatory threat, a rubber snake.

Table 3.8 Component loadings of the measures in the rubber snake test. Eigenvalues for and proportion of variance accounted for each component. (n=44)

| | Rotated Component Loadings | |
|---|---|---|
| Measures | **Component 1 'Emotionality'** | **Component 2 'Coping Strategy'** |
| Average distance | **.919** | .123 |
| Stare duration | **-.852** | .030 |
| Locomotion | **-.706** | -.035 |
| Head-cock | **-.638** | .164 |
| Tsik call | .113 | **.912** |
| Tsik-egg call | .038 | **.896** |
| Stare frequency | -.257 | **.726** |
| Eigenvalues | 3.03 | 1.75 |
| % of variance | 43.31 | 25.02 |

*Note*: Component loadings over .40 appear in bold.

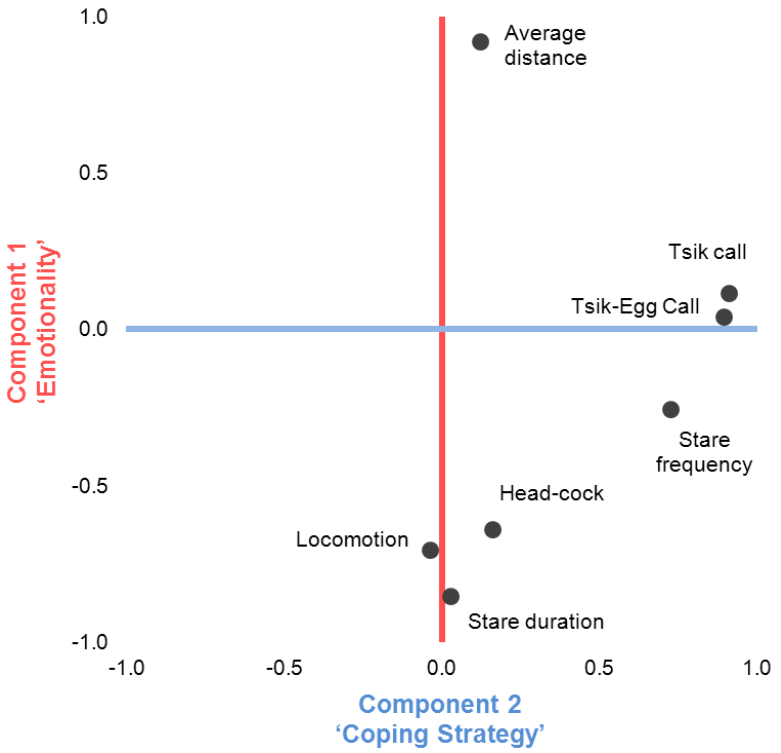



Figure 3.15 Component loadings of the measures in the rubber snake test plotted in rotated space with the axes of 'emotionality' and 'coping strategy'.

Table 3.9 Component score coefficients of the measures in the rubber snake test, from which component scores for individual animals were calculated.

| | Component Score Coefficients | |
|---|---|---|
| Measures | **Component 1 'Emotionality'** | **Component 2 'Coping Strategy'** |
| Average distance | .361 | .063 |
| Stare duration | -.333 | .008 |
| Locomotion | -.276 | -.021 |
| Head-cock | -.248 | .070 |
| Tsik call | .051 | .414 |
| Tsik-egg call | .021 | .407 |
| Stare frequency | -.095 | .327 |

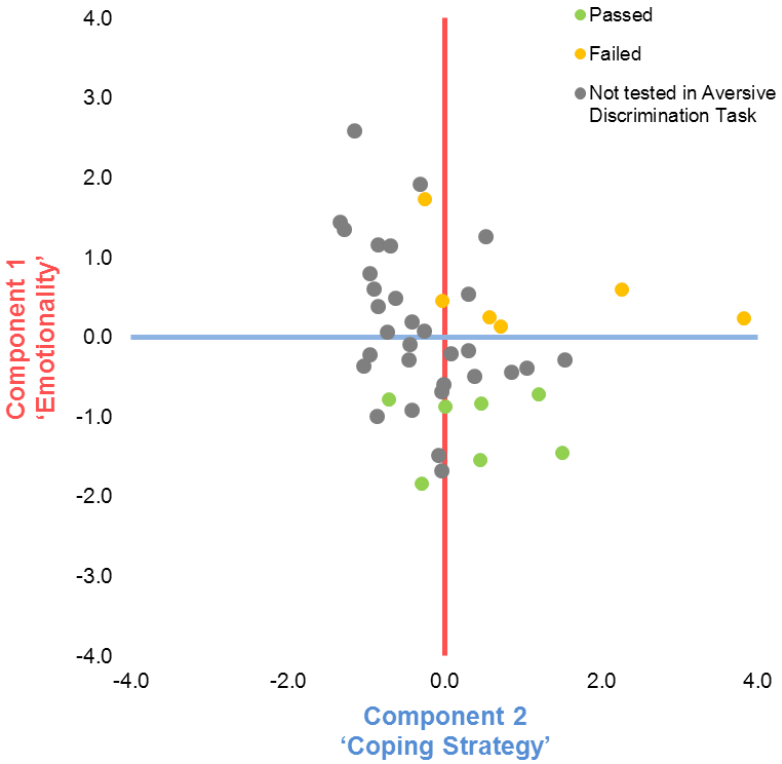



Figure 3.16 Component scores of individual animals plotted on the axes of 'emotionality' and 'coping strategy'. The animals in the 'passed' and 'failed' groups were indicated with green and yellow dots respectively (for the group comparison, refer to section 3.5). (n=44)

## 3.4 How Comparable are the Human Intruder and Rubber Snake Tests of Anxiety?

### 3.4.1 Subjects

The 44 adult common marmosets that received the rubber snake test had also received the human intruder test as described in section 3.3.1.1. Therefore, their scores from the two tests were used for the following correlation and comparison analyses.

### 3.4.2 Statistical Analysis

Statistical analyses were performed using a statistic software SPSS (version 19.0). Individual animal's score from PCA on the human intruder test variables (section 3.2.2.2) and the rubber snake test variables (section 3.3.2.2) were used for Pearson's correlation analysis for parametric data and Spearman's correlation for non-parametric data. Student's *t*-tests were used to compare the high / low responders between the two tests. Pearson's correlations were performed on the variables that were shared between the two tests. The assumption of normality was checked by Kolmogorov-Sminov test and Shapiro-Wilk test. The homogeneity of variance was tested by Leven's test. The data satisfied all assumptions, otherwise noted.

## 3.4.3 Results

### 3.4.3.1 Comparison of the 'Emotionality' and 'Coping Strategy' Component Scores Derived from the Human Intruder Test and Rubber Snake Tests

Both the human intruder test and the rubber snake test were designed to elucidate individual differences in marmoset's anxiety/fear-related responses to potential threat. Whilst, the former used a human whom the animal had never seen before, the latter used a rubber snake which represented marmoset's natural predator. Both stimuli have been used in previous studies and documented as effective stimuli to provoke anxiety/fear responses in primates (Marilia Barros, Boere, et al., 2002; Clara et al., 2008; N Cross & Rogers, 2006; Kalin et al., 2004, 2001; Nelson et al., 2003). However, the human intruder and the rubber snake may have different ecological significances for marmosets and evoke different emotional or attention response. To my knowledge, no study has ever compared psychological impacts evoked between the two stimuli in non-human primate; therefore, the performances of the animals that received both tests (n=44) were directly compared.

Although there were a number of behavioural responses that differentiated the two tests (section 3.2.1.3 and 3.3.1.3), nevertheless, PCA revealed two components in both tests that were composed of similar behavioural variables and which were best described as reflecting 'emotionality' and 'coping strategy' (section 3.2.2.2 and 3.3.2.2). Thus, these two components were directly compared across tests. The analysis revealed a weak but significant positive correlation in the 'emotionality' component [Pearson's $r$=0.36, $p$=0.017] (Figure 3.17 A). Those animals that displayed high emotionality/anxiety response in the presence of the human intruder also tended to behave highly emotionally/anxiously in the presence of the rubber snake. In contrast, no significant correlation was found in the 'coping strategy' component between the two tests [$r$=0.04, $p$=0.808] (Figure 3.17 B). This lack of correlation between the 'coping strategy' scores suggests that the animals that displayed an active coping strategy in response to the human intruder, did not necessarily assume a similar active response in the presence of the rubber snake. The two components were also cross-correlated, but no significant correlation was detected ['emotionality' in the human intruder test × 'coping strategy' in the rubber snake test: $r$=-0.21, $p$=0.183; 'coping strategy' in the human intruder test × 'emotionality' in the rubber snake test: $r$=0.07, $p$=0.676].

These findings were supported by an additional analysis in which the animals identified as high and low on the component spectrum of the human intruder test were compared for their

performance in the rubber snake test. Animals were ordered from the lowest to the highest in their 'emotionality' scores, then the first quartile group and the last quartile group were extracted. Therefore, the groups represent two opposing ends of the 'emotionality' spectrum. When these two quartile groups were compared for their 'emotionality' scores in the rubber snake test, there was a significant difference between the groups (Figure 3.18 A). A Student's *t*-test revealed a significant main effect of group [$t(20)=2.41$, $p=0.026$]. The animals that were highly 'emotional' in response to the intruder also showed high 'emotionality' to the rubber snake. When the same analysis was performed for the 'coping strategy' component, the high and low groups from the human intruder test did not differ significantly in their performance in the rubber snake test (Figure 3.18 B). Since the animals in the high/low groups on the 'coping strategy' component were not the same animals in the high/low groups in the 'emotionality' component, a Student's *t*-test, not a two-way factorial ANOVA, was used to compare the groups. The analysis returned no significant main effect of group [$t(20)=0.16$, $p=0.873$]. The same pattern was also observed when the high and low quartile groups were assembled based on the component spectrum of the rubber snake test and compared against their component scores in the human intruder test (Figure 3.19 A and B). A Student's *t*-test returned a significant main effect of group for the 'emotionality' component [$t(12.7)=2.44$, $p=0.030$ (equal variance not assumed)]. Highly 'emotional' animals in the rubber snake test also displayed high 'emotionality' in the human intruder test. No significant group difference was found in the 'coping strategy' component [$t(20)=-1.33$, $p=0.197$].

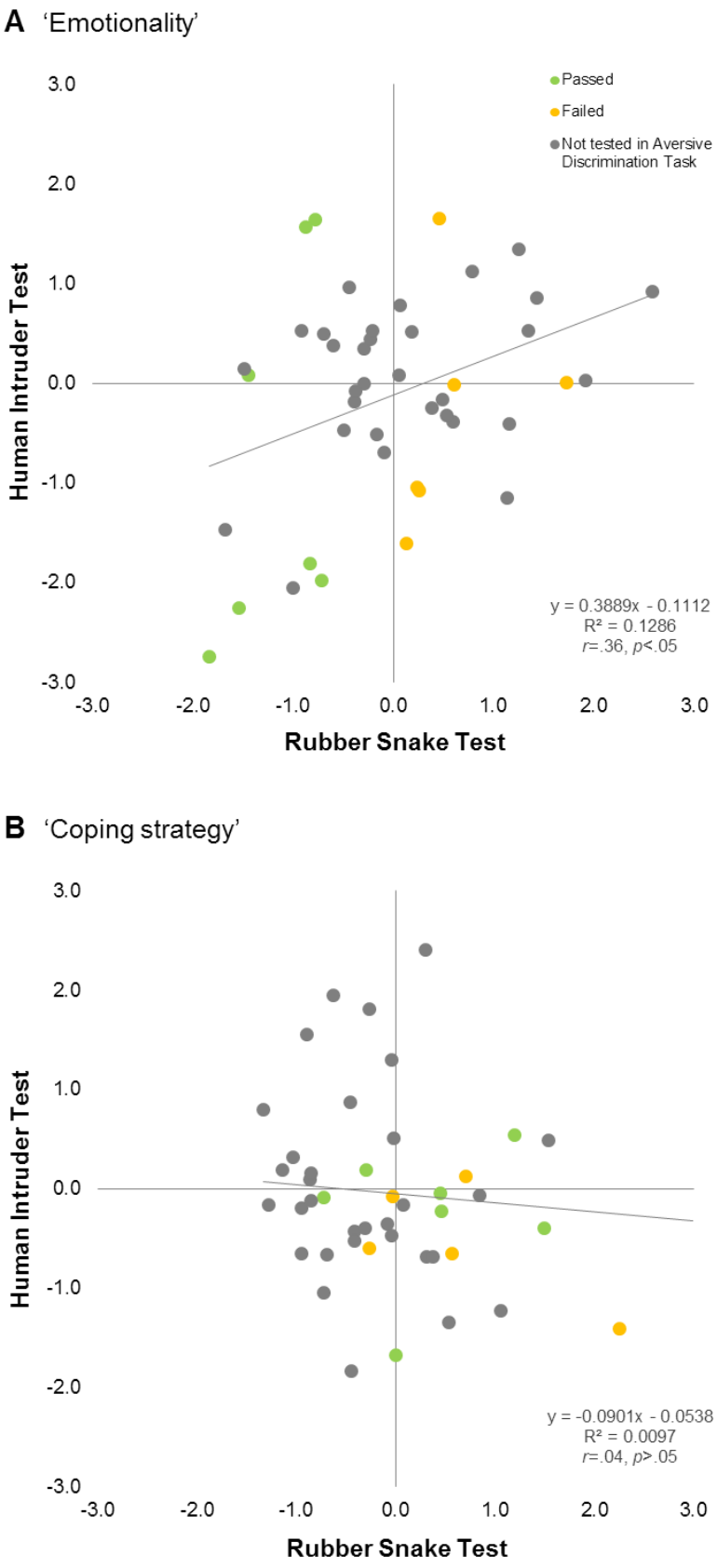



Figure 3.17 Correlations between the human intruder test and the rubber snake test on (A) 'emotionality' component scores and (B) 'coping strategy' component scores derived from PCA (section 3.2.2.2 and 3.3.2.2). The animals in the 'passed' and 'failed' groups were indicated with green and yellow dots respectively (for the group comparison, refer to section 3.5). (n=44)

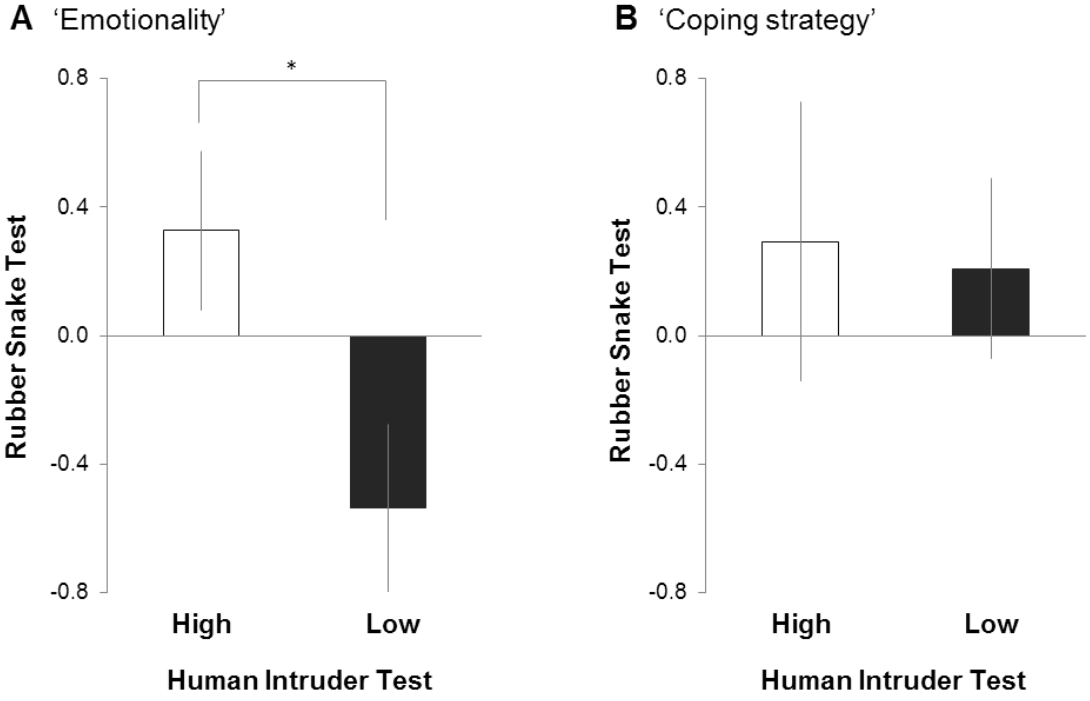



Figure 3.18 Comparison of the first quartile group ('high', n=11) and the last quartile group ('low', n=11) from the human intruder test on their (A) 'emotionality' component and (B) 'coping strategy' component scores in the rubber snake test. Error bars show the standard error for each group. *$p$ <.05

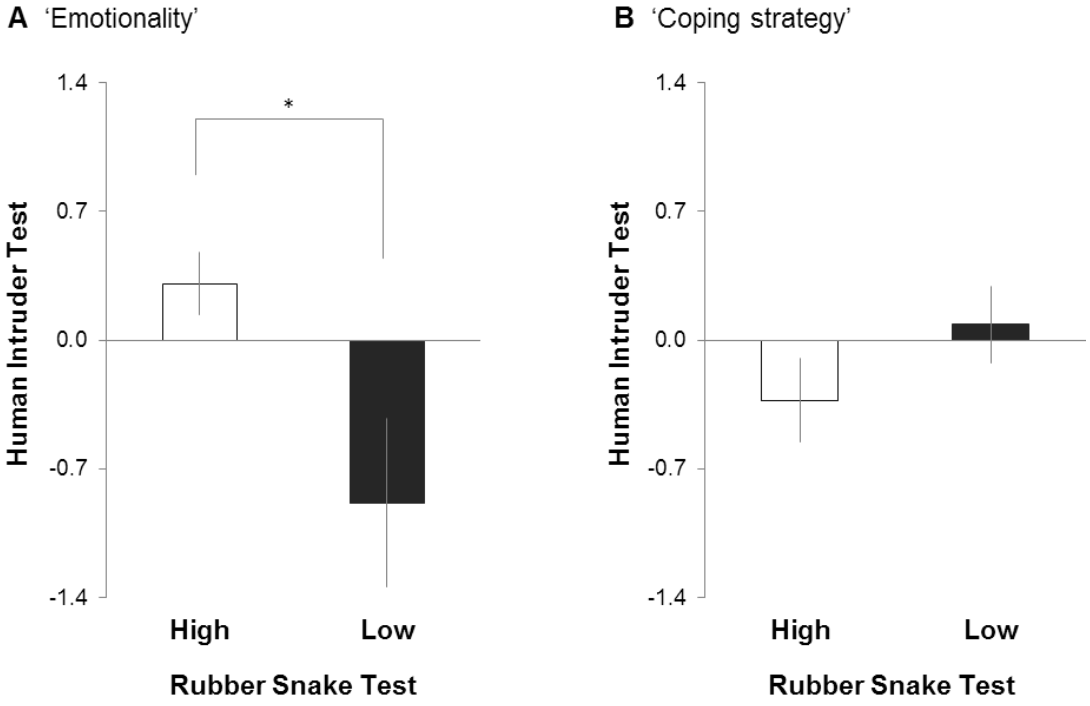



Figure 3.19 Comparison of the first quartile group ('high', n=11) and the last quartile group ('low', n=11) from the rubber snake test on their (A) 'emotionality' component and (B) 'coping strategy' component scores in the human intruder test. Error bars show the standard error for each group. *$p$ <.05

Table 3.10 Correlation statistics between the human intruder test and the rubber snake test for the number of tsik calls, the number of tsik-egg calls, the average distance from the stimulus and the proportion of time spent in locomotion. (n=44)

| | Tsik call | Tsik-egg call | Average distance | Locomotion |
|---|---|---|---|---|
| Correlation coefficient | .24 | -.12 | .27 | .55 |
| *p* value | .112 | .427 | .081 | .000 |

*Note*: Spearman's *ρ*: Tsik call, Tsik-egg call; Pearson's *r*: Average distance, Locomotion

### 3.4.3.2 Correlation of Individual Variables Common to both the Human Intruder and Rubber Snake Tests

The individual behaviours common to both the human intruder test and the rubber snake test were the number of tsik calls, the number of tsik-egg calls, the average distance from the stimulus, and the proportion of time spent in locomotion. Correlation analyses for each of these measures between the two tests revealed a significant positive correlation in locomotion (Table 3.10). The animals that were most active in the presence of the human intruder also showed high activity in the encounter with the rubber snake. No other measurements were significantly correlated between the two tests.

## 3.5 Comparison of the 'Passed' and 'Failed' Groups in the Human Intruder Test and the Rubber Snake Test

### 3.5.1 Subjects

The 13 animals (7 females and 6 males) out of the 27 animals that were tested in the aversive discrimination paradigm (section 2.2) were included in the 63 animals tested in the human intruder test (at the time of the human intruder test, they were aged 2.6 to 4.1 years, average age 3.3 years) and in the 44 animals tested in the rubber snake test (at the time of the rubber snake test, they were aged 2.9 to 4.3 years, average age 3.6 years). Of the remaining 14 animals, one died of unexpected caused before the testing commenced and the rest received an excitotoxic lesion of the prefrontal cortex for another research project and will not be discussed here. Among the 13 animals, seven (4 females and 3 males) successfully satisfied the aversive discrimination criterion therefore identified as the 'passed' group, and six (3 females and 3 males) failed the task therefore identified as the 'failed' group (section 2.2.3). These two groups were compared for their performances in the human intruder test and the rubber snake test.

### 3.5.2 Statistical Analysis

Statistical analyses were performed using a statistic software SPSS (version 19.0). A two-way factorial ANOVA was used to compare the 'passed' and 'failed' groups on their component scores derived from PCAs in the human intruder test (section 3.2.2.2) and the rubber snake test (section 3.3.2.2). A one-way ANOVA with Bonferroni correction was used for post-hoc test. For the comparison of the groups on the individual behavioural measures, a Student's *t*-test (for parametric data) and Mann-Whitney U test (for non-parametric data) were used. Pearson's correlation and a multiple regression analysis were used to investigate the relationship with the predictor measures derived from the early sessions of the aversive discrimination paradigm (i.e. behavioural response to CS's and baseline BP) to the animals' performances in the human intruder test and the rubber snake test. The assumption of normality was checked by Kolmogorov-Sminov test and Shapiro-Wilk test. The homogeneity of variance and sphericity were tested by Leven's test and Mauchly's test respectively. The data satisfied all assumptions, otherwise noted.

## 3.5.3 Results: Human Intruder Test

### 3.5.3.1 Comparison of the 'Passed' and 'Failed' groups on the 'Emotionality' and 'Coping Strategy' Component Scores

In order to examine whether the 'failed' and 'passed' groups, which had been identified with the aversive discrimination paradigm, differed in their responses to the human intruder, the two groups' component scores were compared. Although the means appeared to be different for the 'emotionality' component ('passed' mean: -0.78, SE: 0.70; 'failed' mean: -0.35, SE: 0.48), statistically the two groups did not differ from each other. Also, no difference was found for the 'coping strategy' component ('passed' mean: -0.24, SE: 0.27; 'failed' mean: -0.34, SE: 0.29) (Figure 3.20). A two-way factorial ANOVA comparing the two groups between the two components revealed no main effect of group [$F(1,11)=0.20$, $p=0.661$] nor group × component interaction [$F(1,11)=0.22$, $p=0.647$].

### 3.5.3.2 Comparison of the 'Passed' and 'Failed' Groups on the Individual Behavioural Measurements

Even when the two groups were compared for each of the behavioural measurements separately, no significant difference was detected (Figure 3.21). For the number of calls, the distributions were found to be not normal, [tsik call, 'passed', Shapiro-Wilk's $W=0.65$, $p=0.001$, 'failed', $W=0.79$, $p=0.045$; tsik-egg call, 'passed', $W=0.74$, $p=0.010$, 'failed', $W=0.78$, $p=0.037$; tse & tse-egg call, 'passed', $W=0.53$, $p<0.000$, 'failed', $W=0.61$, $p=0.001$; egg call, 'passed', $W=0.69$, $p=0.003$, 'failed', $W=0.82$, $p=0.085$], a non-parametric Mann-Whitney U test was used to compare the groups. The analysis returned no significant group effect in any of the calls [tsik call, $U=23.50$, $p=0.684$; tsik-egg call, $U=17.00$, $p=0.547$; tse & tse-egg call, $U=25.50$, $p=0.259$; egg call, $U=29.00$, $p=0.250$]. Student's $t$-tests were used to compare the groups for the rest of the measurements. No significant group effect was detected in any of the measures [average distance, $t(11)=-0.58$, $p=0.573$; head-body bobbing, $t(11)=0.04$, $p=0.968$; locomotion, $t(11)=1.06$, $p=0.310$; jump, $t(11)=0.25$, $p=0.805$].

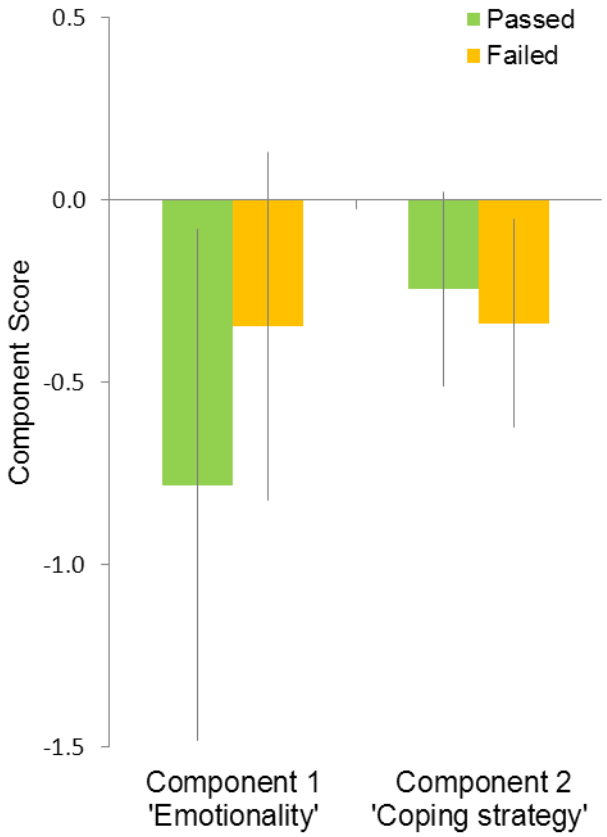



Figure 3.20 Comparison of the component scores between the 'passed' (green bar) and 'failed' (yellow bar) groups. ('passed' n=7; 'failed' n=6)

#### 3.5.3.3 Correlation with the Predictors of Aversive Discriminative Conditioning

In order to examine whether there was any relationship between the two potential anxiety measures in the early sessions of the aversive discrimination paradigm (vigilant behaviour to CS in session 1-3 and baseline BP in sessions 7-9) that predicted the animals' passing or failing of the task (section 2.2.2.2) and their performance in the human intruder test, the scores from those two measures were correlated with the component scores. Pearson's correlation returned no significant relationship for the 'emotionality' component with either of the measures [scanning behaviour to CS, $r$=-0.23, $p$=0.454; baseline BP, $r$=-0.16, $p$=0.599]. Also, no significant correlation was found for the 'coping strategy' with either of the measures [scanning behaviour to CS, $r$=0.16, $p$=0.594; baseline BP, $r$=0.23, $p$=0.356].

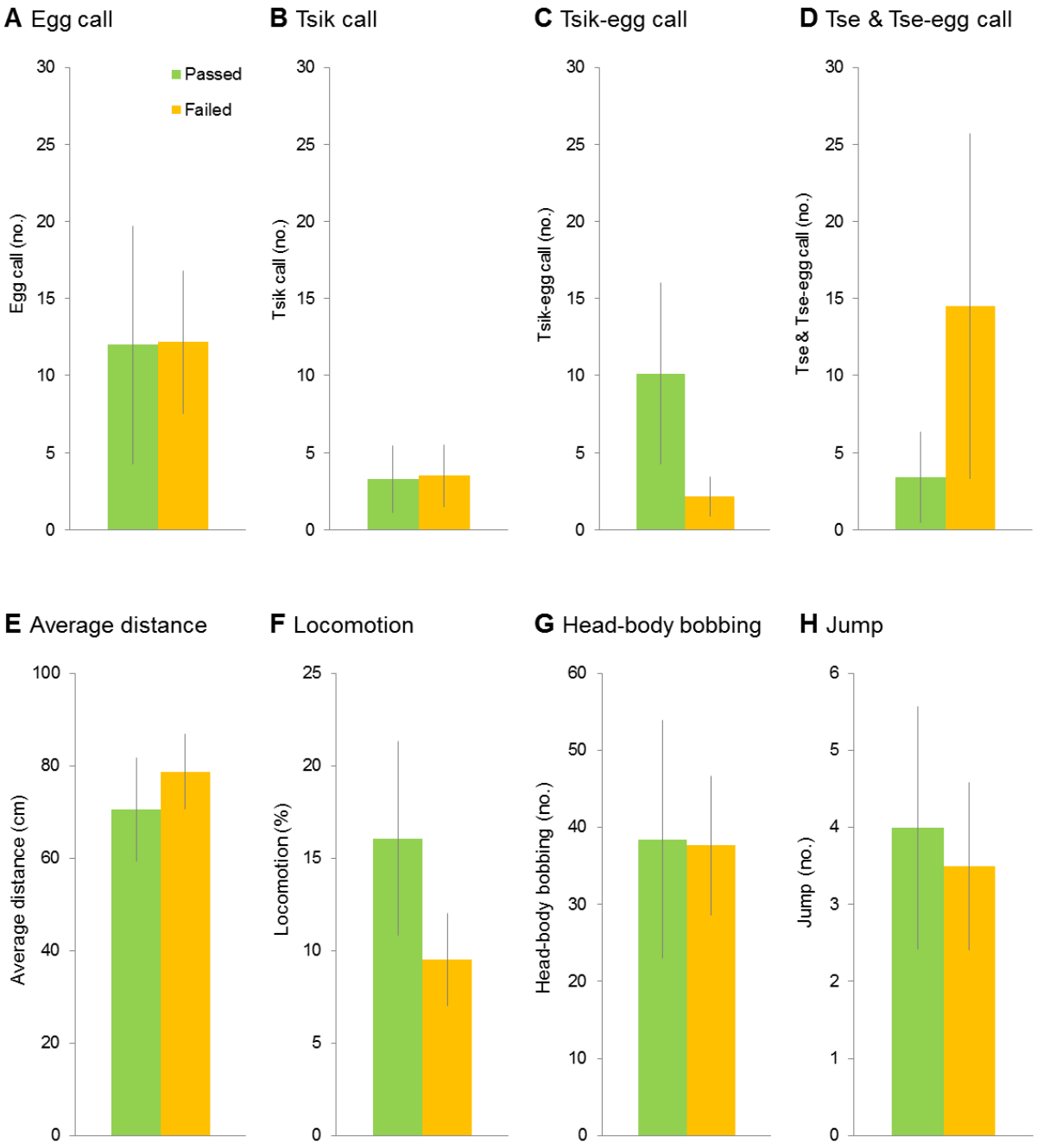



Figure 3.21 Comparison of the 'passed' (green bar) and 'failed' (yellow bar) groups in each of the behavioural measures during the intruder condition. (A) The number of egg calls. (B) The number of tsik calls. (C) The number of tisk-egg calls. (D) The number of tse & tse-egg calls. (E) Average distance from the intruder. (F) Proportion of time spent in locomotion. (G) The number of head-body bobbing. (H) The number of jumps toward the front. Error bars show the standard errors for each group.

## 3.5.4 Results: Rubber Snake Test

### 3.5.4.1 Comparison of the 'Passed' and 'Failed' groups on the 'Emotionality' and 'Coping Strategy' Component Scores

In order to examine whether the 'failed' and 'passed' groups, which had been identified with the aversive discrimination paradigm, differed in their responses to the rubber snake, the two groups' component scores were compared. It was found that the two groups significantly differed in their 'emotionality' response ('passed' mean: -1.15, SE: 0.17; 'failed' mean: 0.57, SE: 0.24) to the rubber snake, but they did not differ in the 'coping strategy' component ('passed' mean: 0.37, SE: 0.30; 'failed' mean: 1.18, SE: 0.63) (Figure 3.22). A two-way factorial ANOVA comparing the 'passed' and 'failed' groups with respect to the two components revealed a significant main effect of group [$F(1, 11)=15.16$, $p=0.003$]. Subsequent pairwise comparison indicated that the 'failed' group showed higher scores than the 'passed' group in 'emotionality' component [$F(1, 11)=35.24$, $p<0.001$] but not in component 2 [$F(1, 11)=1.45$, $p=0.254$]. Thus, the groups differed in their response to the snake, primarily due to the 'failed' group displaying heightened emotionality in comparison to the 'passed' group. Consistent with this, mean scores suggested that the 'failed' group also tended to stay further back in the presence of the empty box following snake exposure ('passed' mean: 44.2cm, SE: 5.63; 'failed' mean: 60.3cm, SE: 7.80). However, there were no differences between groups in the average distance, or in the locomotion, across earlier phases of the test, prior to snake exposure (Figure 3.23). A Student's *t*-tests were performed comparing the two groups in each phase. No significant difference was found between the groups in all phases expect the snake phase for the average distance ['no-box' $t(11)=-0.31$, $p=0.763$; 'pre-snake' $t(11)=-0.29$, $p=0.774$; 'snake' $t(11)=-3.31$, $p=0.007$; 'post-snake' $t(11)=-1.71$, $p=0.116$] nor for the locomotion ['no-box' $t(11)=0.01$, $p=0.993$; 'pre-snake' $t(11)=1.77$, $p=0.105$; 'snake' $t(11)=2.38$, $p=0.037$; 'post-snake' $t(11)=1.06$, $p=0.311$].

### 3.5.4.2 Comparison of the 'Passed' and 'Failed' Groups on the Individual Behavioural Measurements

In addition, when the two groups were compared for each of the behavioural measurements separately, following statistics were obtained. For the number of tsik and tsik-egg calls, the 'passed' and 'failed' groups did not differ significantly (Figure 3.24 A and B). Since the distributions were found not to be normal [tsik call, 'passed', Shapiro-Wilk's $W=0.79$, $p=0.031$, 'failed', $W=0.97$, $p=0.911$; tsik-egg call, 'passed', $W=0.98$, $p=0.932$, 'failed', $W=0.76$, $p=0.022$]; therefore, non-parametric Mann-Whitney U tests were performed to compare the

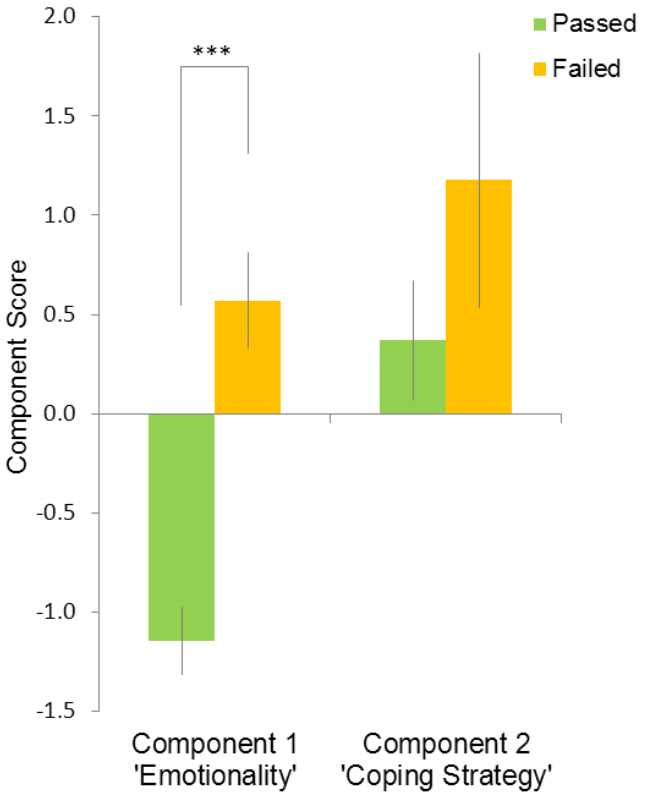



Figure 3.22 Comparison of the 'emotionality' and 'coping strategy' component scores between the 'passed' (n=7, green bar) and 'failed' (n=6, yellow bar) groups. ***$p$<.001

groups. No significant effect of group was found for either call [tsik call, $U$=27.50, $p$=0.352; tsik-egg call, $U$=20.00, $p$=0.886]. As mentioned above, for the average distance and locomotion, the groups differed significantly (Figure 3.24 C and D). The animals in the 'failed' group stayed further away from the rubber snake than the animals in the 'passed' group. The animals in the 'failed' group were significantly less active in the presence of the rubber snake than the animals in the 'passed' group. For the proportion of time spent in staring at the rubber snake, the animals in the 'passed' group were found to be staring at the snake significantly longer than the animals in the 'failed' group (Figure 3.24 E). A Student's $t$-test returned a significant effect of group [$t$(11)=3.76, $p$=0.003]. For the stare frequency measure, although the means indicate that the animals in the 'failed' groups more frequently looked at the snake than the animals in the 'passed' group, no significant statistical difference was found [$t$(11)=-1.59, $p$=0.140] (Figure 3.24 F). Lastly, there was a significant group difference for the number of head-cocks [$t$(11)=4.17, $p$=0.002]. The animals in the 'passed' group displayed significantly greater number of head-cocks than the animals in the 'failed' group (Figure 3.24 G).

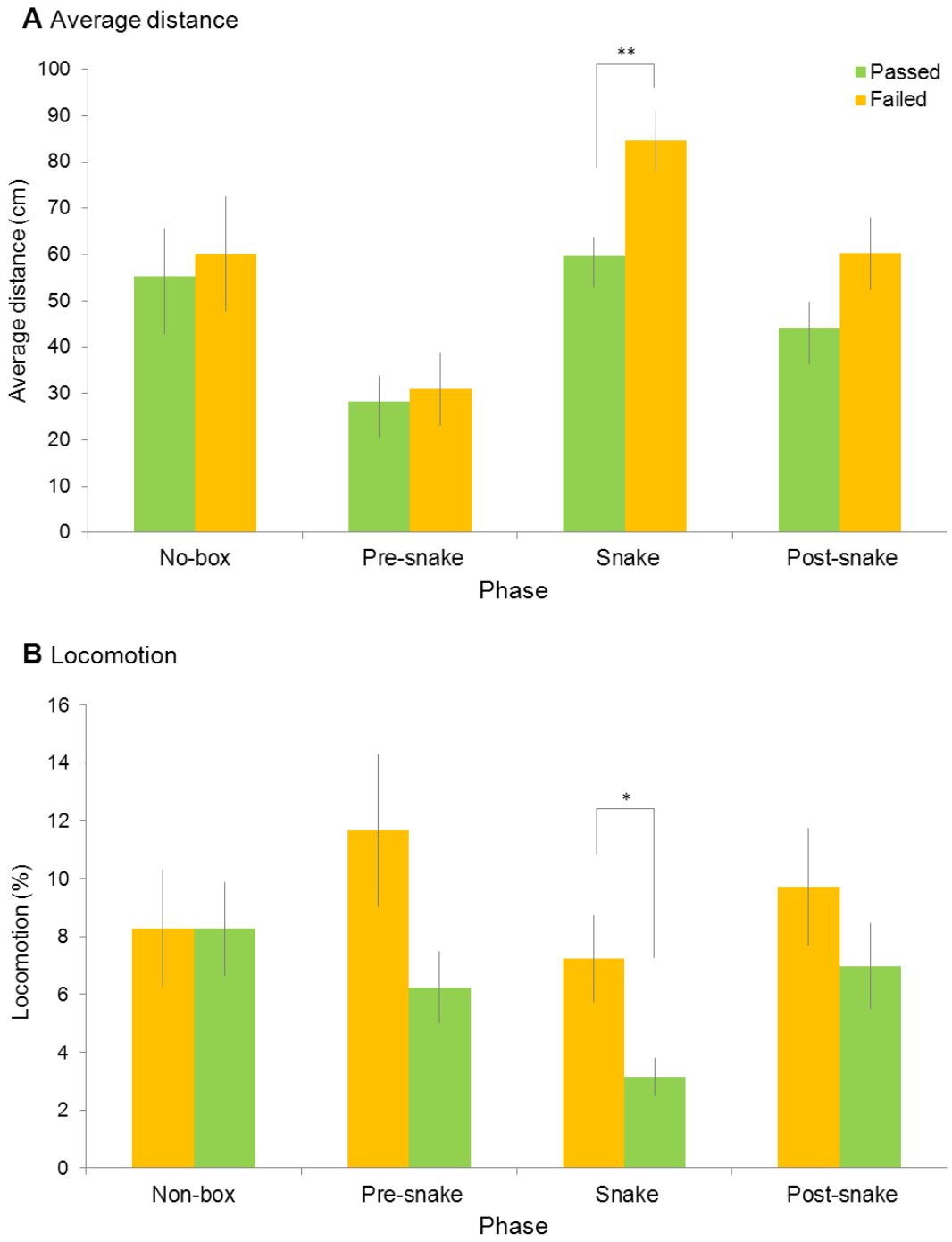



Figure 3.23 Comparison of the 'passed' (green bar) and 'failed' (yellow bar) groups across the four phases: 'no-box', 'pre-snake', 'snake' and 'post-snake', for (A) average distance from the front corner of the cage where the rubber snake was placed, and (B) proportion of time spent in locomotion. Error bars show the standard errors for each group. *$p$<.05, **$p$<.01.

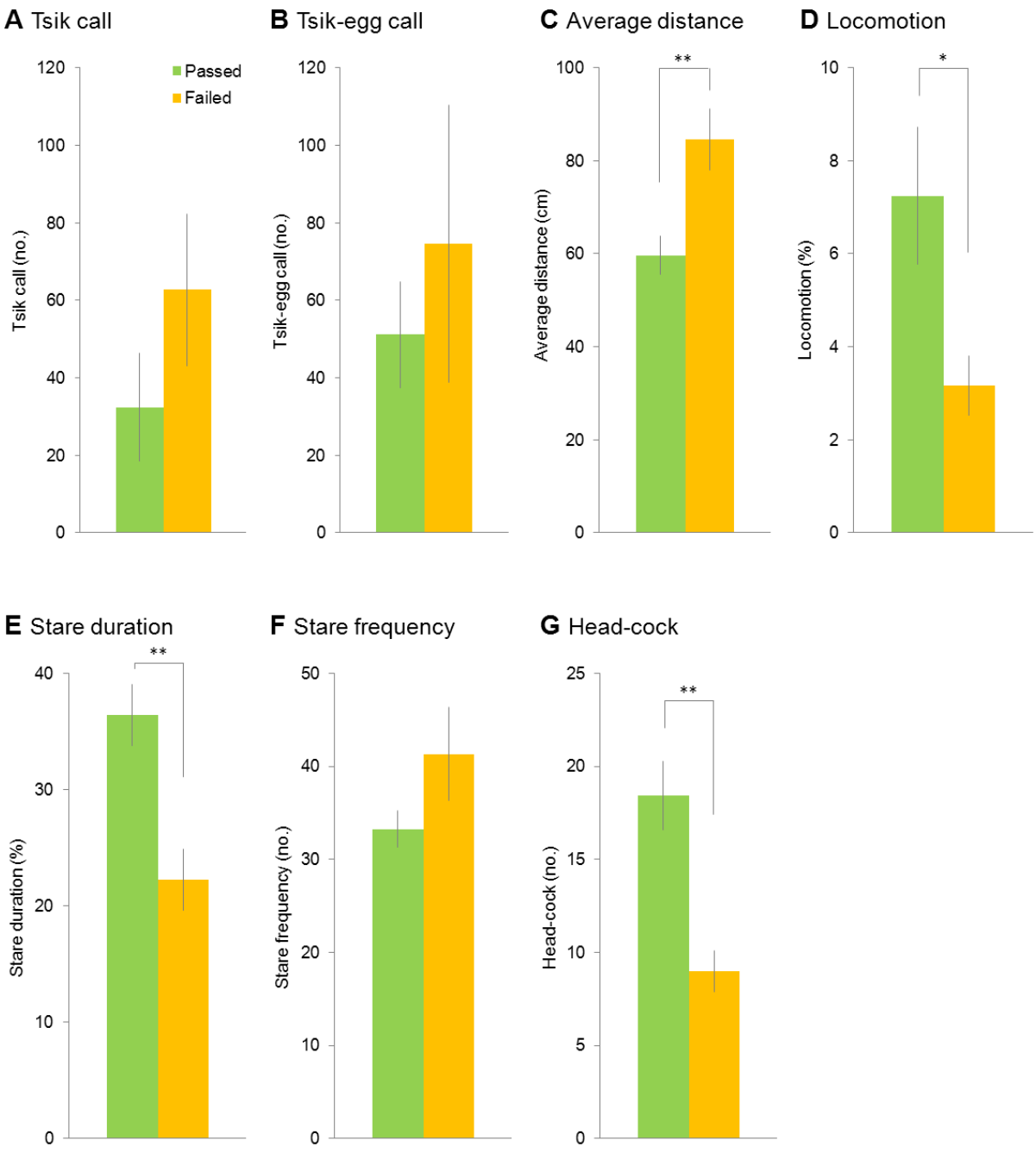



Figure 3.24 Comparison of the 'passed' (green bar) and 'failed' (yellow bar) groups in each of the behavioural measures during the intruder condition. (A) The number of tsik calls. (B) The number of tsik-egg calls. (C) Average distance from the rubber snake. (D) Proportion of time spent in locomotion. (E) Proportion of time spent staring at the rubber snake. (F) The number of occasions looking at the rubber snake. (G) The number of head-cocks. Error bars show the standard errors for each group. *$p$<.05, **$p$<.01.

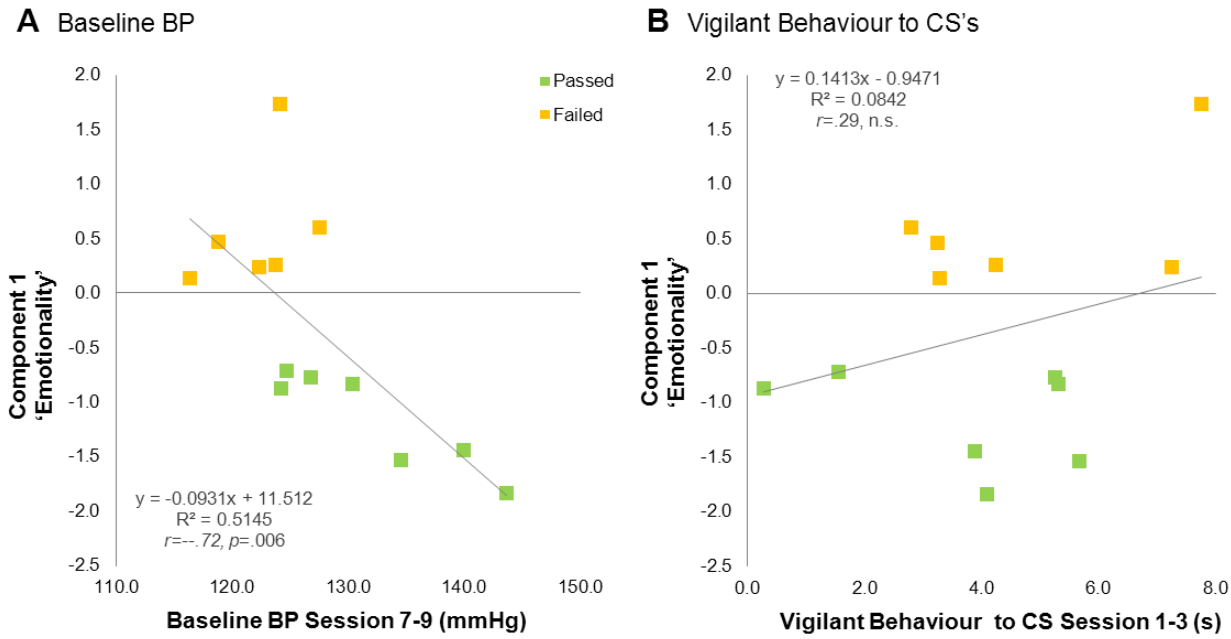



Figure 3.25 Correlations between the 'emotionality' component scores from the rubber snake test and the predictors of the aversive discriminative conditioning: (A) the baseline BP in session 7-9 and (B) vigilant behaviour to CS's in session 1-3.

### 3.5.4.3 Correlation with the Predictors of Aversive Discriminative Conditioning

In order to examine whether there was any relationship between the two predictive measures in the early sessions of the aversive discrimination paradigm (vigilant behaviour to CS's in session 1-3 and baseline BP in sessions 7-9) that predicted the animals' passing or failing of the task (section 2.2.2.2) and performance in the rubber snake test, the predictive measures were correlated with the snake test component scores. Pearson's correlation analysis returned a significant negative correlation between the 'emotionality' component and the baseline BP [$r$=-0.72, $p$=0.006] (Figure 3.25 A), but not with the vigilant behaviour to the CS's [$r$=0.29, $p$=0.336] (Figure 3.25 B). The animals that displayed the greater suppression of BP in the baseline exhibited the stronger emotionality response toward the rubber snake. No significant correlation was found between the 'coping strategy' component and either measure ['baseline BP' $r$=-0.11, $p$=0.715; 'vigilant behaviour to CS' $r$=0.12, $p$=0.697].

### 3.5.4.4 Prediction of the Rubber Snake Test Component Scores

Having found a relationship between the predictive measures in the aversive discrimination paradigm and the emotionality performance in the rubber snake test, whether the former

Table 3.11 Regression coefficients B, standard errors of B, standardized coefficients β and results of *t*-test from a significant model predicting the 'emotionality' component scores from the predictors of the aversive discriminative conditioning: the vigilant behaviour to CS in session 1-3 and the baseline BP in session 7-9.

| Retained predictor | B | Standard Error B | β | *t* | *p* |
|---|---|---|---|---|---|
| Constant | 11.31 | 3.14 | | 3.60 | .005 |
| Vigilant Behaviour to CS's Sessions 1-3 | 0.17 | 0.09 | .36 | 1.88 | .090 |
| Baseline BP Sessions 7-9 | -0.10 | 0.03 | -.75 | -3.94 | .003 |

*Note*: $R^2$= .64 (*p*=.006). Model *F*(2,10)=8.93, *p*=.006.

would predict the latter outcome was investigated by using a multiple regression analysis. Since the two measures (vigilant behaviour to CS's in session 1-3 and baseline BP in session 7-9) had been already selected from a number of variables as potential predictors of anxiety/fear-related response (section 2.2.2.2), a backward stepwise method was selected to investigate their individual contributions to the prediction of the component scores (Field, 2009, p.213). In the backward method, the analysis initially places all predictors in the model, and then calculates the contribution of each one by looking at the significance value of the *t*-test. If the predictor makes a statistically significant contribution to the prediction of the outcome, it is retained in the model; otherwise the predictor is removed from the model. The model continues to be re-assessed until the final model is reached. The analysis was first run with the 'emotionality' component scores as the outcome variable. It reached a significant final model [*F*(2,10)=8.93, *p*=.006] with both the vigilant behaviour to CS's and the baseline BP retained in the model (Table 3.11). The fact that both variables were included in the final model suggests that these measures are better predictors of the outcome when put together than when used singly. Thus, the suppressed baseline BP and the heightened vigilance behaviour to CS's in the early sessions of the aversive discrimination test, together, significantly predicted the greater 'emotionality' response to the rubber snake. When the analysis was run with the 'coping strategy' component, all predictors were removed from the model failing to reach a final model. Neither the vigilant behaviour to CS's nor the baseline BP together, or singly, significantly predicted the 'coping strategy' component scores.

## 3.6 Discussion

A large cohort of marmoset monkeys was tested in two conventional and well-validated tests of anxiety in non-human primates, namely the human intruder test and rubber snake test. Although the behavioural repertoire observed differed slightly between the two tests, the behaviours were in line with previously reported anxiety/fear-related responses in the common marmoset (Marilia Barros, Boere, et al., 2002; Cagni et al., 2009; Carey et al., 1992; Clara et al., 2008; Stevenson & Poole, 1976). The pattern of these behaviours indicated a large individual variation among the subjects, attesting to the strong power of these paradigms in the detection of individual differences across the anxiety response spectrum. By using PCA, two psychological dimensions underlying their behaviour were identified; one was labelled the 'emotionality' component, as the behaviours loading on this component reflected how anxious/fearful the marmosets were. The second was labelled 'coping strategy' as the behaviours loading on this component appeared to reflect whether the marmosets behaviour was passive or active. The 'emotionality' component showed a weak but significant positive correlation between the snake and human intruder test, but there was no correlation between the 'coping strategy' components, suggesting that both stimuli elicited anxiety/fear-related responses but that each of the stimuli may have led to different adaptive strategies.

Comparison of performance of those animals that 'failed' or 'passed' the discrimination paradigm revealed that the 'failed' group displayed significantly greater 'emotionality' scores than the 'passed' group in response to the snake stimulus. This finding is in agreement with the prediction by the fear generalization hypothesis, that animals that show heightened anxiety are less able to discriminate a safety from danger cue in an anxiety-provoking situation. In contrast, no group difference was seen in the 'emotionality' component on the human intruder test or in the 'coping strategy' component on either test. Thus, although these results support the hypothesis that the 'failed' group were more anxious than the 'passed' group, the effect appeared specific to the snake test, an issue that is discussed below. No group difference in the 'coping strategy' component suggests that the aversive discriminative conditioning paradigm is sensitive to the emotional reactivity aspect but not to the coping response aspect of a marmosets psychological profile. The close correspondence between fear discrimination performance and responsivity on the snake test is further highlighted by the significant relationship between the 'emotionality' component score and the behavioural and autonomic predictors of passing or failing on the discrimination test. There was a strong

negative correlation between baseline BP and the 'emotionality' component such that the lower the baseline BP on the discrimination test the higher the 'emotionality' score on the snake test. Moreover, when both discriminative performance predictors were placed into a multiple regression analysis both predictors were retained in the final model, supporting that both suppressed baseline BP and heightened vigilant behaviour to the CS's may be biomarkers of trait anxiety in the common marmoset.

**Comparison of current human intruder study with previous human intruder studies in marmosets and rhesus monkeys**

Although the human intruder paradigm is a well-validated and widely adopted method of testing for anxiety in non-human primates including rhesus macaques and marmoset monkeys (Marilia Barros & Tomaz, 2002), observed behavioural repertoires differ between the species that deserve discussion. The sample size tested in the current study (n=63) is the largest of any studies previously reported using the marmoset human intruder paradigm. Therefore, the behavioural responses described in this chapter should provide a reliable ethogram of the common marmoset upon encountering a human threat. Some of the behaviours that were reported in previous studies (Barnes et al., 1990; Cagni et al., 2009; Carey et al., 1992; Costall et al., 1992; Starr et al., 2007) were rarely observed in the current paradigm, and therefore not included in the statistical analysis. These included tail posture (animal turns its back and raises its tail to expose the anogenital area to the intruder), scent marking (animal rubs its anogenital area against the surface of object), slit stare (animal stares at the intruder with its eyes reduced to slit and ears flattened to its head) and arched pilo (animal arches its back while displaying body piloerection). There are a couple of possible reasons for this discrepancy. First, in previous studies, animals were tested in a pair (Cagni et al., 2009; Carey et al., 1992; Costall et al., 1992; Starr et al., 2007), whilst in the current paradigm the subject animal was separated from its partner prior to exposure to the intruder. Since marmosets are a highly social primate (Stevenson & Poole, 1976), the singly housed condition may have suppressed socially-related behaviours including the above described behaviours. Second, handling prior to testing may have an impact on performance. Many of the studies were designed to test anxiolytic and/or anxiogenic compounds (Barnes et al., 1990; Cagni et al., 2009; Carey et al., 1992; Costall et al., 1992; Starr et al., 2007; Walsh et al., 1995). Inevitably, the protocol involved grabbing the monkey and injecting the animal with vehicle or drug just before testing. In contrast, the procedure in the current study did not involve any direct handling of the animal. Since handling can be highly aversive to the animal (Carey et al., 1992) it may have been responsible for those behaviours that were

rarely observed in the current procedure. Finally, even in those studies that did report these other behaviours, they were summed across the 2 minute test period reflecting the relatively low frequency of any one of them (Barnes et al., 1990; Carey et al., 1992; Costall et al., 1992; Starr et al., 2007), However, it is not clear that they all reflect the same psychological state and so 'summing' may not be appropriate. The slit stare and tail posture have been observed as aggressive behaviours against a conspecific from a different family group (Stevenson & Poole, 1976) whilst scent marking occurs most frequently when animals are moved to a new cage. In fact, it has been suggested that the only reliable behaviour measure consistently sensitive to anxiolytics and anxiogenics is the time spent in the cage's front section (Marilia Barros & Tomaz, 2002). Walsh and colleagues (Walsh et al., 1995) dismissed postures as unreliable and used the time spent at the cage front and locomotion (number of jumps) as the sole behavioural measures for testing the effect of anxiolytics. While it may be reasonable to employ only a couple of established measures to evaluate the effects of anxiolytics or anxiogenics, one of the advantages of ethologically relevant paradigms is a wider range of the observable behavioural responses, which allow more detailed analysis of individual differences in the underlying psychological factors (Marilia Barros & Tomaz, 2002), producing a dilemma between a loss of potentially valuable information and confounding a measure with unwanted variables.

**The use of PCA to extract the underlying psychological dimensions of behaviour in the human intruder test**

The current study took a different approach to this issue. In line with previous reports (Cagni et al., 2009; Costall et al., 1992; Starr et al., 2007; Walsh et al., 1995), the effect of the human intruder was reflected in the significant increase in the animal's average distance from the cage front and the increase in locomotion. The animals stayed further back in the cage and became more active or agitated in the presence of a human intruder in comparison to the immediately preceding separation condition. In addition, there were a number of behaviours and vocalizations that were observed almost exclusively in the human intruder condition. Although these responses may have reflected the animal's psychological state such as aggression, it was more likely that they were, at least partly, influenced by anxiety/fear, the emotion the paradigm was designed to elicit. It is an inherent problem for the paradigms measuring animal's unconditioned responses that the observed behaviours are not only driven by the psychological dimension the experimenter aims to measure, but also by other psychological factors. The current study applied the PCA to elucidate the possible underlying psychological dimensions present in the animal's behavioural repertoire.

PCA is a statistical technique for identifying groups or clusters of variables that are correlated with each other. The existence of clusters of large correlation coefficients between subsets of variables suggests that those variables could be measuring aspects of the same underlying dimensions (Field, 2009). These underlying dimensions can be interpreted as the psychological factors the paradigm aims to measure. By reducing a data set from a group of interrelated variables to a smaller set of components, PCA achieves parsimony by explaining the maximum amount of common variance in a correlation matrix using the smallest number of explanatory constructs. Therefore, PCA is used for understanding the structure of, and the interaction between, a set of variables as well as for reducing a data set to a more manageable size, while retaining as much of the original information as possible (Field, 2009). PCA has been used successfully in rodent models of anxiety, in particular the EPM. Cruz and colleagues (Cruz, Frei, & Graeff, 1994) tested 30 rats on the EPM using not only the standard measures, such as the number of entries into and the length of time spent in the open and closed arms but also a number of additional ethological unconditioned responses, including scanning behaviour, head poking from the closed arm, rearing and self-grooming. When these behavioural variables were placed into a PCA, four components were extracted with every variable loading differentially to each component. The component loading of a variable reflects the amount of contribution of that variable to the component. By examining what behavioural variable contributes most to a component, one can relate the component to a relevant underlying psychological dimension. Through this procedure, the authors labelled component 1 as the index of anxiety, component 2 as locomotion, and components 3 and 4 as factors related to risk-assessment and displacement activity. Drug treatments supported these results since the administrations of anxiolytics and anxiogenics affected the variables loaded highly on component 1, the index of anxiety, in opposing directions. Similar findings have been reported using PCA on mice behaviours in the EPM and open field arena (Carola et al., 2002; Fernández Espejo, 1997; Rodgers & Johnson, 1995).

In the current study, when the behavioural measures were placed into a PCA, the analysis produced two components that accounted substantially for the variance in the data. The behavioural measures that were highly loaded on component 1 were the average distance, locomotion, the number of jumps to the cage front and the number of head-body bobbings. The average distance incorporates the time spent at the cage front, which has been repeatedly shown to be the sensitive measure to anxiolytics and anxiogenics along with the locomotion and jump measures. The head-body bobbing, the behaviour also described as swaying, has been observed in the presence of a threat and associated with apprehensive

alarm behaviour (Marilia Barros, Boere, et al., 2002; Carey et al., 1992). The variables that showed medium to weak loadings were the number of tsik-egg calls, tse & tse-egg calls and egg calls (detailed description of these behaviours and vocalizations were given in section 3.2.1.4). For highly social primates living in dense forests such as the common marmoset, the calls are important means of communications. Marmosets have a complex vocal repertoire of at least 13 different calls (Bezerra & Souto, 2008). Ethological study on the types of marmoset calls reported the association between the egg and tse-related calls with anxiety-related vigilance behaviour in potentially threatening situations (Bezerra & Souto, 2008). The directions and magnitudes of the loaded variables indicate that the animal that scores high on component 1 maintained a greater distance from the cage front, stayed relatively immobile, made fewer jumps to the cage front and made frequent head-body bobbings. Although the lower loadings of the calls reflect less contribution to the component, the pattern indicates relatively high numbers of egg and tse related calls in animals with high component 1 scores. This profile closely fits the description of high anxiety reported in pharmacological studies (Barnes et al., 1990; Cagni et al., 2009; Carey et al., 1992; Costall et al., 1992; Starr et al., 2007; Walsh et al., 1995). Anxiety is the emotional reactivity which the human intruder paradigm is originally designed to elicit. Therefore, component 1 was considered the underlying factor reflecting the animal's 'emotionality'.

Component 2, on the other hand, was loaded on primarily with calls. Notably, tsik call showed the highest contribution, followed by egg and tsik-egg calls. The tsik call is one of the marmoset calls that have been most well profiled. It has been reported that the call is used as a mobbing call to scare away conspecifics from other social groups, unfamiliar humans and potential predators, and it has been observed that the marmosets continued to emit tsik calls and followed the potential predator until it eventually retreated (Bezerra & Souto, 2008). Cross and Rogers (N Cross & Rogers, 2006) suggested that this mobbing vocalization is an animal's coping response in a stressful situation. In their study in which marmosets were exposed to a rubber snake, the number of tsik calls was found to be positively correlated with the magnitude of the decrease in the animal's cortisol level, suggesting that this mobbing call may also serve to reduce the amount of stress experienced by the animal. In addition, Cagni and colleagues (Cagni et al., 2009) reported that tsik calls were not responsive to the treatment of anxiolytics in the human intruder paradigm, suggesting that this call is not related to anxiety reactivity but more likely to a coping response. The relatively high loading of egg calls was inversely related to tsik calls, that is, the animal that made more tsik calls emitted fewer egg calls and vice versa. However, unlike tsik calls, egg calls also

loaded moderately onto the 'emotionality' component indicating that the expression of this call is driven by both underlying dimensions; emotional reactivity and coping response.

The coping response has been defined by Koolhaas and colleagues (Koolhaas et al., 1999) as the behavioural and physiological efforts made by an animal to master the stressful situation and proposed that the coping response spans two opposing styles; at one end is the proactive style and the other, the reactive style. The proactive coping style is linked with aggression or advancement towards a threat whilst the reactive style is associated with defensive or withdrawal behaviours from a threat. Although the approaches differ, under a stressful situation, both styles are aimed at environmental control and avoidance of aversive consequence. For example, rats were first screened with a conspecific confrontation test for aggressiveness and were then presented with an electrified prod in their home cages. Whilst the aggressive rats tended to bury the prod, which was regarded as the proactive coping response, the defensive rats tended to show immobility behaviour, which was seen as the reactive coping response. Regardless of the styles, the successful coping led to the avoidance of further electrical shock (Koolhaas et al., 1999). In the component 2, mobbing tsik calls are the manifestation of aggression toward the human intruder, thus it clearly reflects the proactive coping response. On the other hand, the inversely related egg calls are associated with vigilance behaviours, which potentially lead to a successful escape from the intruder. This suggests that egg calls, though partially, reflect the reactive coping response. Tsik-egg calls are the vocalizations that share both proactive coping tsik and emotionally reactive egg components. This is supported by the fact that tsik-egg calls were moderately and positively loaded on the both components.

Alternative dichotomous styles proposed for coping response are the ones either active or passive. Instead of relating proactive/reactive coping styles to one's tendency to be either aggressive or defensive upon encountering a potential threat (Koolhaas et al., 1999), the stress coping (mis)match hypothesis (J. Homberg, 2011) defines proactive coping as an anticipatory future-oriented and goal-directed act to prevent the effects of stress, and reactive coping as the reaction to aversive events and harm reduction when the evens have occurred. Accordingly, whilst the proactive style is preventive of possible harm, one can only take the reactive response upon encountering a threat, which imply that the behavioural responses observed under the human intruder and snake paradigms may all be reactive coping. Alternatively, the active/passive coping styles are associated with pattern of adaptive behaviours one takes facing an escapable or inescapable stressor (Bandler, Keay, Floyd, & Price, 2000). Active style refers to strategies such as problem-solving and fight/flight

responses, that are adaptive when exposed to escapable stress. Passive style is also adaptive but to the exposure to inescapable stress and entails strategies such as reduction of harm during stress, quiescence and immobility. According to the stress coping (mis)match hypothesis (J. Homberg, 2011), one's coping style is shaped by the interaction between genetic makeup ('nature') and the environment in which he/she grows up with ('nurture'). When the current life situation matches with these factors, one is adaptive to the environmental challenge. However, when they mismatch, for instance one acquiring active style due to exposure to escapable stress during nurture now exposed to inescapable stress, his/her responses are maladaptive in coping with the current stress. Whether an encountering a human threat or snake in the confined but relatively spacious testing space is regarded as an escapable or inescapable stress situation is debatable. However, the utterance of tisk calls can be seen as active coping due to the call's nature as mobbing behaviour and the effect of this call in reducing stress-related cortisol level (Clara et al., 2008). Egg call sits opposite from tsik call on the axis of the component 2. If tsik call is the expression of active coping, the opposing egg call should be considered as part of passive coping style. Whilst active copers display countering behaviours to a threat, passive copers stay immobile and undetected. Therefore, whether emission of any call including vigilant egg call can be considered as passive coping is not clear. Nevertheless, very strong positive loading of tsik call suggests that the positive pole of component 2 represents active coping.

After all, regardless of the interpretation of these calls as proactive/reactive responses or active/passive copings, which suggests these two dimensions of coping styles are not totally different but related (J. Homberg, 2011), the overall pattern the responses loaded on the component 2 suggests that this component most likely represents the type of coping strategy adopted by the marmoset in a potentially threatening situation; thus, it was labelled as the 'coping strategy' component.

**Comparison of snake test with human intruder test**

In the rubber snake test, the short (5-min) confrontation with a rubber snake, in a familiar environment, significantly increased the average distance of the subject from the snake (that was placed in the bottom left hand corner of the cage front) in comparison to the pre-snake phase. This response is not only in agreement with the notion that snakes are major predators of marmosets (Marilia Barros, Boere, et al., 2002; Correa & Coutinho, 1997), possibly having led to evolutionary changes in the primate brain (Isbell, 2006), but also in accordance with the previous reports using the snake paradigm (Marilia Barros, Boere, et al.,

2002; Cagni et al., 2011; Clara et al., 2008). The significant impact of the stimulus on the behaviour lasted into the post-snake phase when many animals appeared cautious to approach the empty box in which the rubber snake was previously placed. During the snake phase, although the observed behavioural responses to the stimulus were slightly different from those in the human intruder test, PCA extracted two similar components. In contrast to the human intruder paradigm, in which there were some discrepancies in the types of behaviours observed between the current study and previous reports, a very similar marmoset behaviour repertoire to the current study was reported in previous studies using the snake stimulus (Marilia Barros, Boere, et al., 2002; Cagni et al., 2011; N Cross & Rogers, 2006). Component 1 was highly loaded on by the average distance of the animal from the snake stimulus, the duration of staring at the snake and locomotion. The number of head-cocks also moderately loaded on this component. Considering the overall similarity of the two paradigms as tests of anxiety and the fact that average distance and locomotion loaded on component 1 in the same direction and a similar magnitude to that of the human intruder paradigm, it is highly plausible that this component reflects the same underlying factor that mediates the 'emotionality' component in the human intruder test. Head-cocks, in which the animal rotates its head about the longitudinal body axis while watching an object (Clara et al., 2008; Stevenson & Poole, 1976), has been reported only in response to a snake stimulus and not to a human intruder (Marilia Barros, Boere, et al., 2002; Clara et al., 2008). This behaviour was positively correlated with the duration of staring at the snake, which supports the description of this behaviour as observational behaviour (Marilia Barros, Boere, et al., 2002). The loadings of these behaviours were inversely related to the average distance, indicating that those animals that display frequent head-cocks and a relatively longer duration of staring at the snake stayed relatively closer to the snake. Even though these behaviours were seen as the animal approaches a snake, they do not appear to be a display of aggression; no correlation was detected with any aggression-related behaviour such as tsik calls. Also, approaching a potential threat is not a typical reactive coping response (Koolhaas et al., 1999). Therefore, it is possible that these observational behaviours are the reflection of less anxiety and more boldness of the subject.

Component 2 was primarily loaded on by the calls. Tsik calls showed the highest loading followed by tsik-egg calls. This pattern is again similar to that of the 'coping strategy' component in the human intruder test. Tsik calls have been invariably observed in previous studies involving the presentation of snake stimuli and described as aggressive mobbing behaviour or a proactive coping response (Marilia Barros, Boere, et al., 2002; Cagni et al., 2011; Clara et al., 2008; N Cross & Rogers, 2006). A tsik-egg call is composed of a tsik

utterance immediately followed by a short egg call. Therefore, as described above, this call may reflect both the response coping mode and the emotional reactivity. However, the tsik-egg calls observed in the snake paradigm are composed of a much stronger tsik component followed by a weaker egg component compared to the ones induced by the human intruder. Thus these tsik-egg calls reflect the coping response much more strongly than the emotional reactivity aspect.

Another behaviour loading on component 2 was the frequency of stares. Whilst the duration of a stare, which mainly loaded on component 1, was the measure of the total time the animal watched the snake stimulus, the stare frequency measures how often the animal shot a glance towards the stimulus. A significant but relatively weak correlation between the two indicates that the duration and frequency of a stare do not necessarily go together. Moreover, the fact that they loaded onto the different components suggests that the duration and the frequency of stare were independently driven by different psychological factors. The high correlation of stare frequency with tsik calls suggests that while the animal was aggressively mobbing the threatening stimulus, it may also have been actively looking for a surface to jump on, seeking an escape route in case of a sudden attack. Alternatively, the animal may be looking for a conspecific since it has been reported that, in the presence of a snake stimulus, the target animal stays closer to its partner, possibly for protection (Cagni et al., 2011). This combination of behaviours may result in the frequent, but often brief glancing towards the snake. Both accounts suggest this vigilant behaviour is a part of animal's coping response. Considering the proactive/reactive dimension of coping strategy described above (Koolhaas et al., 1999), a significant positive correlation with the proactive tsik calls suggests that the stare frequency is more likely a part of the proactive coping style. Alternatively, on the dimension of active/passive coping styles (J. Homberg, 2011), the frequent glance at the snake can be considered as part of active coping since passive copers would instead tend to stay immobile under a threatening situation. Overall, because of the characteristics of the behavioural variables loaded on each component and the similarity in the pattern of these loadings to the components in the human intruder paradigm, these components were also labelled 'emotionality' and 'coping strategy'.

**Why the 'passed' and 'failed' groups differed on the 'emotionality' component in the rubber snake test and not the human intruder test**

One finding in particular that needs further explanation is that the 'passed' and 'failed' groups differed only on the 'emotionality' component of the rubber snake test but not the human

intruder test. A couple of possible explanations can be considered. When the 'emotionality' scores of the large cohort (including those in the 'passed' and 'failed' groups) were correlated between the human intruder and rubber snake tests, a weak but significant positive correlation was found. Having a positive relationship indicates a similarity between the two paradigms, that is, both the human intruder and rubber snake share the same feature that provokes the emotional aspect (in this case, anxiety/fear) of marmoset's mental functions. If the psychological nature of the stimuli is essentially the same, then it is only the intensity that may differ between stimuli. The group difference was detected on the rubber snake test because this stimulus is stronger in its intensity to evoke an emotional reactivity in marmosets than the less powerful human intruder stimulus. However, the cause of this difference in the intensity is not clear. Although the animals had never seen either the rubber snake or the human intruder prior to testing, they had interacted considerably with experimental scientists who had frequented their cages daily during the discrimination testing phase. Since their features resemble much more closely the human intruder than the rubber snake, this prolonged exposure to humans might have habituated the animals and made the anxiety-provoking nature of the human intruder less intense. This is supported by the observation that those that had gone through the discrimination testing phase displayed relatively lower scores on the 'emotionality' component of the human intruder test in comparison to the animals that were experimentally completely naïve prior to the exposure to the intruder.

Another possibility is that the discrepancy between the paradigms is due to the difference in the quality or nature of the stimuli. Given that the correlation between the 'emotionality' scores of the two paradigms was relatively weak, it suggests that differences between the paradigms are greater than the similarities. One possible distinction may lie between the extent to which these two paradigms elicit a state of anxiety or fear. Whilst the unfamiliar human to a marmoset is more ambiguous in terms of its predatory nature, the snake stimulus is more specific and clear threat to the monkey. This uncertainty and specificity may underlie the difference between anxiety and fear respectively (Michael Davis et al., 2010). The notion that the human intruder and snake stimulus are different in their predatory nature is also supported by the lack of correlation in the 'coping strategy' scores between the paradigms. It implies that the coping responses displayed by the animals are specific to each stimulus. If the two stimuli bear the same predatory nature, an animal would undertake very similar approaches to avoid possible harm. However, when the two stimuli represent different types of predator, it is more sensible for the animal to take different survival tactics appropriate for a specific threat. This would lead to greater individual variations since one animal's coping

style to a threat is not necessary the same with another animal's style. Therefore, when compared, the animals' coping responses would not show a linear relationship between different types of predators. Since the predatory natures of the two stimuli differ, possibly the snake being an imminent danger and the human intruder an ambiguous risk, the emotions elicited also differ, fear and anxiety respectively. The aversive discriminative conditioning paradigm detected individual differences in fear but not anxiety and this, in turn, was detected in the rubber snake test as the group difference but not in the human intruder test. This issue is further discussed below along with suggested neural pathways.

**Difference in the anxiety-provoking nature of the human intruder and rubber snake may involve different neural processing circuitry**

If the anxiety-provoking nature differs between the human intruder and snake stimulus resulting in the different emotional and coping responses, it is likely that there is a difference in the neural substrates supporting snake fear versus emotional reactions to an unfamiliar human. There have been a number of studies investigating a role of specific brain regions implicated in the processing of the human intruder and snake stimuli. Because of the anxiety/fear related nature of the stimuli, the amygdala has been the focus along with the orbitofrontal cortex (OFC, aka orbital prefrontal cortex) to which the amygdala has a strong reciprocal projection (Izquierdo & Murray, 2004).

One of the first studies (Meunier et al., 1999) compared the effects of aspiration versus neurotoxic lesions of the amygdala in rhesus monkeys on a variety of emotionally relevant stimuli including a rubber snake and unfamiliar human. Both lesions significantly reduced the freezing response, which is interpreted as a marker of trait-anxiety in rhesus monkeys (Kalin & Shelton, 1989) to the human intruder, and increased approach behaviour to a snake. A subsequent study (Kalin et al., 2001) replicated the effect of amygdala lesions on responsivity to a snake but found no significant difference in the response to a human intruder. The authors attributed this discrepancy to the different nature of the stimuli, proposing that while a snake induces acute fear that is mediated by the amygdala, a human intruder promotes more stable anxiety that is associated with other structures such as the OFC and the bed nucleus of stria terminalis Other studies reported similar results. Whilst the bilateral lesion to the amygdala (Izquierdo et al., 2005), the central nucleus of the amygdala (Kalin et al., 2004) and the unilateral combined removals of the amygdala and the OFC (Izquierdo & Murray, 2004) all reduced the snake fear in rhesus monkeys, the effects of these lesions in emotional response to an unfamiliar human were varied.

Similar to the effect of the amygdala lesion, the OFC lesioned rhesus monkeys also displayed a reduction in the snake fear (Izquierdo et al., 2005; Kalin et al., 2007). However, the same OFC lesioned monkeys, when tested on the human intruder paradigm, exhibited altered emotional responses such as increased mild aggression (Izquierdo et al., 2005) and reduced fear (Kalin et al., 2007). Similarly, in a marmoset study (Agustín-Pavón et al., 2012), in comparison to the controls, the animals with a lesion to the OFC displayed significantly increased anxiety-related response.

In summary, the removal of the amygdala invariably reduces fear response in the snake paradigm but varied effects on the human intruder paradigm with some reporting negative results, whereas the removal of the OFC produces blunted fear response to a snake stimulus and altered fear response to an unfamiliar human. These findings indicate that a snake stimulus and an unfamiliar human are processed on different neural pathways, suggesting a difference in the emotional quality of the two stimuli. Kalin and colleagues (Kalin et al., 2001) proposed that while a snake induces acute fear that is mediated by the amygdala, a human intruder promotes more stable anxiety that is associated with other structures including the OFC.

Considering that a snake stimulus represents a specific predator and elicits innate fear in primates (Isbell, 2006), information of such a specific and evolutionally relevant threatening stimulus should be processed quickly for avoidance of much predicted attack. This suggests that the quick and dirty thalamo-amygdala pathway (J. E. LeDoux, 1995) is a likely neural circuit involved in processing such stimulus. The amygdala is also implicated in expression of phasic fear to a specific cue (Indovina et al., 2011), which is also in line with the above findings that the removal of this subcortical structure abolished fear response to a specific threat, i.e. the snake stimulus. The finding that the lesion to the OFC attenuated the snake fear to the lesser extent of the lesion of the amygdala (Izquierdo et al., 2005) suggests that the OFC may play a role in up-regulating the fear processing in the amygdala under a situation in which a quick response is the matter of survival.

In contrast to the snake stimulus, an unfamiliar human is a social stimulus with which the anticipated consequence of the interaction is less certain. A human to a captive monkey can be a real threat but also be a harmless object or even be a positive subject who provides food. This ambiguity in the anticipated outcome may lead to a mental conflict when the animal is required to make an appropriate action. A well-established function of the OFC is to

modulate goal-directed behaviour based on the assessment of future positive and negative consequences (Bechara, Damasio, Damasio, & Anderson, 1994; Kalin et al., 2007). Therefore, it is plausible that emotionally ambiguous stimulus such as an unfamiliar human is first processed in the OFC, where the stimulus value is assessed and the signals for subsequent actions are sent to lower structures such as the amygdala. Without the functional OFC, the animal would misinterpret the human threat as a positive stimulus with the reduced fear response (Kalin et al., 2007) or as overly dangerous stimulus with the enhanced emotional reactivity (Agustín-Pavón et al., 2012; Izquierdo et al., 2005).

Although those interpretations of the findings support the proposal in which fear and anxiety are differentiated by respective unique neural pathways, the role of the OFC in anxiety is still less clear than the one of the amygdala in fear. Indeed, coherent evidence from all the lesion studies appears to be the important role of the amygdala in mediating emotional responses to a snake stimulus. As mentioned in the Chapter 2 Discussion, the amygdala is also critically involved in a fear conditioning, regardless of the types of conditioning: simple, context or discriminative. Therefore, it is likely that the failure to display differential responses between the $CS^+$ and $CS^-$ in the aversive discriminative conditioning implicates the impaired or abnormal amygdala function. If passing or failing the discriminative conditioning depends, at least partly, on the function of the amygdala, it should be the rubber snake test that is capable of detecting the difference between the 'passed' and 'failed' groups. This, in fact, was what reported in the current study.

In summary, the results from this chapter demonstrate that testing a large cohort of marmosets on the human intruder and rubber snake tests produced a reliable ethogram of marmoset behaviour when confronted with a human intruder or a model snake. The PCA on the behavioural data produced two underlying psychological dimensions, namely 'emotionality' and 'coping strategy', in both tests. When correlated, only the 'emotionality' showed a positive relationship between the tests, suggesting a similarity but also a difference between responsivity to human intruder and snake stimuli. The animals from the 'passed' and 'failed' groups differed significantly on the 'emotionality' component, which was in agreement with the prediction of the fear generalization hypothesis that failure to discriminate is due to enhanced anxiety. However, this group difference was found only for the rubber snake but not the human intruder test. The discrepancy implied a difference in the nature of the stimuli as well as in the neural circuitry involved in processing the two stimuli. Possible impairment in the amygdala function may have led to both the failure in the discriminative

conditioning and the enhanced emotional reactivity in the rubber snake test. Involvement of the prefrontal areas including the OFC in the discriminative ability is suggestive. Although the results from the human intruder test provided no group difference, it was far from being conclusive on the possible involvement of the OFC. In the next chapter, this issue is further investigated by testing the animals from the 'failed' and 'passed' groups on the OFC and lPFC dependent cognitive flexibility tests.

# Chapter 4

## How does trait anxiety affect prefrontal cognitive functionalities? Comparison of high and low anxious groups' performances on the OFC- and lPFC-dependent cognitive flexibility tests

## Abstract

Disruptions of prefrontal executive functions under threatening situations have been widely reported among those with clinical or high trait anxiety (M. Eysenck, Macleod, & Mathews, 1987; Mathews et al., 1989). However, under non-threatening conditions, mixed results have been reported for the effect of trait anxiety on cognitive performances; some reporting superiority in anxious individuals (Sorg & Whitney, 1992; Topçuoğlu et al., 2009). Neuroimaging studies have also been inconclusive; whilst some reported reduced prefrontal activities among those high in trait anxiety (Bishop, 2009), others reported increased activations (Basten, Stelzel, & Fiebach, 2011). Several models have been proposed for the emotion-cognition interplay. Attentional control theory (M. Eysenck et al., 2007) and the dual route model of trait anxiety (Indovina et al., 2011) both assume an unbalanced coupling between subcortical stimulus-driven system and prefrontal cognitive-control system. However, whilst the former predicts operational prefrontal functionality, the latter posits impoverished recruitment of it. The differential susceptibility model (Belsky et al., 2009) proposes that both enhanced anxiety and improved cognition are adaptive responses to changing environments. Trait anxiety has also been associated not only with unbalanced bottom-up and top-down mechanisms but also with altered couplings across the circuits connecting subcortical structures to prefrontal regions (Indovina et al., 2011; Pezawas & Meyer-Lindenberg, 2005), which may also contribute to altered cognition observed among those high in trait anxiety.

In order to investigate the effect of trait anxiety on prefrontal executive functions, the animals defined as either high or low in trait anxiety, based on their performance on an aversive discriminative conditioning (Chapter 2) and rubber snake test (Chapter 3), were tested on the orbitofrontal cortex (OFC) dependent incongruent object discrimination test and the lateral prefrontal cortex (lPFC) dependent detour-reaching rule transfer test. The results revealed that whilst no group difference was found, two proposed biomarkers of enhanced trait anxiety: enhanced cue-specific vigilance and suppressed baseline blood pressure, were inversely and differentially correlated with the perseveration measures from the two tests. This not only indicates that enhanced trait anxiety, specifically the predictors of high trait anxiety were associated with improved prefrontal cognitive function, but also suggest that there may be two distinct neural circuits connecting subcortical structures to prefrontal sub-regions, specifically the cue-sensitive amygdala-OFC and context-sensitive hippocampus-lPFC circuits, contributing to trait anxiety.

## 4.1 Introduction

In the previous chapters it was discussed how the amygdala played an important role in the processing of emotional stimuli and the expression of defensive responses, as demonstrated in both fear conditioning (Phelps & LeDoux, 2005) and unconditioned fear/anxiety response (Kalin & Shelton, 2003) paradigms. Recent investigations into the neural mechanisms underlying trait anxiety also suggest an involvement of the prefrontal cortex (PFC) in emotional regulation including the emotion-cognition interplay, which seem to be impaired in individuals high in trait anxiety. The PFC is crucially involved in cognitive control (Thayer & Hansen, 2009), especially the functions that require a working memory system. Working memory is required for actively keeping information online while further cognitive processing is performed on the information (Topçuoğlu et al., 2009). According to the tripartite working memory model (Baddeley, 1992), the system consists of three components: 1) a central executive, which is involved in the processing of information and having self-regulatory functions (e.g. performance monitoring, planning and strategy selection); 2) a phonological loop for the rehearsal and transient storage of verbal information; and 3) a visuospatial sketchpad for the processing and transient storage of visual and spatial information. Attentional control theory (M. Eysenck et al., 2007), which has been one of the most influential accounts of the relationship between anxiety and cognition, proposes that it is the central executive on which anxiety exerts its main influence. The multidimensional executive functioning model (Miyake et al., 2000) further divides the central executive into three functional components: 1) updating, i.e. actively updating and monitoring working memory representations; 2) set-shifting, i.e. shifting back and forth between multiple tasks, operations or mental sets; and 3) inhibition, i.e. an ability to actively inhibit dominant, automatic or prepotent responses when necessary. All these functions, especially the latter two, require active control of selective attention (Derakshan & Eysenck, 2009), which has been shown to be altered or disrupted among individuals with high trait and pathological anxiety (M. Eysenck et al., 2007). In the following paragraphs, previous studies that investigated an association between the prefrontal controlled cognitive functions, especially the central executive and attentional control, and trait/pathological anxieties are reviewed.

**High trait anxiety and pathological anxiety are related to reduced prefrontal executive functioning: Evidences from emotional-cognitive paradigms**

There have been a relatively large number of studies determining the effect of high trait anxiety and various anxiety disorders on the performance of cognitive behavioural tasks that involve emotional stimuli. For instance, the neural mechanisms underlying selective attention to threat implicate both the amygdala and prefrontal functionality (Bishop, 2008), however, this adaptive mechanisms for allocating attentional resources to biologically relevant threatening stimuli seem to be altered among anxious individuals (Ferreri, Lapp, & Peretti, 2011). The dot probe paradigm has been used for both assessing attentional bias in anxious subjects and treatment for anxiety disorders. The task involves a brief presentation of two images or words, threatening or neutral, followed by a small target probe appearing in the location just occupied by one of the stimuli. The subjects are required to discriminate two types of the probe as fast as possible. Attentional bias towards threat is revealed when subjects are faster to respond to probes that replace threat-related stimuli rather than neutral stimuli. Individuals diagnosed with GAD showed greater attentional bias effect compared to non-anxious controls (Bradley & Mogg, 1999). In addition, by manipulating the paradigm, the attentional bias to threat can be altered, which has led to significant reduction of the symptoms in the individuals with GAD and social anxiety disorder (SAD) (Bar-Haim, 2010).In connection with attentional bias, high anxiety has been associated with an interpretive bias of ambiguous stimuli. Individuals diagnosed with generalized anxiety disorder (GAD) (Mathews et al. 1989) or rated high in trait anxiety score (M. Eysenck et al., 1987) were presented with ambiguous stimuli, specifically, homophones that have both threatening and non-threatening meanings. In comparison to controls, the clinically and trait anxious individuals displayed the tendency to select more threatening interpretations, which was accompanied by greater skin conductance responses. The authors interpreted these findings as the anxious individuals showing an interpretational bias that favours the processing of threatening stimuli.

Attentional bias can also be seen when people have difficulties disengaging attention away from threatening stimuli. Individuals diagnosed with posttraumatic stress disorder (PTSD), when asked to respond to a target word presented together with a threat-related word, showed a difficulty in attentionally disengaging from the threat-related word (Pineles, Shipherd, Mostoufi, Abramovitz, & Yovel, 2009). Individuals high in social anxiety also showed greater difficulty disengaging from negative social cues (disgust faces) but not from positive social cues (happy faces) (Buckner, Maner, & Schmidt, 2010). A modified stroop task (Stroop, 1935) has also been used to demonstrate attentional bias in anxious populations. The subjects were presented with either threatening or non-threatening word in

varying colours and asked to report the colour while ignoring the semantic content of the word. Increased response times to report the colour of threat words compared to non-threatening words are considered an indication of attentional bias. Vietnam combat veterans with PTSD took longer response times towards negative emotional words relative to positive or neutral words whilst the controls showed no difference in response times across word types, indicating a deficit in attentional control among those with PTSD (McNally, Kaspi, Riemann, & Zeitlin, 1990). Similar findings were reported for the individuals high in trait anxiety (Mathews & MacLeod, 1985). Furthermore, in an experiment where subjects were required to inhibit task-irrelevant distractive information, high trait-anxious individuals were slower in responding to a target stimulus when there was either an emotional or non-emotional distractor on the same searching screen (E. Fox, 1994), indicating that high trait anxiety interferes with the inhibition of distractor information under conditions of attentional search. Also, in a task requiring attentional set shifting, individuals high in trait anxiety exhibited a difficulty in switching from a neutral to an emotional stimuli set (Johnson, 2009), suggesting high trait anxiety produces either increased attentional avoidance to emotional stimuli or selective difficulty disengaging from threatening material.

**Altered prefrontal neural activations during emotional-cognitive task performances are related to trait / pathological anxiety: Evidences from neuroimaging studies**

The studies described above illustrate a detrimental effect of pathological / high trait anxieties on cognitive controls that likely involve prefrontal functionalities. According to the attentional control theory, anxiety is experienced when a current goal is threatened. Threat to a current goal causes attention resource to be allocated to detecting its source and deciding how to respond; therefore, anxiety reduces attentional focus on the current task unless it involves threatening stimuli. In other words, anxiety prioritizes the stimulus-driven attentional system over goal-directed attentional control, allowing task-irrelevant anxiety-related information to interfere with executive functioning (M. Calvo & Eysenck, 1998). The attentional control theory further postulates that high levels of motivation to reduce the aversive state may lead to a compensatory enhancement of cognitive effort, in order to maintain a standard level of performance (Fales, Barch, & Burgess, 2008). Such enhanced effort could be associated with increased activation in brain regions associated with cognitive control, such as dlPFC, vlPFC or dACC. The evidences for this proposal have been provided by neuroimaging studies using an emotional-cognitive behavioural paradigm. Eisenberger and colleagues demonstrated that when tested on a task requiring discrepancy detection, individuals high in neuroticism, a personality trait highly comorbid with anxiety disorders (Hansell et al., 2012),

exhibited greater activity in the dorsal anterior cingulate cortex (dACC), a region functionally closely related to the prefrontal cortex (PFC) and higher interoceptive accuracy, relative to individuals low in neuroticism (Eisenberger, Lieberman, & Satpute, 2005). Telzer and colleagues (Telzer et al., 2008) examined a relationship between trait anxiety, attention bias and their neurological correlates by testing young subjects on a modified dot probe task while monitoring event-related neural activity with functional magnetic resonance imaging (fMRI). Subjects were presented with a pair of angry and neutral facial expressions and asked to discriminate the location of a probe that replaced one of the faces. Shorter reaction times when the probe replaced the angry face versus the neutral face was considered an index of attentional bias. The analysis revealed that not only individuals with high trait anxiety displayed greater attentional bias relative to low trait-anxious individuals but also, both trait anxiety and the attentional bias were associated with greater right dorsolateral prefrontal cortex (dlPFC) activation. Additionally, trait anxiety was associated with greater right ventrolateral prefrontal cortex (vlPFC) activation, irrespective of the emotional content of face stimuli. The results from these studies indicate that when individuals high in trait anxiety perform cognitive tasks involving emotional stimuli, their performances, which are usually reduced or impaired, are associated with greater activation of the prefrontal area. This is in consistent with the prediction by the attentional control theory (M. Eysenck et al., 2007).

In contrary to the above hypothesis, Bishop and colleagues (Bishop, Duncan, Brett, et al., 2004) have shown that state anxiety is significantly, inversely correlated with PFC activity during performance of an attentional control task. In their experiment, subjects were presented with two probe pictures (that were either identical or different to one another), along with distractor pictures of fearful facial expressions. The task was to identify if the probe pictures were identical or different. Hence, the subjects were required to selectively attend to the probes while ignoring the threat-related distractors. fMRI data revealed that the rostal ACC was strongly activated in response to the fearful face distractor, consistent with a role for this region in responding to unexpected conflict caused by salient emotional stimuli. However, they also found that the subjects with higher anxiety levels showed lower rostral ACC activity overall and reduced recruitment of lateral PFC (lPFC) as expectancy of threat-related distractors was established. The authors hypothesized that as lPFC is implicated in attentional resource allocation, this region may receive signals from rostral ACC about the ongoing stimulus conflict and selectively allocate attentional resources to task-relevant stimuli. Whist in normal and low anxious individuals this prefrontal 'top-down' control process down-regulates the 'bottom-up' sensory driven mechanism, therefore no negative attentional bias is induced, the reduced recruitment of the PFC control mechanisms among high anxious

individuals may allow emotional salient stimuli to win attentional resource competition, leading to an attentional bias effect. This proposal is further supported by findings of Dolcos and McCarthy (Dolcos & McCarthy, 2006), in which deactivation of dlPFC was observed in response to emotional distractors in a delayed-response working memory task. In this paradigm, subjects were first presented with three different pictures of human face followed by a brief presentation of distractor pictures, either neutral or emotional, and finally a photo of human face was shown. The task was to identify if the last photo was one of the three pictures presented earlier or a new one, therefore testing subject's working memory maintenance. fMRI data revealed that the presentation of emotional distractors evoked strong activity in typical 'bottom-up' emotional processing regions including the amygdala whilst simultaneously evoking relative deactivation of typical working memory regions including lPFC and lateral parietal cortex, and also impairing the task performances.

In order to further investigate the relationship between the impoverished recruitment of PFC 'top-down' control mechanisms and the increased 'bottom-up' emotional circuit centred around the amygdala and high trait anxiety, Indovina and colleagues (Indovina et al., 2011) tested high and low trait anxious individuals on a fear conditioning paradigm involving both cue and context while measuring physiological response (skin conductance response) and event-related neural activity with fMRI. The data revealed 1) that increased amygdala reactivity to phasic fear cues was positively correlated with physiological responses and trait anxiety level, and 2) that heightened trait anxiety and physiological responses were associated with impoverished ventral PFC activation in both phasic (cued) and sustained (contextual) responses. Based on these results, the authors proposed a model for trait vulnerability to anxiety with two neurological dimensions (dual-route model of trait anxiety). In one dimension, elevated trait anxiety is associated with hyper-responsivity of the amygdala to phasic cues, which leads to maladaptive acquisition and excessive expression of the fear response in threat-related situations. In another dimension, elevated trait anxiety is associated with impoverished recruitment of prefrontal down-regulation, which may lead to impaired cognitive control and the loss of control over the 'bottom-up' emotional circuit. Either or both of the two dimensions may contribute to excessive emotional reactivity and reduced executive functionality seen in high trait-anxious individuals.

**Does enhanced trait anxiety contribute to improved or impaired executive functioning in cognitive paradigms absent of emotional stimuli?**

Although the attentional control theory (M. Eysenck et al., 2007) and the dual-route model of trait anxiety (Indovina et al., 2011) both predict detrimental effects of high trait anxiety on cognitive executive functioning due to an enhanced subcortical stimulus-driven emotional system, they differ in how this increased 'bottom-up' input relates to the prefrontal goal-directed/down-regulatory cognitive control mechanism; the former predicts increased PFC activity as a result of cognitive compensatory effort whereas the latter hypothesizes impoverished PFC recruitment allowing the bias of attentional resources towards the stimulus-driven system. Whilst the literatures described so far investigated this relationship between trait anxiety and executive functioning using cognitive tasks involving emotional stimuli, a number of recent studies examined the prefrontal functionality in high trait-anxious individuals on cognitive tasks absent of emotional stimuli. In this latter context the two models provide dissociable predictions. Predictions from the attentional control theory are mixed with some proponents hypothesising that the PFC will be more active in anxious individuals i.e. "working harder" to perform at levels equivalent to those of non-anxious individuals, whilst other proponents suggest that without the pressure from the emotional stimulus-driven system, the PFC may prevail its superiority as improved cognitive efficiency (Fales et al., 2008; Visu-Petra, Miclea, & Visu-Petra, 2012). On the other hand, the dual-route model of trait anxiety (Bishop, 2007; Indovina et al., 2011) assumes reduced PFC functionality among high trait-anxious individuals, thereby leading to impaired cognitive performance even in the absence of emotional stimuli.

The results of the investigations so far have not been conclusive. In support of the former prediction, Sorg and Whitney (Sorg & Whitney, 1992) demonstrated that under non-stressful conditions individuals high in trait anxiety performed better than low trait-anxious individuals on a working memory task in which subjects had to recall the final word of between 2 and 6 sentences. On the contrary, Topcuoglu and colleagues (Topçuoğlu et al., 2009) reported that social phobics who were rated high in trait anxiety were impaired on the Wisconsin Card Sorting Test, a classic test of prefrontal function requiring working memory and set-shifting executive functions. Ansari and colleagues (Ansari, Derakshan, & Richards, 2008) tested individuals who were high or low in trait anxiety on a mixed antisaccade paradigm, a task requiring top-down attentional control, and showed that those high in trait anxiety were less able to efficiently shift attentional resources between an antisaccade and prosaccade version of an eye gaze task than low anxious subjects. Whilst low anxious subjects exhibited expected improvement in antisaccade performance when switching between anti- and

prosaccade trials, high anxious subjects failed to show this improvement. Visu-Petra and colleagues (Visu-Petra et al., 2012) tested individuals rated either high or low in trait anxiety on a cognitive test battery examining three components of central executive functions: inhibition, set-shifting and updating. The results indicated that high trait anxiety was associated with reduced performance efficiency in inhibition and set-shifting tasks; however, on the memory updating task those high in trait anxiety outperformed low trait anxious subjects in terms of performance accuracy. The authors claimed that these results are in agreement with the predictions by the attentional control theory, that when resources are available, high trait anxious individuals could outperform less anxious individuals especially regarding to performance accuracy.

**Whether enhanced trait anxiety is related to increased or impoverished prefrontal activation on cognitive paradigms absent of emotional stimuli input?**

In the same way that behavioural studies comparing the performance of high and low trait anxious individuals on non-emotional cognitive tasks have reported mixed results, neuroimaging studies relating prefrontal activity to cognitive performance in high and low anxious subjects also been inconclusive. Basten and colleagues (Basten et al., 2011) tested high or low trait anxious individuals on the colour-word Stroop test while monitoring subjects' neural activities. The event-related fMRI data revealed that the high trait-anxious individuals exhibited 1) stronger task-related neural activation in the dlPFC, the area implicated in executive control to inhibit irrelevant representations, and 2) reduced functional connectivity between the dlPFC and other regions of a task-relevant cerebral network (inferior frontal junction area, dorsal ACC and left fusiform gyrus). The authors interpreted the findings of increased dlPFC activity in high trait-anxious subjects as reflecting a compensatory effort for a functional handicap resulting from suboptimal connectivity within the cortical network subserving task performance. Although this compensatory effort was not reflected in actual performance (i.e. high anxious subjects made more errors than low anxious counterparts), the authors suggested that this was due to either particularly high levels of anxiety in the sample or the high attentional control demands of the task.

Contrary to these findings, Bishop (Bishop, 2009) demonstrated that high trait anxiety is associated with impoverished prefrontal recruitment when inhibiting distractors, leading to impaired task performance. Here, subjects were tested on a letter-search task, in which they were asked to respond to a target letter in a letter string while ignoring a larger distractor letter appearing just below the letter string. This required the subject to attend selectively to

the letter string while inhibiting processing of the distractor. Perceptual load was manipulated by making the search easier (all letters in the string were targets) or more difficult (only one of the letters was a target). The fMRI data analysis revealed that trait anxiety was inversely related with dlPFC recruitment in response to distractor processing under conditions of low versus high perceptual load, that is, high levels of trait anxiety were associated with reduced task-relevant dlPFC activation under low perceptual load. This was accompanied by behavioural data showing that high trait-anxious subjects were slower to identify the target in the presence of distractor under low perceptual load, relative to low anxious counterparts. The author proposed that high trait anxiety is characterised by deficient attentional control and this deficit is associated with reduced, rather than increased, dlPFC recruitment. Contrary to the account from a previous finding (Basten et al., 2011), the performance was impaired under low attentional demand, suggesting that under high attentional load condition, the distractor information is expelled at an early processing stage; whereas, under low attentional demand, the information can come through to the stage where the prefrontal executive control is required to inhibit the distractor, at which the deficit in high trait-anxious individuals can be detected. In addition, this impoverished prefrontal control seen even in a purely cognitive task may account for broader dysregulation of cognitive functions observed among high trait-anxious individuals (Bishop, 2009).

In an attempt to account for these discrepant views of the relationship between trait anxiety and prefrontal cognitive functionality, Fales and colleagues (Fales et al., 2008) tested high and low trait-anxious individuals on a n-back task requiring working memory, while monitoring both sustained and transient neural activities with fMRI. The results revealed that, compared with low anxious individuals, the high anxious group showed significantly reduced sustained activity in dlPFC but increased transient activation during task trials. The authors interpreted these findings in light of the dual mechanism of control theory (Braver, Gray, & Burgess, 2008), which proposes two types of cognitive control; 1) proactive control, characterised by sustained representation of task requirement throughout the periods of high control demand and more effective top-down control processing; and, 2) reactive control. characterised by transient recruitment of working memory, which is critical for transiently detecting/solving interference when it appears but is susceptible to the influence by bottom-up input. High trait anxiety is associated with reduced proactive control and increased reactive control. This was in line with the observation that dlPFC showed reduced sustained activity throughout the trial period but increased event-related transient activity.

**Aim of experiments**

Since the effects of trait anxiety on prefrontal executive functioning are not conclusive, the present study investigates the relationship between trait anxiety and prefrontal functioning in more detail. Up until now the majority of studies have focussed on attentional and working memory tasks that are associated primarily with dorsolateral regions of prefrontal cortex. We wanted to determine the effects of trait anxiety on other tests of prefrontal function associated with the orbitofrontal cortex. Marmoset monkeys from the 'passed' and 'failed' groups identified based on their performances on the aversive discriminative conditioning (Chapter 2) were tested on two different cognitive flexibility tasks, one dependent upon an intact OFC and the other dependent upon the ventrolateral PFC. It was hypothesized that those animals with an apparent high level of trait anxiety would show reduced performace compared to the 'passed' group.

The first experiment tested both groups of animals on the OFC sensitive incongruent object discrimination test. In this paradigm, the animals were presented with two plastic boxes containing either high or low incentive food objects. Only the choice to the low incentive food box was associated with the delivery of actual food reward. Therefore, the animal was required to inhibit prepotent tendency to reach for the high incentive food box and instead choose the low incentive food box. The OFC plays a role in behavioural flexibility by continually monitoring response outcome with expected reward/punishment and signalling for adaptive control of action (Kringelbach & Rolls, 2004). A study conducted by Man and colleagues(M. S. Man, Clarke, & Roberts, 2009) demonstrated that marmosets that received a lesion of the OFC were impaired on this task, displaying perseverative response to the task-irrelevant high incentive food box.

The second experiment tested the animals on the lPFC sensitive detour-reaching rule transfer task. In this paradigm, the animals were first trained with a black opaque box containing a food reward. One of the sides of the box could be opened for access. However, since the animal could not tell by looking which side was open, it was required to touch each side until an open side was found; once found the animal could detour-reach inside to obtain the reward. After acquiring this rule, the animal was then tested with a transparent box containing the same type of food reward. The only difference from the training condition was that now the food inside was visible to the animal. The detour reach rule stayed the same; therefore, the animal was required to transfer the learned strategy to the new context. Wallis and colleagues (Wallis, Dias, Robbins, & Roberts, 2001) demonstrated that the marmosets that received an excitotoxic lesion to the lPFC were impaired in performing this task. The

lesioned animals perseverated, making more unsuccessful reaches to the front side of the box, along the animal's direct line of sight to the visible food reward.

Those two paradigms were not completely free of emotional component since the food reward provided appetitive aspect to the tasks. However, evidences suggest that the PFC exerts top-down regulatory control on not only negative but also positive emotional mechanisms (M. S. Man et al., 2009; Wallis et al., 2001). Therefore, if trait anxiety is associated with impoverished or increased prefrontal cognitive control, its influence on behaviour could be detected on those paradigms. If, as described previously, enhanced trait anxiety is associated with increased prefrontal activation, the animals that are hypothetically high in trait anxiety (i.e. 'failed' group) may display improved cognitive performances. On the other hand, if enhanced trait anxiety is associated with impoverished recruitment of prefrontal control, those in the 'failed' group are predicted to display greater perseveration or behavioural inflexibility on either or both experiments, in comparison to the animals with normal or low trait anxiety (i.e. 'passed' group).

## 4.2 Incongruent Object Discrimination Test

### 4.2.1 Methods and Materials

#### 4.2.1.1 Subjects

Thirteen adult common marmosets (7 females and 6 males, aged 2.0 to 3.6 years, average age 2.8 years at the onset of testing) were tested on the incongruent object discrimination test. They were among the 27 animals that had received the aversive discrimination test (section 2.2, Table 2.1). Seven animals (4 females and 3 males) had successfully discriminated the CS's ('passed' group) and six (3 females and 3 males) failed to discriminate ('failed' group). The remaining 15 animals of the 27 were not tested in the incongruent object discrimination paradigm as they went on to be subjects in a prefrontal lesion study as accounted in section 3.2.1. All animals were housed in male/female pairs under controlled temperature and humidity conditions on a 12-h light/dark cycle. On weekdays, they were fed 20 g of MP.E1 primate diet food pellets (Special Diet Services, Essex, UK) and two pieces of carrot at 1530 h. This diet was supplemented at the weekends with additional fruit, eggs, bread, marmoset jelly (Special Diet Services), and peanuts. Water was available ad libitum. All procedures were conducted in accordance with the project and personal licenses under the UK animals (Scientific Procedures) Act of 1986.

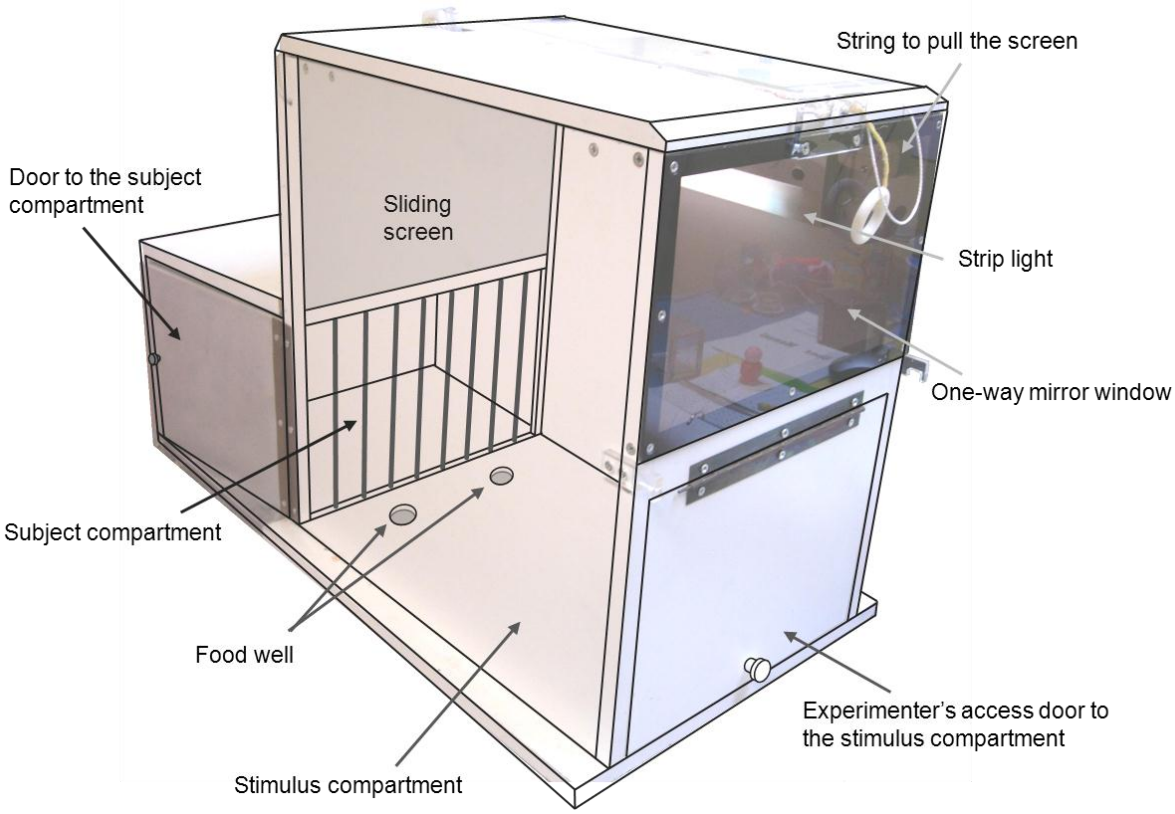



Figure 4.1 A photograph of the modified hand-operated Wisconsin General Test Apparatus for the incongruent object discrimination task, with superimposed schematic illustration of its interior.

#### 4.2.1.2 Test Apparatus

***Wisconsin General Test Apparatus*** Testing took place in a modified hand-operated Wisconsin General Test Apparatus (WGTA, Figure 4.1) in a darkened, sound attenuated room. The apparatus consisted of two compartments (i) a smaller room (23 cm high, 25 cm wide, 35 cm deep) which contained the clear Perspex carry box (18 cm high, 24 cm wide, 20 cm deep) in which the animal was placed and (ii) a larger room (47 cm high, 46 cm wide, 31 cm deep) where stimuli were presented. In the stimulus compartment, there were two food wells (3.0 cm diameter, 1.0 cm deep, spaced 7.0 cm apart, and 2.3 cm from the edge of the subject compartment), located on the right and left sides, in which the food reward, a small piece of syrup malt loaf, was placed. The compartments were separated by an opaque screen. Upon placement of the carry box into the subject compartment, the door of the carry box was removed. The opaque screen was then raised to allow the animal a view of the interior of the stimulus compartment which was lit by two 8W/35A strip lights. The animal could reach toward the food wells through the bars (spaced 2.5 cm apart) placed between

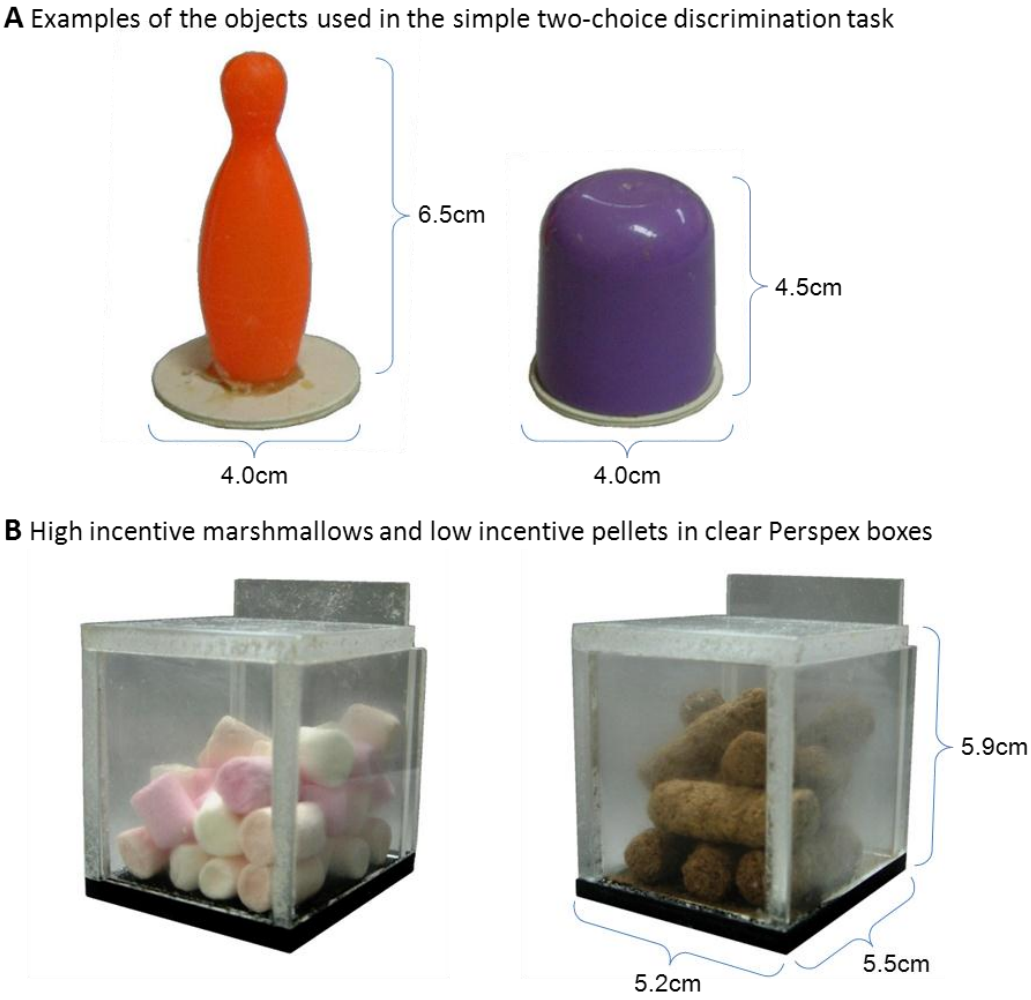



Figure 4.2 (A) Photographs and dimensions of the objects (orange skittle and purple dome) used for the simple two-choice discrimination task and (B) photographs and dimensions of clear Perspex boxes containing high incentive marshmallows and low incentive pellets for the incongruent object discrimination task.

the subject and stimulus compartments. The experimenter could view the stimulus compartment through a one-way mirror and control the sliding screen using pulleys and string. In this way, the animal did not have any visual or physical contact with the experimenter during the test session, avoiding the effect of human presence on the animal's behaviour.

#### 4.2.1.3 Behavioural Procedure

***Habituation to Apparatus and Objects*** On the first day of habituation, the animal was placed in the WGTA with the food reward presented in both food wells. A trial began when

the screen was slid open and the animal was allowed access to the food reward; it ended when the animal obtained the reward and the screen was closed, or, if the animal did not reach toward the food for more than five minutes. When the animal completed about 15 trials within a 20-min session, the animal was considered to be sufficiently habituated to taking the reward from the food well and moved to the next habituation stage. The objects such as the ones depicted in Figure 4.2. A were introduced. They were each attached to a base disk (4.0 cm diameter) that could completely cover the food well. Two objects were initially placed far away from the animal and the food well. Across trials, they were brought closer and closer to the animal, until they eventually were positioned at the edge of the food well. Once the food well was covered, the animal was required to touch the object. The experimenter then revealed the food well by pulling the string attached to the object, allowing the animal access to the reward. Once the animal had completed about 15 trials within a 20-min session during which the food wells were completely covered, the animal was considered to be sufficiently competent on the task to be moved to the training stage.

***Preliminary Training*** Animals were trained to perform simple two-choice object discriminations. In each trial, the food reward was placed, pseudorandomly, in either the left or right food well. Two objects of differing colour and shape were placed over each food well, concealing the reward. Upon raising the opaque screen, an animal was required to touch an object to choose it, whereby the object was retracted by the experimenter pulling on an attached piece of string. This revealed the underlying food well, which, if the choice was correct, contained the reward whilst, if incorrect, revealed an empty food well. Lowering the screen terminated the trial. Animals received up to 30 trials in a daily session. The position of the two objects was allocated, pseudorandomly, to the left and right food wells. If the animal displayed a significant response bias to one side, correction trials, in which only the non-preferred side was rewarded, were inserted until the animal made three correct responses to that side. Criterion for a successful discrimination was set at 90% correct responses within 30 trials (27/30). After completing two pairs of object discriminations, the animals were moved on to the discrimination task involving incongruent incentive objects.

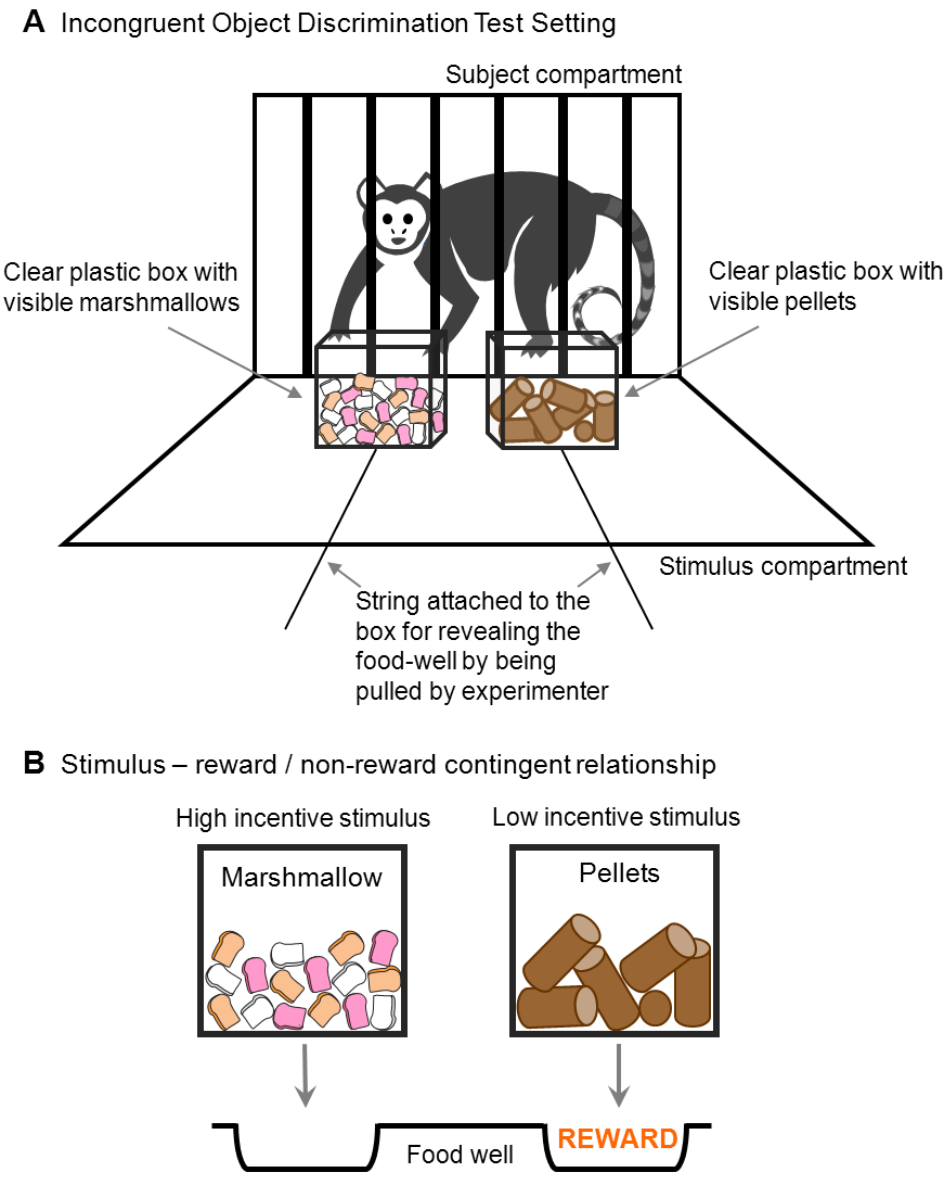



Figure 4.3 (A) Schematic diagram of the test setting for the incongruent object discrimination task and (B) the actual stimulus–reward contingency. By choosing the food box containing low incentive pellets, the animal was rewarded with syrup malt loaf. Diagram was adapted from Man, Clarke, & Roberts (2009).

***Incongruent Object Discrimination Test*** In the incongruent discrimination task, the paradigm was identical to the object discriminations with the exception that the choice objects were now replaced by clear Perspex boxes of identical size (50 × 50 × 50 mm, Figure 4.2 B). One box was half-filled with high incentive marshmallows while the other box was half-filled with low incentive dry food pellets (Caldwell et al., 2009). The food types were clearly visible within the boxes, but the animal did not have access to them. Animals were

required to inhibit their prepotent response of reaching toward the high incentive food and instead choose the low incentive food in order to obtain the reward in the food well underneath (Figure 4.3 A). Therefore, the incentive values of the food objects were incongruent with the reward contingencies. A correct choice led to displacement of the object revealing the reward, whereas an error revealed an empty food well (Figure 4.3 B). The position of the high and low incentive objects was allocated, pseudorandomly, to the left and right food wells and balanced across each block of 10 trials. The response (correct or error) was recorded. The criterion for successful discrimination was set at 90% correct responses within 30 trials (27/30). The animals received up to 30 trials in a daily 10-min session. Testing was video-recorded by a small camera placed in the stimulus compartment and scored by an observer subsequently.

#### 4.2.1.4 Data Acquisition and Analysis

***Behavioural Assessment*** The number of trials the animal took to reach the discrimination criterion, as well as the number of correct and error responses were recorded. By using signal detection theory, a previous study (M. S. Man et al., 2009) demonstrated that damage to the OFC impairs an animal's ability to perform the incongruent object discrimination task (i.e. lesioned animals made greater numbers of perseverative errors than did controls). Therefore, the same analytical technique was used in the current study (see statistical analysis below). Based on signal detection theory, the analysis classified the types of errors into 'perseverative' (i.e. responding to the incorrect high-incentive stimulus significantly above chance) and 'non-perseverative' (i.e. responding to the correct, low-incentive stimulus at, or above, chance) across each block of 10 trials. In addition, as in the previous study mentioned above, the number of errors the animal made before making two correct responses was determined. This is a measure of the ability to first initiate a response away from the high-incentive stimulus and thus inhibit the prepotent response. Two responses were used instead of a single correct response to ensure that the animal really had directed their attention away from the incorrect high-incentive stimulus and had not touched the correct, low-incentive, stimulus unintentionally. This measure also showed sensitivity to OFC damage, that is, the lesioned animals made significantly greater numbers of errors before making two correct responses than did controls.

***Statistical Analysis*** Statistical analyses were performed using statistic software SPSS (version 17.0). In order to characterise the type of errors that were made during the incongruent object discrimination task, sessions were classified as perseverative (where

responding to the previously-correct stimulus was significantly above chance), chance, or learning (where responding to the newly-correct stimulus was at, or above chance respectively). Signal detection theory (Macmillan & Creelman, 2005) was used to establish subjects' ability to discriminate correct from incorrect stimuli independently of any side bias that might have been present. The discrimination measure d′ and the bias measure *c* were calculated and the normal cumulative distribution function (CDF) compared to the criterion values of a two-tailed Z test (each tail $p = 0.05$) to determine the classification of each session (perseveration, chance or learning). Sessions where CDF(d′) < 0.05 were classified as perseverative; sessions where CDF(d′) > 0.95 were classified as learning, and sessions where 0.05 ≤ CDF(d′) ≤ 0.95 were classified as chance. Sessions where CDF(c) < 0.025 or CDF(c) > 0.975 were considered biased, but were not excluded as d′ is still a valid measure of discrimination. Correspondingly, the errors in each session were labelled as perseverative or non-perseverative (chance or learning).

The number of errors to two correct responses, the total number of errors before reaching the discrimination criterion and the number of perseverative / non-perseverative errors were compared between the 'passed' and 'failed' groups identified in the aversive discrimination paradigm by using Student *t*-test, Mann-Whitney U test (for nonparametric data) and a two-way factorial ANOVA. In addition, the relationship between these flexibility measures and the two predictive measures derived from the early sessions of the aversive discrimination paradigm (vigilant behaviour to CS's in session 1-3 and baseline BP in sessions 7-9) was examined with Pearson's correlation analysis which was followed up with a multiple regression analysis. The assumption of normality was checked by Kolmogorov-Sminov test and Shapiro-Wilk test. The homogeneity of variance and sphericity were tested by Leven's test and Mauchly's test respectively. The data satisfied all assumptions, otherwise noted.

## 4.2.2 Results

### 4.2.2.1 Comparison of the 'Passed' and 'Failed' Groups during Preliminary Training

Two pairs of object discrimination training were carried out. One of the objects used in the first discrimination was identical to the one introduced during habituation while the objects used in the second discrimination were both completely novel to the animals; therefore, the performance in the second discrimination was analysed as a measure of their general discrimination learning ability. All animals learnt to discriminate the objects and there was no significant difference in the performance of the groups. Both normality and homogeneity of variance were violated; therefore, nonparametric Mann-Whitney U test was performed comparing the two groups on the number of errors taken to reach the criterion. The analysis returned no significant difference [$U$=15.00, $z$=-0.86, $p$=0.389] between the groups ('passed' mean: 38.14, SE: 20.58; 'failed' mean: 10.67, SE: 4.69). The apparent difference in the mean between the two groups was due to two animals in the 'passed' group making particularly high numbers of errors (151 and 66). All other animals across both groups made fewer than 30 errors. Since the objects used in the training were incentive-neutral, no prepotent response was evident; therefore, a perseverative measure was not analysed.

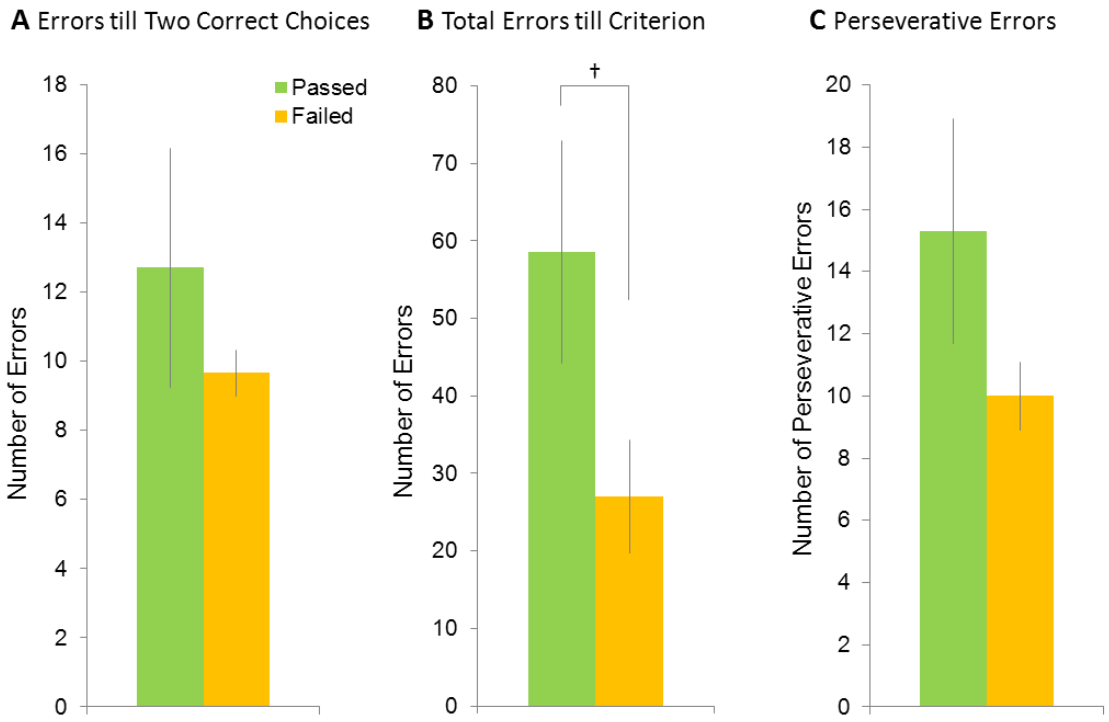



Figure 4.4 Performance on the incongruent object discrimination task of the animals in the 'passed' group (n=7, green bar) and the 'failed' group (n=6, yellow bar) as identified in the aversive discrimination test. (A) The number of errors until two correct choices were made, (B) the total number of errors before reaching the discrimination criterion and (C) the number of perseverative errors before reaching the discrimination criterion. Error bars show the standard error for each group. $p<.10$†

#### 4.2.2.2 Comparison of the 'Passed' and 'Failed' Group on Performance of the Incongruent Object Discrimination Task

All animals showed an initial prepotent response tendency to select the high incentive (marshmallow) food object (Figure 4.4 A), making at least three errors before performing two correct responses to the low incentive food object. However, this measure did not detect any group difference in the leaning to inhibit this prepotent response tendency. A Student *t*-test comparing the groups ('passed' mean: 12.71, SE: 3.46; 'failed' mean: 9.67, SE: 0.67) returned no significant group effect [$t(11)=0.80$, $p=0.440$]. The total number of errors to reach the discrimination criterion returned a trend in the group difference [$t(11)=1.87$, $p=0.089$]. Interestingly, this was due to the animals in the 'failed' group making fewer errors (mean: 27.00, SE: 7.26) than those in the 'passed' group (mean: 58.57, SE: 14.31) (Figure 4.4 B).

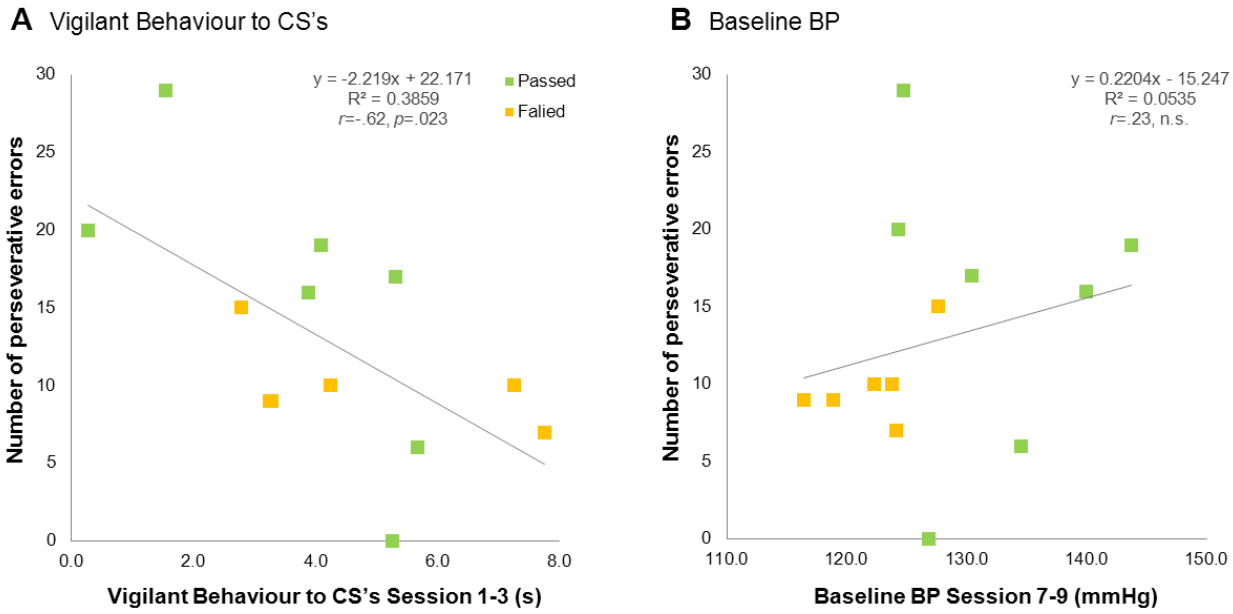



Figure 4.5 Correlations between the number of perseverative errors in the incongruent object discrimination task and the predictors of aversive discriminative conditioning: (A) the vigilant behaviour to CS's in session 1-3 and (B) the baseline BP in session 7-9.

However, the two groups did not differ significantly in the number of perseverative errors (Figure 4.4 C), the measure that has been shown to be affected by orbitofrontal lesions (M. S. Man et al., 2009). A two-way factorial ANOVA comparing the 'passed' and 'failed' groups across perseverative and the non-perseverative errors returned neither a significant group × error type interaction [$F(1,11)=1.75$, $p=0.212$] nor a main effect of group [$F(1,11)=3.36$, $p=0.094$].

#### 4.2.2.3 Relationship of the Performance on the Incongruent Object Discrimination Task with the Predictors of Aversive Discriminative Conditioning

In order to further investigate whether there was any relationship between performance on the incongruent object discrimination task and the predictor responses measured in the aversive discrimination paradigm, correlation analyses were performed between the error measures mentioned above and the two predictors of aversive discriminative conditioning (vigilant behaviour to CS's in session 1-3 and baseline BP in sessions 7-9, section 2.2.2.2). No significant correlation was found between the number of errors until two correct choices and either of the predictors [vigilant behaviour to CS's, Pearson's $r=-0.41$, $p=0.163$; baseline BP, $r=0.26$, $p=0.394$]. The total number of errors to reach the discrimination criterion also did not return any significant correlation with either measures [vigilant behaviour to CS's,

Table 4.1 Regression coefficients B, standard errors of B, standardized coefficients β and results of *t*-test for the initial model (Step 1) and the final model (Step 2) predicting the number of perseverative errors in the incongruent object discrimination task from the predictors of aversive discriminative conditioning: the vigilant behaviour to CS's in session 1-3 and the baseline BP in session 7-9. Note that the vigilant behaviour to CS's was retained in the significant final model whereas the baseline BP was removed.

| | Retained predictor | B | Standard Error B | β | *t* | *p* |
|---|---|---|---|---|---|---|
| Step 1 | | | | | | |
| | Constant | -12.55 | 28.05 | | -0.45 | .664 |
| | Vigilant Behaviour to CS's Sessions 1-3 | -2.31 | 0.83 | -.65 | -2.80 | .019 |
| | Baseline BP Sessions 7-9 | 0.275 | 0.22 | .29 | 1.25 | .240 |
| Step 2 | | | | | | |
| | Constant | 22.17 | 3.93 | | 5.64 | .000 |
| | Vigilant Behaviour to CS's Sessions 1-3 | -2.22 | 0.84 | -.62 | -2.63 | .023 |

*Note*: $R^2$=.47, $F(2,10)$=4.41, $p$=.042 for Step 1; $R^2$=.39, $F(2,10)$=6.91, $p$=.023 for Step 2.

Pearson's $r$=-0.31, $p$=0.303; baseline BP, $r$=0.22, $p$=0.480]. However, there was a significant negative correlation between the number of perseverative errors and the vigilant behaviour to CS's [$r$=-0.62, $p$=0.023] (Figure 4.5 A). The animals that displayed a heightened vigilant response to the CS's in the early sessions of the aversive discrimination paradigm made fewer perseverative errors in the incongruent object discrimination task. With the baseline BP, however, no significant correlation was detected [$r$=0.23, $p$=0.447] (Figure 4.5 B). These findings were supported by subsequent multiple regression analyses which tested whether the two predictors of the aversive discrimination conditioning would also predict the outcome of the incongruent object discrimination performance. As in the regression analysis of the rubber snake test outcome, the backward method was applied (section 3.2.3.5). When the total number of errors to criterion or the number of errors till two correct choices was placed in the analysis as its outcome variable, it failed to produce a significant final model, that is, both variables (vigilant behaviour to CS's and baseline BP) were removed from the model as they were not reliable predictors of the outcome. However, with the number of perseverative errors as the outcome, the analysis reached a significant final model [$F$(1,12)=6.91, $p$=0.023]

with the vigilant behaviour to CS's retained in the model (Table 4.1). The negative regression coefficient B indicates that as the score of the vigilant behaviour to CS's increases by one, the number of perseverative errors is predicted to decrease by 2.22, suggesting that if an animal is seen to be highly vigilant in the aversive discrimination paradigm, this animal is expected to make fewer perseverative errors in the OFC-sensitive incongruent object discrimination task compared to an animal that does not show such vigilance.

#### 4.2.2.4 Correlation with the 'Emotionality' and 'Coping Strategy' Components of the Human Intruder Test and the Rubber Snake Test

Having found an association between cognitive performance and the predictors of failure on the aversive discrimination task, a possible relationship was also sought between the error measures in the incongruent object discrimination task and the psychological components identified in the human intruder test (section 3.2) and the rubber snake test (section 3.3). The total number of errors to reach criterion, the number of errors till two correct choices and the number of perseverative errors were analysed for any correlation with the 'emotionality' and 'coping strategy' components of both human intruder and rubber snake tests. However, no significant correlation was found between any of the error measures and these components (Table 4.2).

Table 4.2 Correlation coefficients and *p* values derived from the Person's correlation analysis between the three error measures (the number of errors till two correct choices made, the total number of errors before reaching criterion and the number of perseverative errors) in the incongruent object discrimination task and the principal components ('emotionality' and 'coping strategy') identified in the human intruder test and the rubber snake test. None of the correlations were significant.

| | | Incongruent object discrimination task | | |
|---|---|---|---|---|
| | | Errors till two correct choices | Total errors till criterion | Perseverative errors |
| Human intruder test | 'Emotionality' | -.23 (.447) | .33 (.269) | .07 (.831) |
| | 'Coping strategy' | .31 (.306) | -.48 (.097) | -.19 (.529) |
| Rubber snake test | 'Emotionality' | -.27 (.376) | -.28 (.355) | -.38 (.204) |
| | 'Coping strategy' | .28 (.351) | -.34 (.254) | .11 (.733) |

*Note*: (*p* value).

# 4.3 Detour Reaching Rule Transfer Test

## 4.3.1 Methods and Materials

### 4.3.1.1 Subjects

All animals, as described in 4.1.2.1, went on to receive the detour reaching rule transfer task (Table 2.1), at least three weeks after performing the incongruent object discrimination test.

### 4.3.1.2 Test Apparatus

***Wisconsin General Test Apparatus*** The WGTA used in the incongruent discrimination task (section 4.2.1.2) was further modified for the detour reaching task (Figure 4.6). Except for the modifications described here, all apparatus accessories stayed the same. In the modified version, instead of bars separating the subject and stimulus compartments, a transparent Perspex screen (20 cm high, 26 cm wide) with a gap (2.5 cm high, 22 cm wide) in the middle and an opaque aluminium plate (9.0 cm high. 26 cm wide) lay between the compartments. An animal could reach through the gap in the transparent screen to a clear Perspex platform (8.5 cm high, 30 cm wide, 24 cm deep) within the stimulus compartment. A small rectangular Perspex box (6.0 cm high, 7.0 cm wide. 5.0 cm deep, Figure 4.7), in which a food reward (half a piece of marshmallow) was placed, could be attached to the platform (1.5 cm from the edge of the subject compartment) with patches of industrial Velcro hook-and-loop fastener. Three of the box sides (front, left and right) acted as doors that were attached to the box frame and could either be locked into position or alternatively, allowed to hang free such that an animal could push the door open and access the food from within. To lock the door, a pin was inserted through the box's ceiling into the top of the door, holding it in place. A fake pin was used for the unlocked door so that the presence of pin did not indicate whether a door was locked or unlocked. The side facing the experimenter was left open for the placement of the reward and the viewing of the animal's reaching action. There were two versions of the box, black and opaque and translucent that were used in the task, described in section 4.3.1.3 'Behavioural Procedure'. As in the incongruent object discrimination paradigm, the animal was placed in the carry box which was fitted into the subject compartment. A trial commenced and was terminated, respectively, with the opening and closure of the opaque screen inserted between the compartments. Testing took place in a darkened, sound attenuated room.

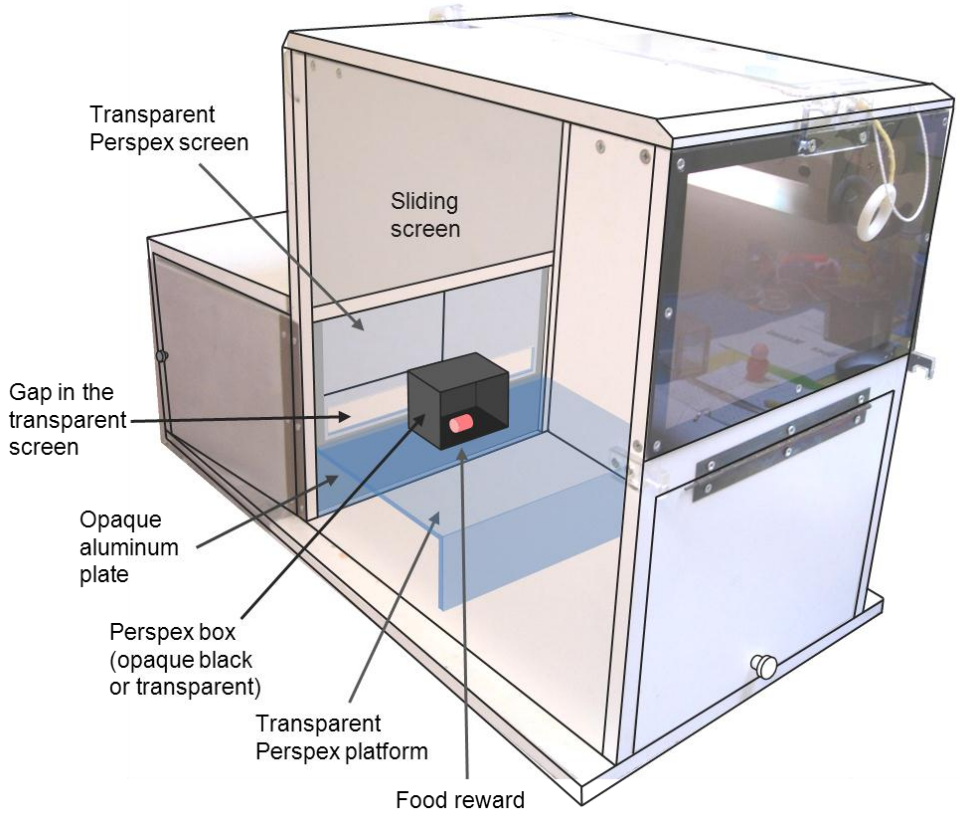



Figure 4.6 A photograph of the modified hand-operated Wisconsin General Test Apparatus used for the detour reach rule transfer task, with superimposed schematic illustration of its interior. The frame of the apparatus is the same as described in Figure 4.1.

#### 4.3.1.3 Behavioural Procedure

***Habituation to Apparatus*** On the first day of habituation, five pieces of marshmallow were placed on the platform. Upon removal of the carry box door and the opening of the opaque screen separating the compartments, the animal could view and reach for the marshmallows. The time that the animal took to retrieve all five pieces was recorded as well as which hand the animal preferred. All animals received two habituation sessions. In the second session, they managed to retrieve all five pieces of marshmallow within one minute. The preferred hand (left or right) was determined as the one that was used more often than the other.

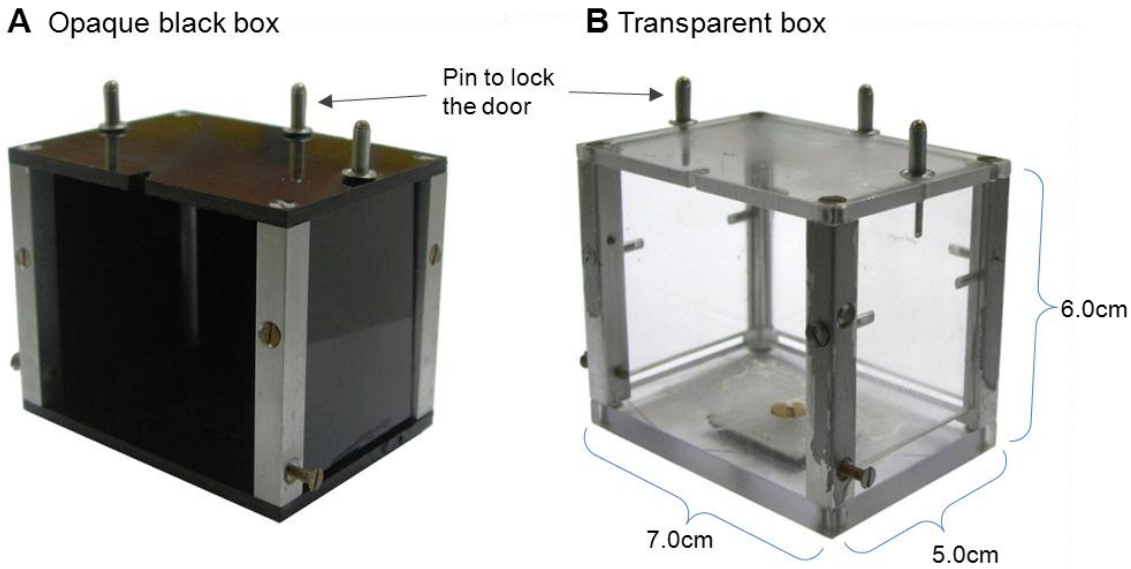



Figure 4.7. Photographs and dimensions of the rectangular Perspex boxes, inside which the food reward was placed. (A) Opaque black version used in the detour reach training and (B) transparent version used in the detour reach rule transfer test.

***Preliminary Training*** Training was divided into three stages. The black opaque Perspex box (Figure 4.7 A; dimension described in section 4.3.1.2) was used throughout the training stages. In the first stage, the animal was trained to reach into the box. For the first five trials, the front door of the box was removed so that the animal could see the food reward (a piece of marshmallow) placed inside. Once the animal was successfully reaching through the front to obtain the reward, the open side was switched to the side of the animal's preferred hand. The box was initially positioned so that the opening was at 45˚ to the animal. A piece of marshmallow was at first placed so that it was sticking out of the opening. When the animal was able to retrieve the reward from this position, the reward was moved further into the box on subsequent trials, first, just 1 cm from the opening, and then to the centre of the box. After four consecutive successful trials with the reward in the centre, the box was positioned so that the opening was at 90˚ to the animal. Thus, in order to obtain the reward, the animal was required to make a reach into the box through the side opening without having a view of the reward (detour reach). After four successful trials, the opening was switched to the un-preferred side and the procedure was repeated. Lastly, at the end of the session the animal was re-tested with the preferred side open, to ensure that the animal had not forgotten how to detour-reach for the reward through the preferred side. Once the animal became competent in making the detour reach through the open sides, they were moved to the second training stage. In this second stage, the doors were attached to the box. First, the

Table 4.3 Summary of the training protocol for stage 1 and stage 2, using the opaque black box.

| **Training stage 1: One of the doors detached** | | | |
|---|---|---|---|
| Position of open side | Angle of the box | Position of the reward | Number of successful trials required |
| Front | 0° | Centre of box | 5 |
| Preferred | 45° | Partly out | 2 |
| Preferred | 45° | 1cm inside | 2 |
| Preferred | 45° | Centre of box | 4 |
| Preferred | 90° | Partly out | 2 |
| Preferred | 90° | 1cm inside | 2 |
| Preferred | 90° | Centre of box | 4 |
| Unpreferred | 45° | Partly out | 2 |
| Unpreferred | 45° | 1cm inside | 2 |
| Unpreferred | 45° | Centre of box | 4 |
| Unpreferred | 90° | Partly out | 2 |
| Unpreferred | 90° | 1cm inside | 2 |
| Unpreferred | 90° | Centre of box | 4 |
| Preferred | 90° | Centre of box | 5 |

| **Training stage 2: One of the doors unlocked** | | | |
|---|---|---|---|
| Position of unlocked side | Door | Position of the reward | Number of successful trials required |
| Front | Partially held open | Partly out | 2 |
| Front | Partially held open | Centre of box | 2 |
| Front | Closed | Centre of box | 5 |
| Preferred | Partially held open | Partly out | 2 |
| Preferred | Partially held open | Centre of box | 2 |
| Preferred | Closed | Centre of box | 3 |
| Unpreferred | Partially held open | Partly out | 2 |
| Unpreferred | Partially held open | Centre of box | 2 |
| Unpreferred | Closed | Centre of box | 3 |
| Preferred | Closed | Centre of box | 3 |

front door was unlocked whilst the side doors were locked. During these trials, the door was initially partially held open and the reward was placed in the gap between the door and the floor of the box. After successful reward retrieval, the reward was moved to the centre of the box and the door was closed (but unlocked). The unlocked door could easily be nudged open in the process of reaching into the box. After five successful trials with the front door unlocked, the unlocked side was switched to the animal's preferred hand side. The above procedure (i.e. the door partially open → door closed) was repeated until the animal made three successful trials with the door closed; then, the door was switched to the un-preferred hand side and the procedure was repeated. Lastly, the preferred hand side was re-tested. The summary of the first and second training stages is given in Table 4.3.

***Over-training on Detour Reach Rule*** The third training stage was conducted to ensure that all animals mastered the detour reach rule, that is, the left and right detour reaches became as reinforced as the direct reach through the front door. A daily 20-min session consisted of 21 trials with the position of the unlocked door (seven trials each for front, left or right door) varying from trial to trial in a pseudorandom manner. Since the animal could not predict which door was unlocked and could not identify it by visual inspection, in order to reach into

the box and retrieve the reward placed in the centre of the box, the animal was required to touch and check each door in turn until the unlocked door was located. A successful trial was defined as when each side of the box was checked once and once only until the unlocked door was found. Therefore, a maximum of three touches (e.g. locked-front → locked-left → unlocked-right) was allowed in any successful trial. If the animal re-tried a locked door that had already been checked on that trial, the trial was terminated and deemed unsuccessful. Also, if the animal did not make any attempt to retrieve the reward for 20 seconds, the trial was terminated. The number of successful trials in a session was determined and provided a strategy score. Criterion for mastering the detour reach rule was set when an animal achieved a strategy score of 16 or higher in four consecutive sessions (Wallis et al., 2001). Once the criterion was fulfilled, the animal was considered to have successfully acquired the detour reach rule and progressed to the detour reach rule transfer test. All sessions were video-recorded.

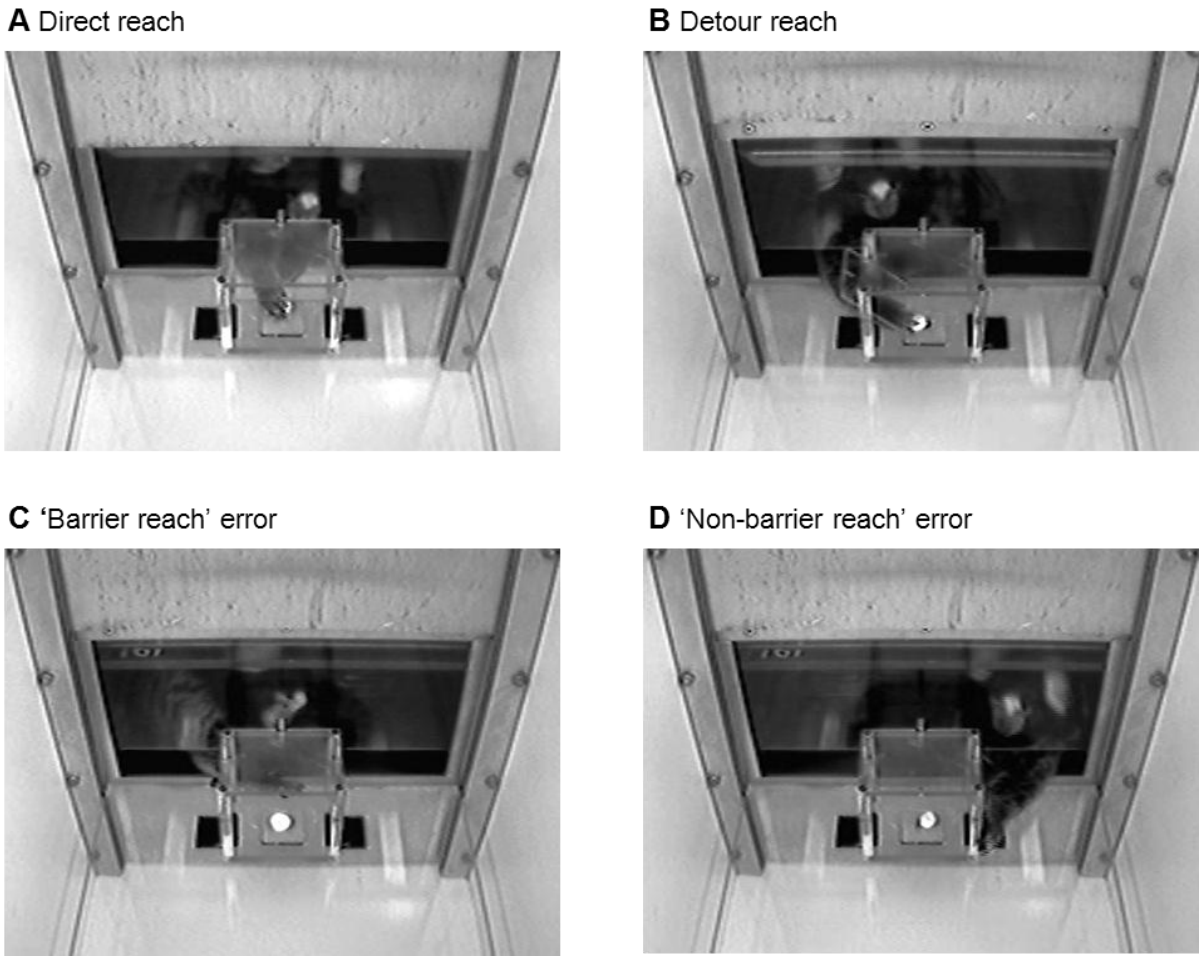



Figure 4.8 Snapshots of the animal making (A) direct reach through the unlocked front door, (B) detour reach through the unlocked left door, (C) 'barrier reach' to the locked front door and (D) 'non-barrier' reach to the locked right door, during the test session with the transparent box.

***Detour Reach Rule Transfer Test*** This paradigm was identical to the training except that the black opaque box was replaced by a transparent box (Figure 4.7 B). Now, for the first time, the animal could 'see' the reward, placed in the centre of the box, along its direct line of sight, but it was required to make a detour reach when the unlocked door was on the left or right side of the box (Figure 4.8 B). When the unlocked door was on the front side, the animal made a direct reach (Figure 4.8 A). In a daily 20-min session of 21 trials, the position of the unlocked door (seven trials each for front, left or right door) was pseudorandamly selected and all animals received them in the same order. The criterion for successful rule transfer from the training condition was set as three consecutive sessions with a strategy score of 16

or higher. The animals were tested until they achieved the criterion. All sessions were video-recorded and each reach was scored subsequently.

#### 4.3.1.4 Data Acquisition and Analysis

***Behavioural Assessment*** For every trial, each reach was recorded. A discrete reach was defined as making contact with the door and then taking the hand away from the box (Wallis et al., 2001). In the training, the animal learnt to make one reach for each door until the unlocked door was located (detour reach rule). Error reach was registered when the animal made more than one reach to the locked doors of the box. For those error reaches, it was noted whether the reach was made along the direct line of sight (i.e. to the front door) which was assigned as 'barrier reach' (Figure 4.8 C), or it was an incorrect detour reach (i.e. to the left or right door) which was assigned as 'non-barrier' reach (Figure 4.8 D). The total number of error reaches was counted for the first and second sessions (Wallis et al., 2001) and these were categorized as 'barrier reach' or 'non-barrier reach'. The total number of trials and sessions the animal took to reach the criterion were also scored.

***Statistical Analysis*** Statistical analyses were performed using statistic software SPSS (version 17.0). Student *t*-test was used to compare the 'passed' and 'failed' groups (as identified in the aversive discrimination paradigm) in the number of trials, the number of sessions and the strategy scores during training and test sessions. A three-way factorial ANOVA was used to compare the groups on the number of 'barrier reach' and 'non-barrier reach' errors across the first and second test sessions. In addition, the relationship between these detour reach rule transfer error measures and the two predictive measures derived from the early sessions of the aversive discrimination paradigm (vigilant behaviour to CS's in session 1-3 and baseline BP in sessions 7-9) was examined with Pearson's correlation analysis which was followed up with a multiple regression analysis. The assumption of normality was checked by Kolmogorov-Sminov test and Shapiro-Wilk test. The homogeneity of variance and sphericity were tested by Leven's test and Mauchly's test, respectively. The data satisfied all assumptions, otherwise noted.

## 4.3.2 Results

### 4.3.2.1 Comparison of the 'Passed' and 'Failed' Groups during Preliminary Training

All animals successfully learnt to make a detour reach with the opaque black box. There was no significant difference between the 'passed' and 'failed' groups on the number of training trials required before the animals were able to successfully use either of the two opaque black boxes, that is either the box without doors (training stage 1), or the box with doors (training stage 2). Student *t*-tests comparing the number of trials between the groups returned no effect of group either in the training stage 1 [$t(11)=-0.71$, $p=0.492$] ('passed', mean: 54.71, SE: 4.21; 'failed', mean: 60.33. SE: 7.01) or in the training stage 2 [$t(11)=-0.80$, $p=0.442$] ('passed', mean: 57.00, SE: 8.13; 'failed', mean: 66.00, SE: 7.64).

### 4.3.2.2 Comparison of the 'Passed' and 'Failed' Groups on the Over-training on the Detour Reach Rule

All animals successfully acquired the detour reach rule. There was no significant difference in the total number of errors the groups made before reaching the criterion. A Student *t*-test comparing the total number of errors between the 'passed' and 'failed' groups returned no significant effect of group [$t(11)=-0.69$, $p=0.506$] ('passed', mean: 64.43, SE: 14.23; 'failed', mean: 92.17, SE: 40.51). In order to determine whether the groups differed in their ability to learn the strategy of checking each door in turn (detour reach rule), the average strategy score over the last four sessions was calculated. This score is the number of trials out of a maximum possible of 21, in which the animal reached to each side of the box once, and once only, until it found the unlocked door and successfully retrieved the reward. A Student *t*-test comparing the mean strategy score over the last four sessions between the groups returned no significant group effect [$t(11)=-0.07$, $p=0.943$] ('passed', mean: 18.00, SE: 0.39; 'failed', mean: 18.04, SE: 0.42). The two groups did not differ in the ability to acquire the detour reach rule.

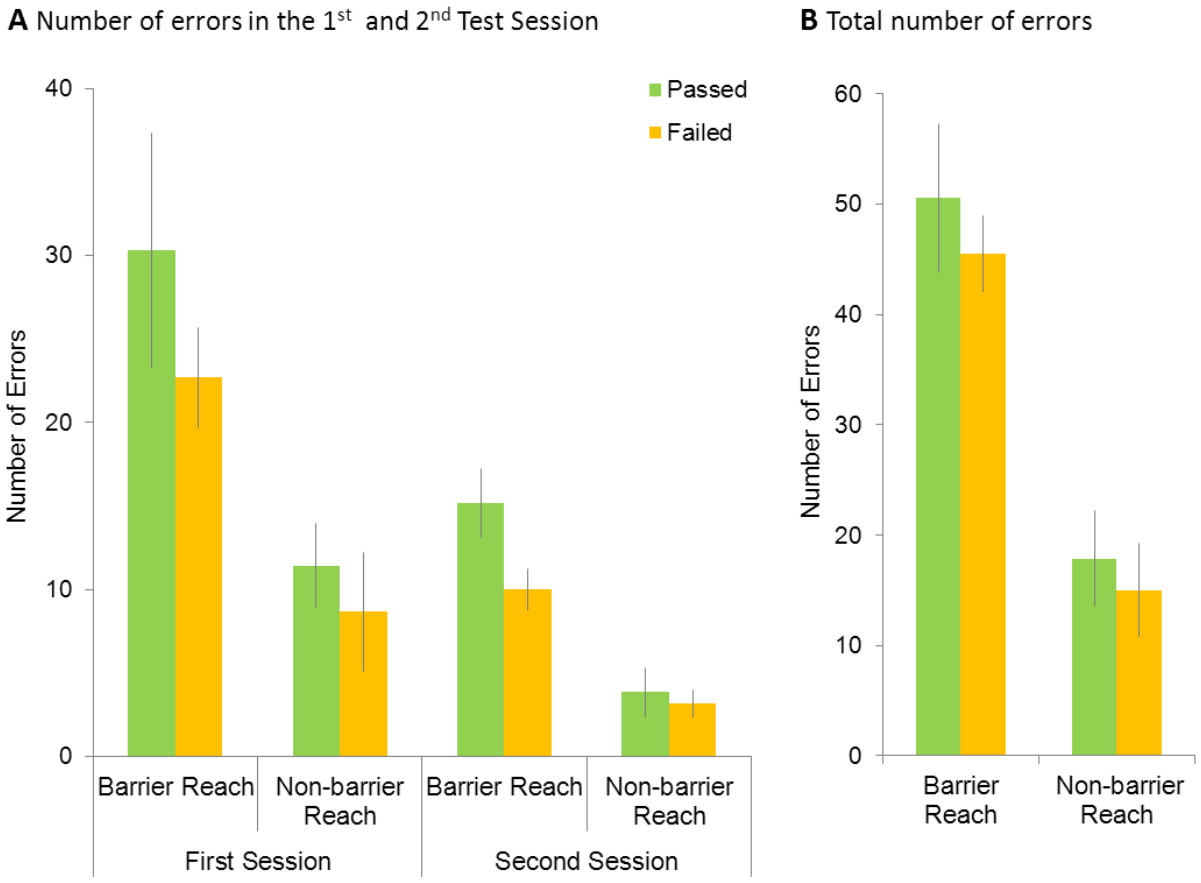



Figure 4.9 Performance on the detour reach rule transfer task of the animals in the 'passed' group (n=7, green bar) and the failed' group (n=6, yellow bar) as identified in the aversive discrimination test. (A) The number of 'barrier reach' and 'non-barrier reach' errors in the first test session, and in the second test session. (B) The total number of 'barrier reach' and 'non-barrier reach' errors until the animal reached the criterion. Error bars show the standard errors for each group.

#### 4.3.2.3 Comparison of the 'Passed' and 'Failed' Groups on Performance of the Detour Reach Rule Transfer Task

When the animals were transferred to the transparent box, all of them initially reached directly towards the now visible food reward before adopting the previously acquired detour reach rule. This tendency was particularly evident in the first and second test sessions. Therefore, the number of 'barrier reach' and 'non-barrier reach' errors from these sessions was compared between the 'passed' and 'failed' groups, similarly to that of Wallis et al. (2001) in which it was shown that those animals that received a lateral PFC excitotoxic lesion made significantly greater number of 'barrier reaches' than the controls. However, when the

'passed' and 'failed' groups were compared across the two sessions, no significant group difference was found on the number of 'barrier reaches' (Figure 4.9 A), despite the 'failed' group looking like they were adapting the detour reach rule quicker than the 'passed' group. A three-way factorial ANOVA comparing the two groups (between-subjects factor) on the number of 'barrier reach' and 'non-barrier reach' (within-subjects factor) across the first and second sessions (within-subjects factor) returned no main effect of group [$F(1,11)=2.31$, $p=0.157$], no significant group × error type ('barrier reach' / 'non-barrier reach') interaction [$F(1,11)=1.29$, $p=0.281$], nor significant group × error type × session interaction [$F(1,11)=0.00$, $p=0.954$]. There was a significant main effect of error type [$F(1,11)=38.55$, $p<0.000$], indicating that all the animals made greater 'barrier reach' than 'non-barrier reach' errors. Also, there was a significant main effect of session [$F(1,11)=11.54$, $p=0.006$], indicating that overall the animals made fewer errors in the second session than the first session. A trend in the error type × session interaction [$F(1,11)=4.64$, $p=0.054$] indicated that the difference between the numbers of 'barrier reach' and 'non-barrier reach' errors was greater in the second session than in the first session, suggesting that the second session might have been more sensitive to the different error types.

In addition to the number of 'barrier reach' and 'non-barrier reach' errors in the first and second sessions, the total number of these errors before the animals reached the criterion was compared between the 'passed' and 'failed' groups. No significant group difference was found in the total number of 'barrier reaches', in the total number of 'non-barrier reaches', nor in the total number of both error types (Figure 4.9 B). A two-way ANOVA comparing the two groups on the number of 'barrier reach' and 'non-barrier reach' errors returned neither significant main effect of group [$F(1,11)=0.50$, $p=0.494$], nor significant effect of group × error type interaction [$F(1,11)=0.06$, $p=0.814$]. There was a significant effect of error type [$F(1,11)=45.51$, $p<0.000$], indicating that overall the animal made more 'barrier reach' errors than 'non-barrier reach' errors.

In order to examine whether there was any difference between the 'passed' and 'failed' groups in their ability to re-acquire the detour reach rule with the transparent box, the number of sessions (each contained 21 trials) the animal took to regain criterion was compared between the groups. A Student *t*-test returned no significant effect of group in the number of sessions to reach the criterion [$t(11)=-1.63$, $p=0.131$] ('passed', mean: 2.43, SE: 0.48; 'failed', mean: 4.67, SE: 1.38). The animals in the two groups did not differ in the number of sessions before re-acquiring the detour reach rule.

#### 4.3.2.4 Relationship of the Performance on the Detour Reach Rule Transfer Task with the Predictors of Aversive Discriminative Conditioning

In order to further investigate whether there was any relationship between performance on the detour reach rule transfer task and the predictor responses measured in the aversive discrimination paradigm, correlation analyses were performed between the number of 'barrier reach' and 'non-barrier reach' errors in the first and second test sessions and the two predictors of aversive discriminative conditioning (vigilant behaviour to CS's in session 1-3 and baseline BP in sessions 7-9, section 2.2.2.2). No correlation was found between the number of 'barrier reaches' in the first session and either of the predictors [vigilant behaviour to CS's, Pearson's $r$=0.04, $p$=0.905; baseline BP, $r$=0.09, $p$=0.780]. Although no significant correlation was found between the number of 'barrier reaches' in the second session and the vigilant behaviour to CS's [$r$=0.18, $p$=0.551] (Figure 4.10 A), there was a significant positive correlation between the number of 'barrier reaches' in the second session and the baseline BP [$r$=0.55, $p$=0.050] (Figure 4.10 B). The animals that displayed higher baseline BP made greater number of 'barrier reaches', conversely the animals that showed suppressed baseline BP made fewer number of 'barrier reaches'. With regard to the number of 'non-barrier reach' errors in the first test session, no significant correlation was found with either predictors [vigilant behaviour to CS's, $r$=-0.21, $p$=0.487; baseline BP, $r$=0.16, $p$=0.608]. Likewise, no significant correlation was found between the number of 'non-barrier reach' errors in the second session and either predictors [vigilant behaviour to CS's, $r$=-0.38, $p$=0.195; baseline BP, $r$=0.08, $p$=0.790].

The relationship found between the number of 'barrier reach' errors and the baseline BP but not with the vigilant behaviour to CS's was supported by subsequent multiple regression analyses which tested whether the two predictors of aversive discriminative conditioning would also predict the performance outcome of the detour reach rule transfer task. As in the regression analysis of the incongruent object discrimination task performance with the predictors of aversive discriminative conditioning, the backward method was applied (section 4.2.2.3). When the number of 'barrier reaches' in the first test session was placed in the analysis as the outcome variable, the regression failed to produce a significant final model as it was not a reliable predictor of the outcome. However, with the number of 'barrier reach' in the second test session as the outcome, the analysis reached a significant final model [$F$(1,11)=4.85, $p$=0.050] with the baseline BP retained in the model (Table 4.4). The positive regression coefficient B indicates that as the baseline BP (mmHg) increases by one unit, the number of 'barrier reaches' is predicted to increase by 0.36, suggesting that if an animal is seen to display reduced blood pressure during the baseline in the aversive discrimination

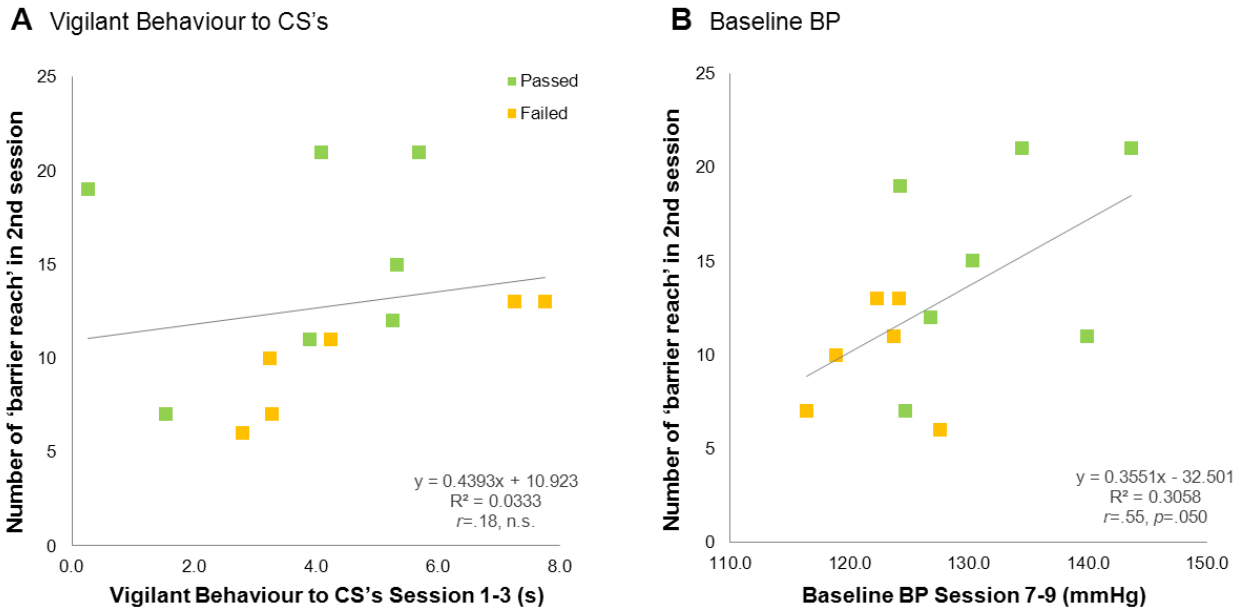



Figure 4.10 Correlations between the number of 'barrier reach' errors in the second test session of the detour reach rule transfer task and the predictors of aversive discriminative conditioning: (A) the vigilant behaviour to CS's in session 1-3 and (B) the baseline BP in session 7-9.

paradigm, this animal is expected to commit less 'barrier reach' errors in the lateral PFC-sensitive detour reach rule transfer task compared to an animal that shows no such blood pressure suppression.

Table 4.4 Regression coefficients B, standard errors of B, standardized coefficients β and results of *t*-test for the initial model (Step 1) and the final model (Step 2) predicting the number of 'barrier reach' errors in the second test session of the detour reach rule transfer task from the predictors of aversive discriminative conditioning: the vigilant behaviour to CS's in session 1-3 and the baseline BP in session 7-9. Note that the baseline BP was retained in the significant final model whereas the vigilant behaviour to CS's was removed.

| | Retained predictor | B | Standard Error B | β | *t* | *p* |
|---|---|---|---|---|---|---|
| Step 1 | | | | | | |
| | Constant | -32.88 | 21.34 | | -1.54 | .154 |
| | Vigilant Behaviour to CS's Sessions 1-3 | 0.32 | 0.63 | .13 | 0.51 | .619 |
| | Baseline BP Sessions 7-9 | 0.35 | 0.17 | .54 | 2.07 | .065 |
| Step 2 | | | | | | |
| | Constant | -32.50 | 20.60 | | -1.58 | .143 |
| | Baseline BP Sessions 7-9 | 0.36 | 0.16 | .55 | 2.20 | .050 |

*Note*: $R^2$=.32, $F(2,10)$=2.39, $p$=.142 for Step 1; $R^2$=.30, $F(2,10)$=4.85, $p$=.050 for Step 2.

#### 4.3.2.5 Correlation with the 'Emotionality' and 'Coping Strategy' Components of the Human Intruder Test and the Rubber Snake Test

Having found an association between cognitive performance and the predictors of aversive discriminative Pavlovian conditioning, a possible relationship was also sought between the 'barrier reach' error measure in the detour reach rule transfer task and the psychological components identified in the human intruder test (section 3.2) and the rubber snake test (section 3.3). The numbers of 'barrier reach' errors in the first test session and the second session were analysed for any correlation with the 'emotionality' and 'coping strategy' components of both human intruder and rubber snake tests. However, no significant correlation was found between the 'barrier reach' error measures and these components (Table 4.5).

Table 4.5 Correlation coefficients and *p* values derived from the Person's correlation analysis between the number of 'barrier reach' errors in the first and second sessions of the detour reach rule transfer task and the principal components ('emotionality' and 'coping strategy') identified in the human intruder test and the rubber snake test. None of the correlations were significant.

| | | Detour reach rule transfer task | |
|---|---|---|---|
| | | 'Barrier reach' error in 1st session | 'Barrier reach' error in 2nd session |
| Human intruder test | 'Emotionality' | .00 (.994) | -.07 (.833) |
| | 'Coping strategy' | -.14 (.646) | .37 (.215) |
| Rubber snake test | 'Emotionality' | -.32 (.288) | -.45 (.124) |
| | 'Coping strategy' | -.17 (.582) | -.35 (.241) |

*Note*: (*p* value).

## 4.4 Discussion

Marmosets defined as either high or low in trait anxiety, based on their performance on an aversive discriminative conditioning (Chapter 2) and rubber snake test (Chapter 3) were compared on two different cognitive flexibility tasks; an OFC-dependent incongruent object discrimination task and a lPFC-dependent detour reaching rule transfer task. Previously, lesions of the OFC (M. S. Man et al., 2009) and lPFC (Wallis et al., 2001) in marmosets had been shown to increase the number of perseverative errors on one or other of these tests. Whilst the overall mean of perseverative errors in the 'failed' group was lower than that of the 'passed' group, this did not reach significance. However, when the two predictors of passing or failing the aversive discrimination, namely the vigilant behaviour to CS's and the baseline BP during the early sessions, were correlated with an individual's perseverative errors on the two tests, significant relationships were found. Specifically, there was a significant negative correlation between the perseverative measure on the OFC-dependent test and the vigilant behaviour to CS's and a significant positive relationship between the number of 'barrier reach' errors on the lPFC-dependent test and baseline BP. The animals that displayed enhanced vigilant scanning to CS's in the early sessions of the aversive discrimination were less perseverative on the OFC-dependent test than those that had shown less vigilance. In contrast, the animals that had displayed suppressed baseline BP made fewer 'barrier reach' errors on the lPFC-dependent test and thus were less perseverative than those whose BP had not been suppressed. Given that the heightened vigilant behaviour to CS's and the reduced baseline BP may be biological markers of high trait anxiety, the findings imply that those marmosets that were potentially higher in trait anxiety were better performers on the OFC- and lPFC- dependent cognitive flexibility tests, respectively, in comparison to those that were low trait-anxious. It should be noted though that heightened vigilant behaviour to the CS did not correlate with the lPFC-dependent test and vice versa for the reduction in baseline BP. Moreover, subsequent multiple regression analysis revealed that only vigilant behaviour to the CS's and not baseline BP was retained as a significant predictor of the animal's perseveration on the OFC-dependent test and only reductions in baseline BP and not CS vigilance, was retained as a significant predictor of the animals perseveration on the lPFC dependent test.

Overall, the current findings suggest that the high trait-anxious individuals displayed superior prefrontal-dependent cognitive flexibility. The results also indicate that the functions of the OFC and lPFC are dissociably associated with, or even predicted by, each of the two

proposed biomarkers of enhanced trait anxiety in common marmosets. Whilst the heightened vigilant behaviour to CS cues predicted less perseveration or better inhibition of pre-potent responses in the OFC-dependent cognitive flexibility task, the suppressed baseline BP predicted less perseveration or more rapid rule transfer from training to the new situation in the lPFC-dependent cognitive flexibility task. Together these results suggest that the individual differences in cue driven vigilant behaviour may be related to alterations in OFC-related circuitry whereas individual differences in the baseline-related cardiovascular response may be related to alterations in lPFC-related circuitry.

**Significance of the correlations between the physiological predictors and perseverative performance**

According to multiple regression analysis, the two physiological predictors of discrimination failure (heightened vigilant behaviour to cues and suppressed baseline BP) together significantly predicted the 'failure' to discriminate. Each of these measures ($p$=0.070 and 0.049 respectively, refer to Chapter 2) also substantially contributed to the prediction. This suggests that the two measures differ between animals and are somewhat independent of one another. The finding in the present chapter that the two biomarkers were also differentially correlated with performance on the OFC- and lPFC-dependent cognitive flexibility tests strengthens this hypothesis. Moreover, the latter also suggests that these biomarkers may be associated with the functioning of two distinct neural circuits, one involving the lPFC and the other, the OFC. If so, this may explain why there was no overall difference between the 'passed' and 'failed' groups because the high trait anxious group is made up of two phenotypes or dimensions, and not one, that are differentially related to one or other of the prefrontal regions, but not both. This issue will be discussed further in the next section. The other possibility as to why the 'passed' and 'failed' groups did not differ significantly from one another may have been due simply to the relatively small sample sizes, thereby weakening the statistical model. Whilst 27 animals were analysed for the aversive discriminative conditioning (20 'passed' and 7 'failed'; detail in Chapter 2), only 13 of them were used for the present cognitive flexibility study, the rest being used in a lesion study that is not reported here (Agustín-Pavón et al., 2012).

The finding that the behavioural and autonomic predictors of enhanced trait anxiety were associated independently with two distinct prefrontal circuits is of considerable interest. Firstly, when considering the enhanced vigilant response to cues, it has been proposed that the amygdala is the central structure mediating cue-specific phasic fear responses in fear

conditioning (Michael Davis et al., 2010; Indovina et al., 2011; J. LeDoux, 2000); therefore, the enhanced vigilance to cues may reflect a hyper-responsive amygdala. The amygdala sends robust bidirectional projections to the OFC, through which they influence each other's functionality (R. J. Davidson, 2002; Ghashghaei & Barbas, 2002; Roberts, Tomic, et al., 2007). The OFC has been shown to both up-regulate (Izquierdo et al., 2005; Kalin et al., 2007; Machado & Bachevalier, 2008) and down-regulate (Agustín-Pavón et al., 2012; Gottfried & Dolan, 2004; M. S. Man et al., 2009) stimulus-driven amygdala responsivity. Thus, changes in the connectivity between the OFC and amygdala may underlie the enhanced vigilant scanning in the high trait anxious animals. Such changes could be brought about by individual differences in genotype or exposure to environmental stressors or an interaction of the two. Certainly, it has been shown that repeated exposures to stressful events cause morphological changes in both the amygdala and the OFC, i.e. significant enhancements in dendritic arborisation primarily in pyramidal and stellate neurons (Liston et al., 2006; Vyas, Mitra, Shankaranarayana Rao, & Chattarji, 2002). These structural changes may reflect increased reactivity of the amygdala in response to adverse events and the OFC's compensatory efforts to down-regulate the amygdala responses. How this morphological change might affect cognitive flexibility and other executive functions subserved by the OFC is unclear. But, from the finding that stress-induced structural changes in the mPFC are associated with altered attentional set-shifting ability (Liston et al., 2006), it can be speculated that the increased dendritic length in the OFC may underlie the improved cognitive performances displayed by those anxious individuals under non-stressful conditions. In contrast with the strong amygdala-OFC connection, the amygdala does not directly project to the lPFC (Ghashghaei & Barbas, 2002), therefore both functionally and physiologically the lPFC may not be as strongly affected by the neural activities in the amygdala. Hence, no significant correlation was detected between the enhanced vigilant behaviour to cues and the lPFC dependent cognitive perseveration measure.

In contrast, the suppressed BP response was observed during the baseline during which no explicit cue was present. Such alterations during the baseline have been interpreted as an anxiety-related response acquired to the background context (Grillon, 2002b). Contextual fear conditioning is dependent upon the hippocampus, which has been shown to play an important role in the integration of background information and the subsequent transmission of the information to the amygdala for the acquisition and expression of fear responses (Otto & Poon, 2006; Phillips & LeDoux, 1992). The hippocampus has also been implicated in the functioning of the central executive in concert with the PFC through reciprocal connections (Goldman-Rakic, Selemon, & Schwartz, 1984). Especially relevant to the current study is the

involvement of the hippocampus in set-shifting. i.e. the ability to alter a behavioural response mode in the face of changing contingencies (Monchi, Petrides, Petre, Worsley, & Dagher, 2001). Neuroimaging studies using a Wisconsin card sorting task, a traditional test for set-shifting ability which requires subjects to sort cards using a rule that changes over time, demonstrated an increased hippocampal activation when subjects established the identity of the rule and a decreased hippocampal activation during the generation of new rule, suggesting that through a reciprocal communication with the dorsolateral PFC, the hippocampus may play a role in updating contingency memory (Berman, Ostrem, & Randolph, 1995; Graham et al., 2009). A rodent study also showed that neonatal removal of the ventral hippocampus caused an impairment on a set-shifting task resulting in the lesioned animals making significantly greater number of perseverative errors than the controls (Brady, 2009; Brooks et al., 2012). The authors suggested that the disconnection of hippocampus-PFC pathway caused abnormal functioning of the PFC network that led to an inability to suppress a previously learned rule. These findings indicate the functional involvement of the hippocampus in the processing of contextual information and updating contingency rules, both of which were required in the lPFC dependent detour-reaching rule transfer task. Therefore, if the hippocampus, via the functional connectivity with the lPFC, was involved in these operations, it is plausible that those animals that showed increased responsivity to the context in aversive discriminative conditioning, also showed improved performances on the rule transfer task. In contrast, the OFC dependent object discrimination task required the animals to focus on the cues rather than the context which did not require much hippocampal involvement; therefore, no correlation was found with its performance and the baseline BP. Together with the interpretation of the significant correlation between the vigilance to cues and the OFC-dependent cognitive flexibility task, it can be speculated that the cue sensitive amygdala-OFC circuit and the context sensitive hippocampus-lPFC circuit independently contribute to trait anxiety. This is further supported by recent findings from my laboratory that excitotoxic lesions of either the antOFC or lPFC enhance anxiety responses on the human intruder (Agustín-Pavón et al., 2012) and the snake test (unpublished findings) demonstrating that dysfunctioning in either the lPFC and antOFC contributes independently to the regulation of anxiety responses.

**Why did high trait-anxious animals exhibit fewer perseverative errors than lower trait-anxious animals on prefrontal dependent cognitive flexibility tasks?**

Two predictions regarding the relationship between trait anxiety and prefrontal functioning have been made. The first is that high trait anxiety is associated with an increase in

prefrontal top-down control mechanisms. Thus, when placed on a task dependent upon prefrontal function, high trait anxious subjects may display improved cognitive performance. This prediction is based on an influential theoretical account of the anxiety-cognition interplay, the attentional control theory (M. Eysenck et al., 2007). This theory postulates that external, potentially threatening stimuli or internal worrying thoughts activate a stimulus-driven attentional system that preferentially allocates attentional resources to detecting the source of threat and deciding how to respond. In addition, there is a counteracting goal-directed attentional system that directs attentional focus to targets relevant to the on-going cognitive task. The two systems bi-directionally influence each other to allocate attentional resources for adaptive behaviour; however, anxiety disrupts the balance between them. Anxiety prioritizes the stimulus-driven attentional system over the goal-directed attentional control system, causing interference to executive functioning from task-irrelevant anxiety-related stimuli (M. Calvo & Eysenck, 1998). At the same time anxiety/worry has a motivational function which increases effort to minimize the aversive anxiety state and maintain cognitive performance (M. Eysenck et al., 2007; Visu-Petra et al., 2012). This motivation is reflected as a compensatory effort exerted by the goal-directed attentional system to focus attention on task-relevant information. The goal-directed attentional system includes the focus, allocation and maintenance of attention that are key features of the central executive and is centred around the prefrontal regions (Dolcos & McCarthy, 2006). This could account for the increased prefrontal activation in individuals high in trait anxiety performing emotional-cognitive tasks (Eisenberger et al., 2005; Telzer et al., 2008). Proponents of the attentional control theory further predict that on a cognitive task that does not involve threatening stimuli or invoke worrying thoughts, without the pressure from the stimulus-driven attentional system, the "hard-working" PFC in trait-anxious individuals may exercise superior cognitive ability over those that are less trait-anxious (Fales et al., 2008; Visu-Petra et al., 2012). Based on this theory, the animals from the 'failed' group that displayed trait anxiety-related responses in the paradigms with threatening stimuli (refer to Chapter 2 and 3) were predicted to show better performance, relative to those less anxious, on the PFC dependent cognitive flexibility tasks that did not involve threatening stimuli, consistent with the present findings.

The second prediction is that those high in trait anxiety will have impoverished recruitment of top-down prefrontal control; therefore, even on a cognitive task that does not involve anxiety-related stimuli, they will display poor performances, in contradiction to the present findings. This prediction is based on a more recently proposed account of the anxiety-cognition interaction, the dual route model for trait anxiety (Bishop, 2007, 2009; Indovina et al., 2011). This hypothesis was derived from neuro-functional observations in fear conditioning. Both

lesion studies in rodents and neuroimaging research in humans implicate the amygdala in the acquisition and expression of cued fear (Maren & Quirk, 2004; Phelps & LeDoux, 2005). On the other hand, PFC circuitry has been implicated in the extinction of conditioned fear and emotion regulation which involves top-down control or inhibition of amygdala output (Bishop, 2007; Delgado, Nearing, Ledoux, & Phelps, 2008; K. Ochsner & Bunge, 2002; K. N. Ochsner & Gross, 2008). It has been suggested that anxiety biases this amygdala-prefrontal interplay by up-regulation of the amygdala response and/or down-regulation of prefrontal control mechanisms (Bishop, 2007). This has led to the proposal that there are two, at least partially, independent dimensions of neuro-cognitive functions that are associated with elevated trait vulnerability to anxiety. One dimension consists of a hyper-responsive 'bottom-up' threat detection system centred on the amygdala, and the other dimension is the impoverished recruitment of 'top-down' prefrontal cognitive control mechanisms. Enhanced trait anxiety is associated with either both or just one or the other of these dimensions, which influences not only associative but also attentional and interpretative processes of threat-related stimuli. This model accounts for the reports showing amygdala hyper-responsivity in combination with frontal hypo-responsivity in both clinical and trait anxious individuals (Bremner et al., 2005; Indovina et al., 2011; L M Shin et al., 2001; Lisa M Shin, Rauch, & Pitman, 2006). Since enhanced trait anxiety is associated with impoverished prefrontal functionality, the model predicts that even on a purely cognitive task, those high in trait anxiety would display diminished attentional control and poor cognitive performance (Bishop, 2009). Accordingly, in the present study, the animals from the 'failed' group that were hypothetically high in trait anxiety might be predicted to show poor performance in comparison to those less anxious on the PFC dependent cognitive tasks.

However, given that the model states that enhanced trait anxiety may be characterised not only by both a hyper-responsive amygdala and impoverished recruitment of prefrontal control but also either of them alone, then not all trait anxiety will be associated with impoverished prefrontal cortex. The trait-anxious animals in the current sample may have been associated with just one of the dimensions, the hyper-responsive amygdala. In anxiety-provoking situations such as the aversive discriminative conditioning paradigm and the rubber snake test, their over-responding amygdala made them excessively anxious. In contrast, the appetitive discriminative conditioning paradigm (Chapter 2) and the PFC dependent cognitive flexibility tasks were not only without any threatening stimuli but also involving appetitive stimuli. Recent evidences indicate that the amygdala becomes aroused not only by anxiety/fear-related stimuli but also by appetitive stimuli information, and may even facilitate positive associative learning through an interaction with the PFC, especially with the OFC

(M.-S. Man et al., 2011; Murray, 2007; Roberts, Reekie, & Braesicke, 2007; Salzman & Paton, 2007). Therefore, extrapolating from the model, the hyper-responsive amygdala, assuming their prefrontal cognitive function was at or above normal level, might have produced positive synergistic effect with the operational PFC, which resulted in the successful appetitive discrimination by all the animals and their improved performances in the PFC dependent cognitive flexibility tests.

Another hypothesis relevant to our findings is, the recently proposed differential susceptibility model (Belsky et al., 2009) which considers altered cognition, especially improved performance, as part of an adaptive strategy with which anxious individuals actively adjust to changing environments. This hypothesis has been developed primarily to account for inconsistent observations in psychiatric genetics, especially the difficulty in replicating associative studies determining the relationships between certain genetic polymorphisms and specific psychological and behavioural conditions. For instance, the short (s), low-expressing variant of the serotonin transporter-linked polymorphic region (5-HTTLPR) has been associated with anxiety-like traits and increased risk for depression in a high-stress context, with the long (l) allele functioning protectively. However, association studies have often failed to find this relationship (Risch, Herrell, & Lehner, 2009; Schinka, Busch, & Robichaux-Keene, 2004; Sen, Burmeister, & Ghosh, 2004). The hypothesis argues that the 5-HTTLPR is not the 'vulnerability gene' for psychological disturbances but a 'plasticity gene' that makes the carriers more susceptible or responsive to both negative and positive environmental conditions. Thus, the very genes that seem to make individuals disproportionately vulnerable to adversity may, simultaneously, confer on them an advantage when it comes to benefitting from exposure to environmental support or enrichment, including the absence of adversity. This differential susceptibility model accounts for the mixed association study reports, and for the recent reports that the 's' allele carriers demonstrate superior abilities in a wide range of cognitive executive functions (J. R. Homberg & Lesch, 2011) including response inhibition (Roiser, Müller, Clark, & Sahakian, 2007), reversal learning (Jedema et al., 2010) and attentional set-shifting (Borg et al., 2009). Since the 's' allele is associated with higher sensitivity to motivationally relevant environmental cues, it makes the carriers highly responsive to threat. However, in the absence of adverse stimuli, this sensitivity to environmental cues may confer increased processing and integration of task relevant stimuli, which is expressed as improved cognition (J. R. Homberg & Lesch, 2011). The 's' allele carriers showing increased amygdala reactivity during an emotional task (Hariri et al., 2002) and increased prefrontal activation during a cognitive task (Fallgatter & Jatzke, 1999), as well as increased functional connectivity

between the PFC and amygdala (Heinz et al., 2005) thereby suggesting enhanced prefrontal-amygdala circuitry as the neural underpinning of this hypothesis.

Although the current findings investigating trait anxiety cannot be linked directly to the studies with the 5-HTTLPR polymorphism, it is plausible to apply the conceptual framework of the hypothesis to trait anxiety. The responses displayed by the high trait-anxious animals may reflect increased plasticity rather than vulnerability. In their wild habitat, marmoset monkeys live under constant threats from a diverse range of predators (Marilia Barros, Boere, et al., 2002); therefore, it may be evolutionarily adaptable to develop defensive behaviours that can be considered in a different context, excessive emotional responses. This was reflected as the over-generalization of fear in the aversive discriminative conditioning task and enhanced emotional reactivity in the rubber snake test. The increased sensitivity to environmental cues in turn provides the anxious animals superior cognitive processing of task-relevant information under the conditions absent of dangers. This may have been reflected in the tendency to commit fewer perseverative errors and thus improved performance in the cognitive flexibility tasks.

In addition to the overall observation that the trait-anxious animals displayed improved cognitive flexibilities, as noted above, the current findings also suggest that there are at least two underlying neural circuits, namely the amygdala-OFC and hippocampus-lPFC, independently associated with trait anxiety. Alteration of subcortical-prefrontal circuitry has been suggested as a common neurobiological mechanism underlying enhanced trait anxiety and cognitive biases (Bishop, 2007). However, this change in connectivity may be more complex than the suggested model of up-regulation of the amygdala/hippocampus- and down-regulation of the prefrontal-centred circuits (Indovina et al., 2011). For instance, Pezawas and colleagues (Pezawas & Meyer-Lindenberg, 2005) reported functional uncoupling between rostral ACC and amygdala in 's' allele carriers, which also accounted for relatively large amounts of variance in temperamental anxiety, suggesting that increased amygdala reactivity seen in the 's' allele carriers is due to altered top-down regulation. However, the same study also noted increased functional coupling between the amygdala and ventromedial PFC among the 's' allele carriers, which has also been reported elsewhere (Heinz et al., 2005). On the other hand, reduced and uncoupled functional connectivity between the amygdala and mPFC including OFC has been associated with high trait anxiety (Kim & Whalen, 2009) and social anxiety disorder (Hahn et al., 2011). These studies suggest that there are more than one circuits connecting the amygdala to different sub-regions of the PFC. The balance across these circuits may be independently altered among those high in

trait anxiety; some show increased couplings but others are uncoupled. Indovina and colleagues (Indovina et al., 2011) reported reduced ventral PFC-hippocampal connectivity associated with contextual fear among the individuals high in trait anxiety, suggesting that reduced ventral PFC regulatory function contributes to increased contextual fear. However, the current results imply increased activities in both hippocampus and lPFC. As with the amygdala-prefrontal connections, the hippocampus may also be implicated in unbalanced and dissociable connectivities with the PFC, which give rise to trait vulnerability to anxiety.

In summary, the present study determined whether individuals with enhanced trait anxiety would display improved or impaired cognitive function associated with increased or impoverished prefrontal functionality. The results indicate that enhanced trait anxiety, specifically the predictors of high trait anxiety were associated with improved prefrontal cognitive function. This association may be explained by either the attentional control theory or dual route model of trait anxiety. The present results also raise the possibility that there may be two independent neural pathways that contribute to trait anxiety, the amygdala-OFC pathway and hippocampus-lPFC pathway. This is based on the finding that the two hypothesized biomarkers of high trait anxiety, namely cue-specific vigilant scanning and baseline BP, correlated independently with the OFC and lPFC-dependent cognitive flexibility measures, respectively.

# Chapter 5

## Conclusion

## 5.1 Summary

The main aim of the presented thesis project has been to develop a new non-human primate model of trait anxiety, using a new world monkey, the common marmoset. The project was carried out through three steps, corresponding to Chapters 2, 3 and 4 respectively. The studies in each step not only followed but also integrated the findings of the previous steps. Therefore, the results and implications have been inclusively discussed throughout the chapters. Here, the main findings and issues discussed are briefly summarised.

The starting point of the project was to identify individual differences in the behavioural phenotype of trait anxiety in this species of monkey. Humans that are high in trait anxiety tend to over-generalise their anxiety/fear responses under stressful situations. This detrimental effect of high trait anxiety is called fear generalization (Lissek et al., 2010). Hypothesizing that high trait-anxious marmosets would also demonstrate over-generalization of fear, a marmoset aversive discriminative conditioning paradigm was designed. In the paradigm, the animal was presented with auditory CS's that were paired with either aversive loud noise or a neutral event involving a brief episode of lights-off. Out of 27 animals tested, 20 (74%) successfully developed discriminative heart rate and vigilant behavioural responses ('passed' group); however, seven animals (26%) failed to discriminate between the cues ('failed' group). A regression analysis of the measures taken during early training sessions that differentiated the 'passed' and 'failed' groups revealed two potential biomarkers of enhanced trait anxiety. Specifically, a suppression of baseline BP and hyper cue-specific vigilance together predicted the failure of the discrimination. In order to rule out the possibility that the failure was due to impaired perception or general learning ability, the 'failed' group was subsequently tested on an appetitive discriminative conditioning paradigm using the same CS's as that used in the aversive discriminative conditioning paradigm. Their successful discrimination in this non-stressful situation indicated that their failure in the aversive discrimination was due likely to the effect of high trait anxiety.

Two hypotheses have been proposed to account for the mechanisms underlying the over-generalization of fear. The fear inhibition hypothesis postulates that high anxiety interferes with the learning of a 'safety' cue. Thus, an anxious individual fails to inhibit a fear response in the presence of a safety signal (Lissek et al., 2009). Comparing the animal's responses between $CS^+$ and $CS^-$ revealed that the failure to acquire the differential responses was not due to a reduced response to the danger cue but to an increased response to the safety cue.

The animals in the 'failed' group were unable to inhibit a fear response to the safety signal, supporting the hypothesis. Alternatively, a contextual fear hypothesis proposes that high anxiety disproportionately enhances the conditionability to the context, which prevents cue-specific learning (Grillon, 2002b). Changes in the baseline period of a Pavlovian discrimination paradigm very often reflect the impact of contextual cues on an animal's performance. Since the 'failed' group displayed significantly suppressed BP during the baseline, this autonomic response quite likely reflects a species specific anxiety-related response to the aversive context; the context being the training box in which the animal received the aversive loud noise. This enhanced response to the context among the 'failed' group supports the alternative hypothesis. Therefore, the present results support both hypotheses. Indeed, the finding that both a hyper vigilance response to cues and a lowered baseline BP together predicted failure on the discrimination task better than either on its own suggests that both may contribute, as well as independently, to the heightened anxiety. Alternatively, they may be interactive, since a failure to learn the safety signal makes the aversive US more unpredictable, which leads to the animals showing stronger conditioning to the context.

The aversive discriminative conditioning paradigm bears several advantages over more conventional models of trait anxiety. Firstly, whilst the measures in the unconditioned anxiety tests are often confounded by task irrelevant measures such as individual differences in exploration, the variables in the aversive discriminative conditioning paradigm are more clearly defined as specific responses to fear/anxiety inducing stimuli. Secondly, whilst the conventional models define high or low anxiety groups by arbitrarily dividing continuous variables, the aversive discriminative conditioning paradigm provides the binary categorical variable, i.e. 'passed' or 'failed', giving more objective boundary between low/normal and high/pathological effects of trait anxiety.

If the fear generalization in marmosets is the reflection of enhanced trait anxiety as hypothesised, those in the 'failed' group would display enhanced anxiety response in more conventional tests of anxiety, which would also verify the validity of the newly developed paradigm as a model of anxiety. Therefore, the next step in the project was to test the animals in the 'passed' and 'failed' groups on well-verified classical tests of anxiety in primates, namely the human intruder and rubber snake tests.

Based on the paradigms described in the neurobiological and pharmacological literatures, the human intruder and rubber snake paradigms that fit the need of the current project and

the settings of the colony/facility were designed. In order to characterise animal's responses, a large normal cohort of marmosets was first tested on the paradigms. The human intruder and rubber snake induced a number of anxiety-related behavioural changes, which markedly varied between individuals. In order to identify psychological dimensions underlying these behaviours, principal component analysis (PCA) was performed. The analysis produced two components in both tests. The first component was labelled 'emotionality' as the behaviours that loaded on this component reflected how anxious/fearful the subject was. The second component was labelled 'coping strategy' as the loaded behaviours reflected an active/passive management of the situation. The 'emotionality' component showed a weak but significantly positive correlation between the human intruder and snake tests, but there was no correlation in the 'coping strategy' component between the tests, suggesting that both stimuli elicited anxiety/fear-related responses but that each of the stimuli may have induced different adaptive strategies.

Subsequently, the 'passed' and 'failed' groups were compared on their performances. The 'failed' group displayed significantly greater 'emotionality' responses than the 'passed' group in response to the snake stimulus, confirming the association between the over-generalization of fear and heightened levels of trait anxiety. On the other hand, no group difference was found in the 'emotionality' component of the human intruder test or in the 'coping strategy' component of either test. In addition, the proposed two biomarkers of enhanced trait anxiety, the suppressed baseline BP and hyper cue-specific vigilance, predicted the 'emotionality' scores in the snake test, further supporting the close correspondence between over generalisation in the fear discrimination task and unconditioned anxiety responsivity to the emotional stimulus.

Two possible accounts were discussed to explain the discrepancy between the group difference in the 'emotionality' scores in response to the snake but not to the human threat. Firstly, although both stimuli invoked negative emotional responses, as indicated by the positive correlation between the tests, the snake stimulus was stronger in emotional intensity than the human threat. Daily handling by caretakers may have habituated the animals, reducing the impact of an encounter with a strange human. Therefore, the human intruder test may be less sensitive in detecting the difference in anxiety levels between the groups. Alternatively, the responses induced by the two stimuli may differ in emotional quality. Whilst the snake is a specific predatory threat to marmosets, the unfamiliar human represents more ambiguous threat. This threat specificity versus uncertainty may underlie the difference between fear and anxiety, respectively. Whist a fear response is phasic and intense, an

anxiety response is sustained and diffuse (Michael Davis et al., 2010). Both responses may be influenced by trait anxiety. The over-generalization of fear may reflect an enhanced fear response associated with high trait anxiety, which may be detected by a fear-inducing snake stimulus but not by an anxiety-inducing human threat.

Suppose that the snake and human threats differentially induce fear and anxiety, respectively, the neural circuits processing the stimuli and responses may also differ. Evidence so far strongly supports the critical role of the amygdala in the expression of fear-related responses to snake stimuli (Izquierdo & Murray, 2004; Izquierdo et al., 2005; Kalin et al., 2004). The short thalamo-amygdala pathway is responsible for processing quick responses to biologically relevant threat such as snakes (J. E. LeDoux, 1995). In contrast, the literature varies somewhat with respect to identifying the structures involved in processing human threats, but the involvement of cortical structures such as the orbitofrontal cortex (OFC) as well as the amygdala have been suggested (Agustín-Pavón et al., 2012; Izquierdo et al., 2005; Kalin et al., 2007). The OFC may up- or down-regulate the amygdala activity when facing uncertain anxiety situations. Given that the amygdala is also critically involved in discriminative fear conditioning (J. E. LeDoux, 1995), the failure in the aversive discrimination task may have been due to a hyper responsive amygdala. Therefore, the enhanced emotional reactions in response to the snake stimulus may have also reflected the hyper responsive amygdala among those in the ‘failed’ group. On the other hand, the neural circuits processing more ambiguous stimuli such as the human threat may not have been altered in those individuals; therefore, no group difference was detected in the human intruder paradigm.

Having developed and verified the new marmoset model of trait anxiety, the next step was to investigate the neural underpinnings of trait anxiety, especially contributions of the prefrontal cortex. The animals in both the ‘failed’ and ‘passed’ groups were tested on two cognitive flexibility tests: the OFC-dependent incongruent object discrimination test and the lateral prefrontal cortex (lPFC)-dependent detour reaching rule transfer test (M. S. Man et al., 2009; Wallis et al., 2001). Whilst the overall mean of perseverative errors in the ‘failed’ group was lower than that of the ‘passed’ group in both tests, this did not reach significance. However, two proposed biomarkers of high trait anxiety were inversely and differentially correlated with the perseverations in the two tests. Specifically, the hyper cue-sensitive vigilance was related to less perseveration in the OFC- but not lPFC-dependent task, and the suppressed baseline BP corresponded to less perseveration in the lPFC- but not OFC-dependent task. Moreover, regression analyses revealed that the sole predictor of OFC-dependent task performance

was cue-sensitive vigilance; likewise, the only predictor of the lPFC dependent task performance was the suppression of baseline BP.

The differential associations between the two biomarkers of high trait anxiety and the OFC-/lPFC-dependent task performance imply two distinct prefrontal-subcortical circuits underlying trait anxiety. The first is the amygdala-OFC connection. The amygdala is the central structure mediating cue-specific fear (Michael Davis et al., 2010; Indovina et al., 2011; J. E. LeDoux, 2000). The amygdala has robust bidirectional projections to the OFC, and it is hypothesised that the OFC, may up-/down-regulate the activity of the amygdala (Barbas, 2000; Gottfried & Dolan, 2004; Kalin et al., 2007; Machado & Bachevalier, 2008; M. S. Man et al., 2009; Schoenbaum, Roesch, & Stalnaker, 2006). In contrast, the amygdala does not directly project to the lPFC (Ghashghaei & Barbas, 2002), thus functionally and physiologically the lPFC may not be as strongly affected by the amygdala activity (however, in marmoset brain the amygdala-lPFC connection may be more robust: Roberts, Tomic, et al., 2007). The second is the hippocampus-lPFC connection. The BP responses during the baseline most likely reflects conditioning to the context, which is dependent upon the hippocampus (Otto & Poon, 2006; Phillips & LeDoux, 1992). The hippocampus is also involved in set-shifting, the ability required for the rule-transfer task, via a reciprocal communication with the dorsolateral PFC (Berman et al., 1995; Graham et al., 2009).

The overall finding that animals that were more likely to over-generalise on the fear discrimination task actually performed, if anything, better on the prefrontal flexibility tasks than those animals that were less likely to over-generalise, is inconsistent with the currently available literature which suggests that trait anxious individuals display impaired prefrontal cognitive ability. A couple of explanations were proposed. Based on the attentional control theory (M. Eysenck et al., 2007), anxiety results from an imbalance between the subcortical stimulus-driven attentional system and the prefrontal goal-directed attentional system. In trait-anxious individuals, the over-activated former system overwhelms one's mental functions despite the latter system's compensatory effort. However, without the emotional stimuli the former system remains silent, and the 'hard-working' PFC can exercise superior cognitive ability (Fales et al., 2008; Visu-Petra et al., 2012). Therefore, the individuals high in trait anxiety perform better on cognitive tasks in non-stressful situations than less trait-anxious individuals.

Alternatively, the dual route model of trait anxiety (Bishop, 2007; Indovina et al., 2011) postulates that the hyper-responsive amygdala and impoverished recruitment of prefrontal

control together or independently contribute to enhanced trait anxiety. It may be that the trait-anxious individuals in the current sample were associated with one of the dimensions, the hyper-responsive amygdala. Since the amygdala is activated both in emotionally negative and positive situations (M.-S. Man et al., 2011; Murray, 2007; Roberts, Reekie, et al., 2007), the hyper-responsive amygdala may have induced exaggerated fear responses in threatening situations such as the aversive discriminative conditioning paradigm and rubber snake test, whereas in cognitive tasks involving appetitive stimuli, the active amygdala together with the operational PFC may have facilitated the task-relevant performances.

Another explanation is suggested based on the differential susceptibility model (Belsky et al., 2009), which considers altered cognition, especially improved performance, as part of an adaptive strategy with which anxious individuals actively adjust to changing environments. Enhanced trait anxiety is associated with increased sensitivity to environmental cues, which is responsible for an individuals heightened anxiety in the presence of threatening stimuli but also provides them superior cognitive processing of task-relevant information under conditions absent of dangers. Whilst the former corresponds to the over-generalisation of fear and increased 'emotionality' in response to the snake, the latter is reflected as the improved performances on the cognitive flexibility tasks.

With the current data, it is difficult to determine which model underlies the findings. These models, however, suggest that there are two systems involved in emotional-cognitive interplay, namely the subcortical stimulus-driven mechanism and prefrontal cognitive control mechanism. The connections between them are altered in high trait anxious individuals. The presented results suggest that there may be more than one circuit connecting the two systems, e.g. the amygdala-OFC and hippocampus-lPFC circuits. The balance across these circuits may be independently altered among the individuals high in trait anxiety; some connections showing increased couplings but others being uncoupled.

## 5.2 Limitations

The binary categorisation of high and low trait-anxious animals, namely 'Passed' and 'Failed' grouping, was a merit of the newly developed mild aversive Pavlovian discriminative conditioning paradigm over other conventional anxiety paradigms, i.e. the new paradigm provides a clear boundary where the high anxiety exerts the detrimental effect whilst other tests, such as EPM, give only arbitrary definition of high versus low anxiety. However, the categorization does not take account the continuous expression of the phenotypic traits such as seen in Figure 2.9. This can be especially problematic when one tries to correlate the measures with other continuous variables, which may restrict the types and extent of statistical analysis. Also, though in retrospect, it would be interesting to investigate the neural correlates of this continuous expression of behavioural and cardiovascular traces as markers of anxiety trait. In the presented project however, the findings of the two possible biomarkers of trait anxiety, namely the cue-associated hyper-vigilance and context-associated hypotension, both of which were continuous variables, allowed subsequent correlation analyses with the measures from other tests. Although using the binary outcome has the obvious advantage as described above, it carries a risk of missing more subtle distribution of anxiety traits, this may have been captured by the two potential anxiety-related biomarkers.

Another limitation worth noting, is the potential effect of the different amount of experiences that the animals had. For the human intruder and rubber snake tests, in order to provide a comprehensive characterisation of the patterns of the behaviours, a large sample of naïve marmosets was tested, along with the animals that had experienced the mild aversive Pavlovian discriminative conditioning paradigm. Those that had gone through the aversive conditioning experienced repeated exposures to the aversive loud noise in the non-escapable condition. This additional experience might have influenced their performances in the subsequent testing. In retrospect (hindsight), it would have been ideal for both experimental and statistical procedures if those animals had been tested on the human intruder and rubber snake tests, along with other experimentally naïve animals, before they were tested on any other paradigms. Currently in my laboratory, all the experimental animals are first screened for their anxiety levels on the human intruder and rubber snake tests before they go on to their respective projects.

Finally, in Chapter 4, the implications of altered neural circuits specifically involving the OFC and lPFC were extrapolated purely from the findings in the behavioural measures. The paradigms on which the animals were tested had been shown to be selectively sensitive to damage in those prefrontal brain regions, therefore it is rational to relate the changes in the

behavioural measures to the neural mechanisms associate with these regions. However, these are just correlations, and other brain regions, connected to the prefrontal regions, are likely part of a neural circuit involved in the performance of these tasks. Thus, it is possible that alterations in these other nodes of the neural circuit may be affected in high trait anxiety, not the prefrontal regions themselves. Ultimately we need to identify changes in these prefrontal regions associated with trait anxiety and then perform actual neurobiological manipulations to determine whether alterations in these prefrontal regions can change a high trait anxious animal into a low trait anxious animal and vice versa.

## 5.3 Future Directions

Anxiety and mood disorders are among the most common causes of disabilities. Approximately one in five people worldwide are reported to experience a clinical level of anxiety within their lifetimes (R. C. Kessler et al., 2005; Somers, Goldner, Waraich, & Hsu, 2006). However, developments of more efficacious treatments, whether based on drugs, psychology or surgery, have been hindered primarily because of poor understanding of the neural mechanisms underlying the aetiology of these psychiatric disorders. Decades of works in rodent models, especially using fear conditioning paradigms have elucidated major neural circuits involved in adaptive emotional responses to environmental threats (J. LeDoux, 2000; Phillips & LeDoux, 1992). Although these studies are informative in how we process and respond to fearful stimuli, they do not explain what makes one more vulnerable to anxiety disorders, than others. Only recently has research in human neuroimaging and genetics begun to elucidate the neurobiological mechanisms of trait vulnerability to anxiety (Bishop, 2007; Indovina et al., 2011; K. P. Lesch et al., 1996; Sandi & Richter-Levin, 2009). My PhD project was initiated with an intention to develop a non-human primate model that could reach the gap between the rodent and human researches.

As described in the summary, the project was successful in developing a new aversive discriminative conditioning paradigm that serves to identify individuals high or low in trait anxiety, and further suggested possible neural circuitries that may be altered in high trait anxious individuals. The project also highlighted several unanswered issues. Particularly, the long-debated distinction between fear and anxiety is of importance because cue-elicited phasic responses and context-elicited sustained apprehension may reflect different neural mechanisms underlying different types of anxiety disorders. For instance, the former may be related to the disorders characterised by exaggerated reactivity to specific fear cues, such as specific phobia and social anxiety disorder, whereas the latter may be linked to the symptoms with long-lasting global uneasiness to uncertain situations, such as general anxiety disorder. The observed discrepancy between the snake and human intruder tests suggested that marmosets may also express differential responses depending on the type of threat.

Another issue is how environmental stress interacts with trait anxiety to produce behavioural and cognitive endophenotypes. The improved cognitive performance in non-stressful conditions demonstrated by high trait-anxious individuals suggests an importance of environmental factors in modulating cognition-emotion interplay. A model that enables the

control of the amount of stress imposed, e.g. comparison of cognitive performance whilst in the presence of more or less emotionally stressful stimuli, with the physiological measures of the stressful experience, e.g. HPA axis response, may further elucidate the mechanism underlying the interaction between environmental stress and trait anxiety.

As shown with the research on the serotonin transporter (5-HTT) gene polymorphism (Caspi & Moffitt, 2006; K. Lesch & Mössner, 1998), genetic makeup, together with developmental environment, makes a substantial contribution to the individual variability in trait vulnerability to anxiety. An on-going project in my laboratory has identified a double nucleotide polymorphism in the marmoset 5-HTT gene (Santangelo et al., unpublished findings). The investigations into the functional effects of this polymorphism at the molecular and behavioural levels are now under way. Such research provides insights into the mechanisms of how genetic traits, i.e. genotype, are expressed as neurobiological and biochemical traits that eventually give rise to behavioural endophenotypes.

Although my thesis project did not conduct any neurobiological manipulations, the results were suggestive of the presence of at least two neural circuits connecting the subcortical emotional and prefrontal cognitive systems, specifically the amygdala-OFC and hippocampus-lPFC. Altered couplings across these circuits and/or between the two systems may underlie individual differences in trait vulnerability to anxiety. My laboratory has already shown that excitotoxic lesions of either the OFC or lPFC induce heightened anxiety and alterations in fear regulation (Agustín-Pavón et al., 2012), so the next step would be to neurobiologically manipulate these networks and determine the differential contribution of these networks to anxiety, focussing on cue versus contextual emotional processing. The infusion of neuro-modulator agonist/antagonist into target brain regions both within and between the neural circuits may up- or down-regulate specific neural activities which may result in observable phenotypic changes, i.e. high anxious individuals becoming low anxious and vice versa. Similarly, but more precisely, with techniques such as optogenetics and the designer receptors exclusively activated by designer drugs (DREADD), it is possible to switch on and off specific neural populations and projection pathways with much finer temporal precision, which allows neurobiological manipulation at a molecular/cellular level. These investigations would not only provide more accurate understanding of the neural circuits underlying trait anxiety but also contribute to the development of more effective pharmaceutical agents that can aim at specific molecular sites.

The finding of an emotion-cognition interplay, especially the improved cognitive flexibility among those characterised with the signs of high trait anxiety, was very intriguing. As discussed in Chapter 4, there are conflicting hypotheses accounting for contrasting reports. The next phase of the research should be to investigate whether similar behavioural patterns can be observed when human trait-anxious individuals presented with such cognitive tasks. Together, with simultaneous brain scanning with fMRI or PET and subsequent analysis for functional connectivity between neural circuits, the investigation would provide clues about the mechanisms underlying the emotion-cognition interaction. Elucidating how individuality in emotional traits, especially trait vulnerability to anxiety and depression, affects one's cognitive functionality may lead to the development of more accurate diagnostic tools using cognitive tasks specifically sensitive to different types of anxiety disorders.

Overall, the project was fruitful in accomplishing the initial aim of developing a new marmoset model of trait anxiety and providing further insights into behavioural and psychological components of threat-related stimuli processing and anxiety-related responses, related to enhanced trait anxiety. Furthermore, the findings suggested underlying neural networks that may be altered among those high in trait anxiety. Meanwhile, the research has raised many interesting questions. Finding answers to these questions will certainly require multi-disciplinary approaches from various scientific domains including cognitive/behavioural psychology, neurobiology, developmental neuroscience and molecular biology. Ultimately, having an integrative understanding of the complex process of how behavioural/cognitive traits are constructed through the interaction between environmental factors and genes at every developmental and anatomical level, will benefit multiple facets of human society, among them particularly, the prevention and treatment of complex psychiatric disorders.

## References


Adolphs, R., Tranel, D., Damasio, H., & Damasio, A. R. (1995). Fear and the human amygdala. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *15*(9), 5879–91. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3030206&tool=pmcentrez&rendertype=abstract

Agustín-Pavón, C., Braesicke, K., Shiba, Y., Santangelo, A. M., Mikheenko, Y., Cockroft, G., Asma, F., et al. (2012). Lesions of Ventrolateral Prefrontal or Anterior Orbitofrontal Cortex in Primates Heighten Negative Emotion. *Biological Psychiatry*. doi:10.1016/j.biopsych.2012.03.007

Aldea, M. A., Geffken, G. R., Jacob, M. L., Goodman, W. K., & Storch, E. A. (2009). Further psychometric analysis of the Florida Obsessive-Compulsive Inventory. *Journal of anxiety disorders*, *23*(1), 124–9. doi:10.1016/j.janxdis.2008.05.001

Amaral, D. G. (2002). The primate amygdala and the neurobiology of social behavior: implications for understanding social anxiety. *Biological psychiatry*, *51*(1), 11–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11801227

Andrade, L., Caraveo-Anduaga, J. J., Berglund, P., Bijl, R. V., De Graaf, R., Vollebergh, W., Dragomirecka, E., et al. (2003). The epidemiology of major depressive episodes: results from the International Consortium of Psychiatric Epidemiology (ICPE) Surveys. *International journal of methods in psychiatric research*, *12*(1), 3–21. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12830306

Andreatini, R., & Bacellar, L. F. S. (2000). ANIMAL MODELS : TRAIT OR STATE MEASURE ? THE TEST-RETEST RELIABILITY OF THE ELEVATED PLUS-MAZE AND BEHAVIORAL DESPAIR. *Progress in Neuro-Psychopharmacology and …*, *24*, 549–560. Retrieved from http://www.sciencedirect.com/science/article/pii/S0278584600000920

Anguelova, M., Benkelfat, C., & Turecki, G. (2003). A systematic review of association studies investigating genes coding for serotonin receptors and the serotonin transporter: I. Affective disorders. *Molecular psychiatry*. Retrieved from http://www.nature.com/mp/journal/v8/n6/abs/4001328a.html

Ansari, T. L., Derakshan, N., & Richards, A. (2008). Effects of anxiety on task switching: Evidence from the mixed antisaccade task. *Cognitive, Affective, & Behavioral Neuroscience*, *8*(3), 229–238. doi:10.3758/CABN.8.3.229

Armony, J. L., Servan-Schreiber, D., Romanski, L. M., Cohen, J. D., & LeDoux, J. E. (1997). Stimulus generalization of fear responses: effects of auditory cortex lesions in a computational model and in rats. *Cerebral cortex (New York, N.Y. : 1991)*, *7*(2), 157–65. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9087823

Asghari, V., & Sanyal, S. (1995). Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. *Journal of …*. Retrieved from http://onlinelibrary.wiley.com/doi/10.1046/j.1471-4159.1995.65031157.x/full

Baas, J M P, van Ooijen, L., Goudriaan, A., & Kenemans, J. L. (2008). Failure to condition to a cue is associated with sustained contextual fear. *Acta psychologica*, *127*(3), 581–92. doi:10.1016/j.actpsy.2007.09.009

Baas, Johanna M P, Grillon, C., Böcker, K. B. E., Brack, A. A., Morgan, C. A., Kenemans, J. L., & Verbaten, M. N. (2002). Benzodiazepines have no effect on fear-potentiated startle in humans. *Psychopharmacology*, *161*(3), 233–47. doi:10.1007/s00213-002-1011-8

Baddeley, A. (1992). Working memory. *Science*, *255*(5044), 556–559. doi:10.1126/science.1736359

Ballaz, S. J., Akil, H., & Watson, S. J. (2007). Previous experience affects subsequent anxiety-like responses in rats bred for novelty seeking. *Behavioral neuroscience*, *121*(5), 1113–8. doi:10.1037/0735-7044.121.5.1113

Bandler, R., Keay, K. A., Floyd, N., & Price, J. (2000). Central circuits mediating patterned autonomic activity during active vs. passive emotional coping. *Brain research bulletin*, *53*(1), 95–104. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11033213

Bar-Haim, Y. (2010). Research review: Attention bias modification (ABM): a novel treatment for anxiety disorders. *Journal of child psychology and psychiatry, and allied disciplines*, *51*(8), 859–70. doi:10.1111/j.1469-7610.2010.02251.x

Barlow, D. H. (2001). *Anxiety and Its Disorders: The Nature and Treatment of Anxiety and Panic* (2nd ed., p. 704). The Guilford Press. Retrieved from http://www.amazon.com/Anxiety-Its-Disorders-Second-Edition/dp/1572304308

Barnes, J. M., Barnes, N. M., Costall, B., Domeney, A. M., Johnson, D. N., Kelly, M. E., Munson, H. R., et al. (1990). The differential activities of R (+)- and S(-)-zacopride as 5-HT3 receptor antagonists. *Pharmacology, biochemistry and behavior*, *37*(4), 717–727.

Barros, M, Boere, V., Huston, J. P., & Tomaz, C. (2000). Measuring fear and anxiety in the marmoset (Callithrix penicillata) with a novel predator confrontation model: effects of diazepam. *Behavioural brain research*, *108*(2), 205–11. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10701664

Barros, M, Mello, E. L., Huston, J. P., & Tomaz, C. (2001). Behavioral effects of buspirone in the marmoset employing a predator confrontation test of fear and anxiety. *Pharmacology, biochemistry, and behavior*, *68*(2), 255–62. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11267630

Barros, Marilia, Boere, V., Mello, E. L., & Tomaz, C. (2002). Reactions to Potential Predators in Captive-born Marmosets ( Callithrix penicillata ). *International Journal*, *23*(2), 443–454.

Barros, Marilia, De Souza Silva, M. A., Huston, J. P., & Tomaz, C. (2002). Anxiolytic-like effects of substance P fragment (SP(1-7)) in non-human primates (Callithrix penicillata). *Peptides*, *23*(5), 967–73. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12084529

Barros, Marilia, Giorgetti, M., Souto, A. A. V., Vilela, G., Santos, K., Boas, N. V., & Tomaz, C. (2007). Persistent anxiety-like behavior in marmosets following a recent predatory stress condition: reversal by diazepam. *Pharmacology, biochemistry, and behavior*, *86*(4), 705–11. doi:10.1016/j.pbb.2007.02.016

Barros, Marilia, Mello, E. L., Maior, R. S., Müller, C. P., de Souza Silva, M. a, Carey, R. J., Huston, J. P., et al. (2003). Anxiolytic-like effects of the selective 5-HT1A receptor antagonist WAY 100635 in non-human primates. *European Journal of Pharmacology*, *482*(1-3), 197–203. doi:10.1016/j.ejphar.2003.09.064

Barros, Marilia, & Tomaz, C. (2002). Non-human primate models for investigating fear and anxiety. *Neuroscience and biobehavioral reviews*, *26*(2), 187–201. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11856558

Barros, Marilia, de Souza Silva, M. A., Huston, J. P., & Tomaz, C. (2004). Multibehavioral analysis of fear and anxiety before, during, and after experimentally induced predatory stress in Callithrix penicillata. *Pharmacology, biochemistry, and behavior*, *78*(2), 357–67. doi:10.1016/j.pbb.2004.04.008

Basten, U., Stelzel, C., & Fiebach, C. J. (2011). Trait anxiety modulates the neural efficiency of inhibitory control. *Journal of cognitive neuroscience*, *23*(10), 3132–45. doi:10.1162/jocn_a_00003

Bechara, A., Damasio, A. R., Damasio, H., & Anderson, S. W. (1994). Insensitivity to future consequences following damage to human prefrontal cortex. *Cognition*, *50*(1-3), 7–15. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8039375

Belsky, J., Jonassaint, C., Pluess, M., Stanton, M., Brummett, B., & Williams, R. (2009). Vulnerability genes or plasticity genes? *Molecular psychiatry*, *14*(8), 746–54. doi:10.1038/mp.2009.44

Belzung, C., & Griebel, G. (2001). Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behavioural brain research*, *125*(1-2), 141–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11682105

Berman, K., Ostrem, J., & Randolph, C. (1995). Physiological activation of a cortical network during performance of the wisconsisn card sorting test: A positron emission tomography study. *Neuropsychologia*, *33*(8), 1027–1046. Retrieved from http://www.sciencedirect.com/science/article/pii/0028393295000352

Berntson, G. G., Sarter, M., & Cacioppo, J. T. (1998). Anxiety and cardiovascular reactivity: the basal forebrain cholinergic link. *Behavioural brain research*, *94*(2), 225–48. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9722275

Bezerra, B. M., & Souto, A. (2008). *Structure and Usage of the Vocal Repertoire of Callithrix jacchus*. *International Journal of Primatology* (Vol. 29, pp. 671–701). doi:10.1007/s10764-008-9250-0

Bishop, S. J. (2007). Neurocognitive mechanisms of anxiety: an integrative account. *Trends in cognitive sciences*, *11*(7), 307–16. doi:10.1016/j.tics.2007.05.008

Bishop, S. J. (2008). Neural Mechanisms Underlying Selective Attention to Threat. *Annals of the New York Academy of Sciences*, *152*, 141–152. doi:10.1196/annals.1417.016

Bishop, S. J. (2009). Trait anxiety and impoverished prefrontal control of attention. *Nature neuroscience*, *12*(1), 92–8. doi:10.1038/nn.2242

Bishop, S. J., Duncan, J., Brett, M., & Lawrence, A. D. (2004). Prefrontal cortical function and anxiety: controlling attention to threat-related stimuli. *Nature neuroscience*, *7*(2), 184–8. doi:10.1038/nn1173

Bishop, S. J., Duncan, J., & Lawrence, A. D. (2004). State anxiety modulation of the amygdala response to unattended threat-related stimuli. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *24*(46), 10364–8. doi:10.1523/JNEUROSCI.2550-04.2004

Bittner, A., Goodwin, R. D., Wittchen, H.-U., Beesdo, K., Höfler, M., & Lieb, R. (2004). What characteristics of primary anxiety disorders predict subsequent major depressive disorder? *The Journal of clinical psychiatry*, *65*(5), 618–26, quiz 730. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15163247

Black, D. W., Noyes, R. J., Goldstein, R. B., & Blum, N. (1992). A family study of obsessive-compulsive disorder. *Archives of general psychiatry*, *49*, 362–368.

Blanchard, D. C., Griebel, G., Rodgers, R. J., & Blanchard, R. J. (1998). Benzodiazepine and serotonergic modulation of antipredator and conspecific defense. *Neuroscience and biobehavioral reviews*, *22*(5), 597–612. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9662722

Blechert, J., Michael, T., Grossman, P., Lajtman, M., & Wilhelm, F. H. (2007). Autonomic and respiratory characteristics of posttraumatic stress disorder and panic disorder. *Psychosomatic medicine*, *69*(9), 935–43. doi:10.1097/PSY.0b013e31815a8f6b

Blumstain, D. T., Daniel, J. C., Evans, C. S., & Blumstein, D. T. (2011). JWatcher. Retrieved from http://www.jwatcher.ucla.edu/

Booth-Kewley, S., & Friedman, H. S. (1987). Psychological predictors of heart disease: a quantitative review. *Psychological bulletin*, *101*(3), 343–62. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3602244

Borg, J., Henningsson, S., Saijo, T., Inoue, M., Bah, J., Westberg, L., Lundberg, J., et al. (2009). Serotonin transporter genotype is associated with cognitive performance but not regional 5-HT1A receptor binding in humans. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, *12*(6), 783–92. doi:10.1017/S1461145708009759

Bourin, M., & Hascoët, M. (2003). The mouse light/dark box test. *European Journal of Pharmacology*, *463*(1-3), 55–65. doi:10.1016/S0014-2999(03)01274-3

Bourin, M., Petit-Demoulière, B., Dhonnchadha, B. N., & Hascöet, M. (2007). Animal models of anxiety in mice. *Fundamental & clinical pharmacology*, *21*(6), 567–74. doi:10.1111/j.1472-8206.2007.00526.x

Bouton, M. E., & Bolles, R. C. (1979). Role of conditioned contextual stimuli in reinstatement of extinguished fear. *Journal of experimental psychology. Animal behavior processes*, *5*(4), 368–78. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/528893

Bradley, B., & Mogg, K. (1999). Attentional bias for emotional faces in generalized anxiety disorder. *British Journal of …*. Retrieved from http://onlinelibrary.wiley.com/doi/10.1348/014466599162845/abstract

Brady, A. M. (2009). Neonatal ventral hippocampal lesions disrupt set-shifting ability in adult rats. *Behavioural brain research*, *205*(1), 294–8. doi:10.1016/j.bbr.2009.07.025

Braesicke, K., Parkinson, J. A., Reekie, Y., Man, M.-S., Hopewell, L., Pears, A., Crofts, H., et al. (2005). Autonomic arousal in an appetitive context in primates: a behavioural and neural analysis. *The European journal of neuroscience*, *21*(6), 1733–40. doi:10.1111/j.1460-9568.2005.03987.x

Braver, T. S., Gray, J. R., & Burgess, G. C. (2008). Explaining the many varieties of working memory variation: Dual mechanisms of cognitive control. In A. Conway, C. Jarrold, M. Kane, A. Miyake, & J. Towse (Eds.), *Variation in working memory* (pp. 76–106). Oxford: Oxford University Press. doi:10.1093/acprof:oso/9780195168648.001.0001

Bremner, J. D., Vermetten, E., Schmahl, C., Vaccarino, V., Vythilingam, M., Afzal, N., Grillon, C., et al. (2005). Positron emission tomographic imaging of neural correlates of a fear acquisition and extinction paradigm in women with childhood sexual-abuse-related post-traumatic stress disorder. *Psychological Medicine*, *35*(6), 791–806. doi:10.1017/S0033291704003290

Broks, P., Young, A. W., Maratos, E. J., Coffey, P. J., Calder, A. J., Isaac, C. L., Mayes, A. R., et al. (1998). Face processing impairments after encephalitis: amygdala damage and recognition of fear. *Neuropsychologia*, *36*(1), 59–70. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9533388

Brooks, J. M., Pershing, M. L., Thomsen, M. S., Mikkelsen, J. D., Sarter, M., & Bruno, J. P. (2012). Transient Inactivation of the Neonatal Ventral Hippocampus Impairs Attentional Set-Shifting Behavior: Reversal with an α7 Nicotinic Agonist. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 1–11. doi:10.1038/npp.2012.106

Brunetti, M., Sepede, G., Mingoia, G., Catani, C., Ferretti, A., Merla, A., Del Gratta, C., et al. (2010). Elevated response of human amygdala to neutral stimuli in mild post traumatic stress disorder: neural correlates of generalized emotional response. *Neuroscience*, *168*(3), 670–9. doi:10.1016/j.neuroscience.2010.04.024

Buckner, J. D., Maner, J. K., & Schmidt, N. B. (2010). Difficulty Disengaging Attention from Social Threat in Social Anxiety. *Cognitive therapy and research*, *34*(1), 99–105. doi:10.1007/s10608-008-9205-y

Burt, J. M. (2006). Syrinx-PC software. University of Washington, Seattle, WA, USA. Retrieved from www.syrinxpc.com

Bush, D., Sotres-Bayon, F., & Ledoux, J. E. (2007). Individual differences in fear: isolating fear reactivity and fear recovery phenotypes. *Journal of traumatic …*, *20*(4), 413–422. doi:10.1002/jts.

Cagni, P., Gonçalves, I., Ziller, F., Emile, N., & Barros, M. (2009). Humans and natural predators induce different fear/anxiety reactions and response pattern to diazepam in marmoset monkeys. *Pharmacology, biochemistry, and behavior*, *93*(2), 134–40. doi:10.1016/j.pbb.2009.04.020

Cagni, P., Sampaio, A. C., Ribeiro, N. B., & Barros, M. (2011). Immediate, but no delayed, behavioral response to a snake model by captive black tufted-ear marmosets. *Behavioural processes*, *87*(3), 241–5. doi:10.1016/j.beproc.2011.04.002

Caine, N. G. (1998). Cutting Costs in Response to Predatory Threat by Geoffroy ' s Marmosets ( Callithrix geoffroyi ). *American Journal of Primatology*, *196*(February), 187–196.

Calder, A. (1996). Facial Emotion Recognition after Bilateral Amygdala Damage : Differentially Severe Impairment of Fear. *Cognitive Neuropsychology*, (July 2012), 699–745. Retrieved from http://www.tandfonline.com/doi/abs/10.1080/026432996381890

Caldwell, C. a., Watson, C. F. E., & Morris, K. D. (2009). Exploiting flavour preferences of common marmosets to increase palatability of a dry pellet diet. *Applied Animal Behaviour Science*, *116*(2-4), 244–249. doi:10.1016/j.applanim.2008.09.001

Calvo, M., & Eysenck, M. (1998). Cognitive bias to internal sources of information in anxiety. *International Journal of Psychology*, *33*(4), 287–299. Retrieved from http://www.tandfonline.com/doi/abs/10.1080/002075998400321

Calvo, M. G., & Cano-vindel, A. (1997). The Nature of Trait Anxiety : Cognitive and Biological Vulnerability. *European Psychologist*, *2*(4), 301–312.

Campeau, S., & Davis, M. (1995). Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *15*(3 Pt 2), 2301–11. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7891168

Cannistraro, P. A., Wright, C. I., Wedig, M. M., Martis, B., Shin, L. M., Wilhelm, S., & Rauch, S. L. (2004). Amygdala responses to human faces in obsessive-compulsive disorder. *Biological psychiatry*, *56*(12), 916–20. doi:10.1016/j.biopsych.2004.09.029

Canteras, N. S., & Swanson, L. W. (1992). Projections of the ventral subiculum to the amygdala, septum, and hypothalamus: a PHAL anterograde tract-tracing study in the rat. *The Journal of comparative neurology*, *324*(2), 180–94. doi:10.1002/cne.903240204

Cardinal, R. N., & Aitken, M. R. F. (2010). Whisker: a client-server high-performance multimedia research control system. *Behavior research methods*, *42*(4), 1059–71. doi:10.3758/BRM.42.4.1059

Carey, G. J., Costall, B., Domeney, A. M., Jones, D. N., & Naylor, R. J. (1992). Behavioural effects of anxiogenic agents in the common marmoset. *Pharmacology, biochemistry, and behavior*, *42*(1), 143–53. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1528937

Carola, V., D'Olimpio, F., Brunamonti, E., Mangia, F., & Renzi, P. (2002). Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. *Behavioural brain research*, *134*(1-2), 49–57. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12191791

Carrive, P. (2000). Conditioned fear to environmental context: cardiovascular and behavioral components in the rat. *Brain research*, *858*(2), 440–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10708699

Caspi, A., & Moffitt, T. E. (2006). Gene-environment interactions in psychiatry: joining forces with neuroscience. *Nature reviews. Neuroscience*, *7*(7), 583–90. doi:10.1038/nrn1925

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*(5), 587–607. doi:10.1016/j.janxdis.2003.09.001

Chan, C. K. Y., & Lovibond, P. F. (1996). Expectancy bias in trait anxiety. *Journal of Abnormal Psychology*, *105*(4), 637–647. Retrieved from <Go to ISI>://A1996VQ84000016

Charney, D., & Drevets, W. (2002). The neurobiological basis of anxiety disorders. In D. KL (Ed.), *Generation of Progress* (5th ed.). Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:NEUROBIOLOGICAL+BASIS+OF+ANXIETY+DISORDERS#0

Chen, C., & Barnes, D. (2011). Generalized vs. stimulus-specific learned fear differentially modifies stimulus encoding in primary sensory cortex of awake rats. *Journal of*, 3136–3144. doi:10.1152/jn.00721.2011.

Cheney, D. L., & Wrangham, R. (1987). Predation. In B. B. Smuts, D. L. Cheney, R. M. Seyfarth, R. W. Wrangham, & T. T. Struhsaker (Eds.), *Primate societies* (pp. 227–239). Chicago: Chicago University Press.

Cilia, J., & Piper, D. C. (1997). Marmoset conspecific confrontation: an ethologically-based model of anxiety. *Pharmacology, biochemistry, and behavior*, *58*(1), 85–91. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9264075

Clara, E., Tommasi, L., & Rogers, L. J. (2008). Social mobbing calls in common marmosets (Callithrix jacchus): effects of experience and associated cortisol levels. *Animal cognition*, *11*(2), 349–58. doi:10.1007/s10071-007-0125-0

Clark, L., Watson, D., & Mineka, S. (1994). Temperament, personality, and the mood and anxiety disorders. *Journal of abnormal psychology*. Retrieved from http://psycnet.apa.org/journals/abn/103/1/103/

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, *142*(1-2), 169–196. doi:10.1007/s10681-005-1681-5

Corcoran, C. A., Pierre, P. J., Haddad, T., Bice, C., Suomi, S. J., Grant, K. A., Friedman, D. P., et al. (2012). Long-term effects of differential early rearing in rhesus macaques: behavioral reactivity in adulthood. *Developmental psychobiology*, *54*(5), 546–55. doi:10.1002/dev.20613

Correa, H. K. M., & Coutinho, P. E. G. (1997). Fatal Attack of a Pit Viper , Bothrops jararaca , on an Infant Buffy-tufted Ear Marmoset ( Callithrix aurita ). *Natural History*, *38*(April), 215–217.

Costall, B., Domeney, A., Farre, A., & Kelly, M. (1992). Profile of action of a novel 5-hydroxytryptamine1A receptor ligand E-4424 to inhibit aversive behavior in the mouse, rat and marmoset. *The journal of pharmacology and experimental therapeutics*, *262*(1), 90–98. Retrieved from http://jpet.aspetjournals.org/content/262/1/90.short

Courtin, J., & Herry, C. (2011). Lesions of the ventral medial prefrontal cortex enhance contextual fear generalisation in C57BL6/J mice. *European Neuropsychopharmacology*, *21*, S38–S39. doi:10.1016/S0924-977X(11)70045-1

Cross, N, & Rogers, L. J. (2006). Mobbing vocalizations as a coping response in the common marmoset. *Hormones and behavior*, *49*(2), 237–45. doi:10.1016/j.yhbeh.2005.07.007

Cross, Nicola, & Rogers, L. J. (2004). Diurnal cycle in salivary cortisol levels in common marmosets. *Developmental psychobiology*, *45*(3), 134–9. doi:10.1002/dev.20023

Cruz, A. P., Frei, F., & Graeff, F. G. (1994). Ethopharmacological analysis of rat behavior on the elevated plus-maze. *Pharmacology, biochemistry, and behavior*, *49*(1), 171–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7816869

Davidson, R., & Irwin, W. (1999). The functional neuroanatomy of emotion and affective style. *Trends in cognitive sciences*, *3*(1), 11–21. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10234222

Davidson, R. J. (2002). Anxiety and affective style: role of prefrontal cortex and amygdala. *Biological psychiatry*, *51*(1), 68–80. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11801232

Davis, M. (1992). The role of the amygdala in fear and anxiety. *Annual review of neuroscience*, *15*, 353–75. doi:10.1146/annurev.ne.15.030192.002033

Davis, M, & Whalen, P. J. (2001). The amygdala: vigilance and emotion. *Molecular psychiatry*, *6*(1), 13–34. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11244481

Davis, Michael, Walker, D. L., Miles, L., & Grillon, C. (2010). Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *35*(1), 105–35. doi:10.1038/npp.2009.109

Deacon, R. M. J. (2011). Hyponeophagia: a measure of anxiety in the mouse. *Journal of visualized experiments : JoVE*, (51), 2–5. doi:10.3791/2613

Delgado, M. R., Nearing, K. I., Ledoux, J. E., & Phelps, E. a. (2008). Neural circuitry underlying the regulation of conditioned fear and its relation to extinction. *Neuron*, *59*(5), 829–38. doi:10.1016/j.neuron.2008.06.029

Deloache, J. S., & Lobue, V. (2009). The narrow fellow in the grass: human infants associate snakes and fear. *Developmental science*, *12*(1), 201–7. doi:10.1111/j.1467-7687.2008.00753.x

Depue, R., Luciana, M., & Arbisi, P. (1994). Dopamine and the structure of personality: relation of agonist-induced dopamine activity to positive emotionality. *Journal of Personality …*. Retrieved from http://psycnet.apa.org/journals/psp/67/3/485/

Derakshan, N., & Eysenck, M. W. (2009). Anxiety, Processing Efficiency, and Cognitive Performance New Developments from Attentional Control Theory. *European Psychologist*, *14*(2), 168–176. doi:10.1027/1016-9040.14.2.168

Dielenberg, R. A., Carrive, P., & McGregor, I. S. (2001). The cardiovascular and behavioral response to cat odor in rats: unconditioned and conditioned effects. *Brain research*, *897*(1-2), 228–37. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11282383

Dolcos, F., & McCarthy, G. (2006). Brain systems mediating cognitive interference by emotional distraction. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *26*(7), 2072–9. doi:10.1523/JNEUROSCI.5042-05.2006

Dunsmoor, J. E., Prince, S. E., Murty, V. P., Kragel, P. A., & LaBar, K. S. (2011). Neurobehavioral mechanisms of human fear generalization. *Neuroimage*, *55*(4), 1878–1888. doi:DOI 10.1016/j.neuroimage.2011.01.041

Duvarci, S., Bauer, E. P., & Pare, D. (2009). The Bed Nucleus of the Stria Terminalis Mediates Inter-individual Variations in Anxiety and Fear. *Journal of Neuroscience*, *29*(33), 10357–10361. doi:Doi 10.1523/Jneurosci.2119-09.2009

Ebstein, R., Novick, O., Umansky, R., & Priel, B. (1996). Dopamine D4 receptor (D4DR) exon III polymorphism associated with the human personality trait of novelty seeking. *Nature …*. Retrieved from http://www.nature.com/ng/journal/v12/n1/abs/ng0196-78.html

Eisenberger, N. I., Lieberman, M. D., & Satpute, A. B. (2005). Personality from a controlled processing perspective: an fMRI study of neuroticism, extraversion, and self-consciousness. *Cognitive, affective & behavioral neuroscience*, *5*(2), 169–81. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16180623

Eley, T., Tahir, E., & Angleitner, A. (2003). Association analysis of MAOA and COMT with neuroticism assessed by peers. *American Journal of …*. Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/ajmg.b.20046/full

Escorihuela, R. M., Fernández-Teruel, A., Gil, L., Aguilar, R., Tobeña, A., & Driscoll, P. (1999). Inbred Roman high- and low-avoidance rats: differences in anxiety, novelty-seeking, and shuttlebox behaviors. *Physiology & behavior*, *67*(1), 19–26. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10463624

Etkin, A., Klemenhagen, K. C., Dudman, J. T., Rogan, M. T., Hen, R., Kandel, E. R., & Hirsch, J. (2004). Individual differences in trait anxiety predict the response of the basolateral amygdala to unconsciously processed fearful faces. *Neuron*, *44*(6), 1043–55. doi:10.1016/j.neuron.2004.12.006

Evans, J., Battersby, S., & Ogilvie, A. (1997). Association of short alleles of a VNTR of the serotonin transporter gene with anxiety symptoms in patients presenting after deliberate self harm. …. Retrieved from http://www.sciencedirect.com/science/article/pii/S0028390897000270

Eysenck, H. (1991). DIMENSIONS OF PERSONALITY - 16, 5 OR 3 - CRITERIA FOR A TAXONOMIC PARADIGM. *Personality and individual differences*, *12*(8), 773–790. Retrieved from http://www.sciencedirect.com/science/article/pii/019188699190144Z

Eysenck, M. (2000). A cognitive approach to trait anxiety. *European Journal of Personality*, *476*(February), 463–476. Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/1099-0984(200009/10)14:5<463::AID-PER393>3.0.CO;2-3/abstract

Eysenck, M., Derakshan, N., Santos, R., & Calvo, M. (2007). Anxiety and cognitive performance: attentional control theory. *Emotion (Washington, D.C.)*, *7*(2), 336–53. doi:10.1037/1528-3542.7.2.336

Eysenck, M., Macleod, C., & Mathews, A. (1987). Cognitive-Functioning and Anxiety. *Psychological Research-Psychologische Forschung*, *49*(2-3), 189–195. Retrieved from <Go to ISI>://A1987J695700017

Fales, C. L., Barch, D. M., & Burgess, G. C. (2008). Anxiety and cognitive efficiency: differential modulation of transient and sustained neural activity during a working memory task. *Cognitive, Affective, & …*, *8*(3), 239–253. doi:10.3758/CABN.8.3.239

Fallgatter, A., & Jatzke, S. (1999). Serotonin transporter promoter polymorphism influences topography of inhibitory motor control. *The International …*, (1999). Retrieved from http://journals.cambridge.org/production/action/cjoGetFulltext?fulltextid=52418

Fendt, M., & Fanselow, M. S. (1999). The neuroanatomical and neurochemical basis of conditioned fear. *Neuroscience and biobehavioral reviews*, *23*(5), 743–60. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10392663

Fernández Espejo, E. (1997). Structure of the mouse behaviour on the elevated plus-maze test of anxiety. *Behavioural brain research*, *86*(1), 105–12. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9105588

Ferreri, F., Lapp, L., & Peretti, C. (2011). Current research on cognitive aspects of anxiety disorders. *Current Opinion in Psychiatry*, *24*, 49–54. doi:10.1097/YCO.0b013e32833f5585

Field, A. P. (2009). *Discovering statistics using SPSS : (and sex, drugs and rock "n" roll)* (3rd ed.). Los Angeles: SAGE Publications. Retrieved from http://www.uk.sagepub.com/field3e/main.htm

File, S. E., & Seth, P. (2003). A review of 25 years of the social interaction test. *European Journal of Pharmacology*, *463*(1-3), 35–53. doi:10.1016/S0014-2999(03)01273-1

Finn, D. A., Rutledge-Gorman, M. T., & Crabbe, J. C. (2003). Genetic animal models of anxiety. *Neurogenetics*. Retrieved from http://www.springerlink.com/index/MC8MDGUFUDJPM4Q1.pdf

Flint, J. (2002). Genetic effects on an animal model of anxiety. *FEBS letters*. Retrieved from http://www.sciencedirect.com/science/article/pii/S0014579302031903

Flint, Jonathan. (2003). Animal models of anxiety and their molecular dissection. *Seminars in cell & developmental biology*, *14*(1), 37–42. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12524005

Fox, A. S., Shelton, S. E., Oakes, T. R., Converse, A. K., Davidson, R. J., & Kalin, N. H. (2010). Orbitofrontal cortex lesions alter anxiety-related activity in the primate bed nucleus of stria terminalis. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *30*(20), 7023–7. doi:10.1523/JNEUROSCI.5952-09.2010

Fox, E. (1994). Attentional bias in anxiety: A defective inhibition hypothesis. *Cognition & Emotion*, (August 2012), 37–41. Retrieved from http://www.tandfonline.com/doi/abs/10.1080/02699939408408934

Freud, S. (1921). *A general introduction to psychoanalysis. (8th ed.).* (8th ed.). Oxford, England.

Fyer, A. J., & Mannuzza, S. (1995). Specificity in familial aggregation of phobic disorders. *Archives of General …*, *52*, 564–573. Retrieved from http://psycnet.apa.org/psycinfo/1996-01584-001

Fyer, A. J., Mannuzza, S., Chapman, T. F., Lipsitz, J., Martin, L. Y., & Klein, D. F. (1996). Panic disorder and social phobia: effects of comorbidity on familial transmission. *Anxiety*, *2*(4), 173–8. doi:10.1002/(SICI)1522-7154(1996)2:4<173::AID-ANXI3>3.0.CO;2-P

Gaburro, S., Stiedl, O., Giusti, P., Sartori, S. B., Landgraf, R., & Singewald, N. (2011). A mouse model of high trait anxiety shows reduced heart rate variability that can be reversed by anxiolytic drug treatment. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, *14*(10), 1341–55. doi:10.1017/S1461145711000058

Gaudry, E., Vagg, P., & Spielberger, C. (1975). Validation of the state-trait distinction in anxiety research. *Behavioral Research*, *10*(3), 331–341. Retrieved from http://www.tandfonline.com/doi/abs/10.1207/s15327906mbr1003_6

Gentile, C. G., Jarrell, T. W., Teich, A., McCabe, P. M., & Schneiderman, N. (1986). The role of amygdaloid central nucleus in the retention of differential pavlovian conditioning of bradycardia in rabbits. *Behavioural brain research*, *20*(3), 263–73. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3741587

Gentili, C., Gobbini, M. I., Ricciardi, E., Vanello, N., Pietrini, P., Haxby, J. V., & Guazzelli, M. (2008). Differential modulation of neural activity throughout the distributed neural system for face perception in patients with Social Phobia and healthy subjects. *Brain research bulletin*, *77*(5), 286–92. doi:10.1016/j.brainresbull.2008.08.003

Gestel, S. V., & Broeckhoven, C. V. (2003). Genetics of personality: are we making progress? *Molecular Psychiatry*. Retrieved from http://www.nature.com/mp/journal/v8/n10/abs/4001367a.html

Gewirtz, J., Mcnish, K., & Davis, M. (1998). Lesions of the bed nucleus of the stria terminalis block sensitization of the acoustic startle reflex produced by repeated stress, but not fear-potentiated startle. *Progress in Neuro-Psychopharmacology …*. Retrieved from http://www.sciencedirect.com/science/article/pii/S0278584698000281

Ghashghaei, H. T., & Barbas, H. (2002). Pathways for emotion: interactions of prefrontal and anterior temporal pathways in the amygdala of the rhesus monkey. *Neuroscience*, *115*(4), 1261–79. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12453496

Goes, T. C., Antunes, F. D., & Teixeira-Silva, F. (2009). Trait and state anxiety in animal models: Is there correlation? *Neuroscience letters*, *450*(3), 266–9. doi:10.1016/j.neulet.2008.11.037

Gogos, J., & Morgan, M. (1998). Catechol-O-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. *Proceedings of the …*. Retrieved from http://www.pnas.org/content/95/17/9991.short

Goldman-Rakic, P., Selemon, L., & Schwartz, M. (1984). DUAL PATHWAYS CONNECTING THE DORSOLATERAL PREFRONTAL CORTEX WITH THE HIPPOCAMPAL FORMATION AND PARAHIPPOCAMPAL CORTEX IN THE RHESUS MONKEY. *Neuroscience*, *12*(3). Retrieved from http://www.sciencedirect.com/science/article/pii/0306452284901660

Gomez, R., & Francis, L. M. (2003). Generalised Anxiety Disorder: relationships with Eysenck's, Gray's and Newman's theories. *Personality and Individual Differences*, *34*(1), 3–17. doi:10.1016/S0191-8869(02)00020-X

Gottfried, J. A., & Dolan, R. J. (2004). Human orbitofrontal cortex mediates extinction learning while accessing conditioned representations of value. *Nature neuroscience*, *7*(10), 1144–52. doi:10.1038/nn1314

Graham, S., Phua, E., Soon, C. S., Oh, T., Au, C., Shuter, B., Wang, S.-C., et al. (2009). Role of medial cortical, hippocampal and striatal interactions during cognitive set-shifting. *NeuroImage*, *45*(4), 1359–67. doi:10.1016/j.neuroimage.2008.12.040

Griebel, G., Sanger, D. J., & Perrault, G. (1996). Further evidence for differences between non-selective and BZ-1 (omega 1) selective, benzodiazepine receptor ligands in murine models of "state" and "trait" anxiety. *Neuropharmacology*, *35*(8), 1081–91. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9121611

Grillon, C. (2002a). Associative learning deficits increase symptoms of anxiety in humans. *Biological Psychiatry*, *51*(11), 851–858. Retrieved from <Go to ISI>://000175651900001

Grillon, C. (2002b). Startle reactivity and anxiety disorders: Aversive conditioning, context, and neurobiology. *Biological Psychiatry*, *52*(10), 958–975. Retrieved from <Go to ISI>://000179238300006

Grillon, C., & Ameli, R. (2001). Conditioned inhibition of fear-potentiated startle and skin conductance in humans. *Psychophysiology*, *38*(5), 807–15. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11577904

Grillon, C., & Morgan, C. A. (1999). Fear-potentiated startle conditioning to explicit and contextual cues in Gulf War veterans with posttraumatic stress disorder. *Journal of abnormal psychology*, *108*(1), 134–42. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10066999

Gross, C., Santarelli, L., & Brunner, D. (2000). Altered fear circuits in 5-HT1A receptor KO mice. *Biological …*. Retrieved from http://directory.umm.ac.id/Data Elmu/jurnal/B/Biological Psichatry/Vol48.Issue12.2000/6906.pdf

Haas, B. W., Omura, K., Constable, R. T., & Canli, T. (2007). Emotional conflict and neuroticism: personality-dependent activation in the amygdala and subgenual anterior cingulate. *Behavioral neuroscience*, *121*(2), 249–56. doi:10.1037/0735-7044.121.2.249

Hahn, A., Stein, P., Windischberger, C., Weissenbacher, A., Spindelegger, C., Moser, E., Kasper, S., et al. (2011). Reduced resting-state functional connectivity between amygdala and orbitofrontal cortex in social anxiety disorder. *NeuroImage*, *56*(3), 881–9. doi:10.1016/j.neuroimage.2011.02.064

Handley, S. L., McBlane, J. W., Critchley, M. a, & Njung'e, K. (1993). Multiple serotonin mechanisms in animal models of anxiety: environmental, emotional and cognitive factors. *Behavioural brain research*, *58*(1-2), 203–10. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8136047

Hansell, N. K., Wright, M. J., Medland, S. E., Davenport, T. a, Wray, N. R., Martin, N. G., & Hickie, I. B. (2012). Genetic co-morbidity between neuroticism, anxiety/depression and somatic distress in a population sample of adolescent and young adult twins. *Psychological medicine*, *42*(6), 1249–60. doi:10.1017/S0033291711002431

Hariri, A. R., Mattay, V. S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., Egan, M. F., et al. (2002). Serotonin transporter genetic variation and the response of the human amygdala. *Science (New York, N.Y.)*, *297*(5580), 400–3. doi:10.1126/science.1071829

Heinz, A., Braus, D. F., Smolka, M. N., Wrase, J., Puls, I., Hermann, D., Klein, S., et al. (2005). Amygdala-prefrontal coupling depends on a genetic variation of the serotonin transporter. *Nature neuroscience*, *8*(1), 20–1. doi:10.1038/nn1366

Heisler, L., & Chu, H. (1998). Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice. *Proceedings of the …*. Retrieved from http://www.pnas.org/content/95/25/15049.short

Heisler, L., Zhou, L., & Bajwa, P. (2007). Serotonin 5-HT2C receptors regulate anxiety-like behavior. *Genes, brain, and behavior*. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1601-183X.2007.00316.x/full

Henderson, A. S., Korten, A. E., Jorm, A. F., Jacomb, P. A., Christensen, H., Rodgers, B., Tan, X., et al. (2000). COMT and DRD3 polymorphisms, environmental exposures, and personality traits related to common mental disorders. *American journal of …*, *96*(1), 102–107. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10686561

Henniger, M. S., Ohl, F., Hölter, S. M., Weissenbacher, P., Toschi, N., Lörscher, P., Wigger, a, et al. (2000). Unconditioned anxiety and social behaviour in two rat lines selectively bred for high and low anxiety-related behaviour. *Behavioural brain research*, *111*(1-2), 153–63. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10840141

Hettema, J. M. (2001). A review and meta-analysis of the genetic epidemiology of anxiety disorders. *American Journal of …*, (October), 1568–1578. Retrieved from http://ajp.psychiatryonline.org/article.aspx?articleID=175066

Hishinuma, E. S., Miyamoto, R. H., Nishimura, S. T., Goebert, D. A., Yuen, N. Y., Makini, G. K., Andrade, N. N., et al. (2001). Prediction of anxiety disorders using the state-trait anxiety inventory for multiethnic adolescents. *Journal of anxiety disorders*, *15*(6), 511–33. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11764310

Hitchcock, J., & Davis, M. (1986). Lesions of the amygdala, but not of the cerebellum or red nucleus, block conditioned fear as measured with the potentiated startle paradigm. *Behavioral neuroscience*, *100*(1), 11–22. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3954873

Hoehn-saric, R. (1989). Somatic Manifestations in Women With Generalized Anxiety Disorder. *Archives of general psychiatry*, *46*, 1113–1119.

Hoehn-saric, R., & Hipsley, P. (1995). Is hyperarousal essential to obsessive-compulsive disorder? *Archives of general psychiatry*, *52*, 688–693.

Holland, P. C. (1977). Conditioned stimulus as a determinant of the form of the Pavlovian conditioned response. *Journal of experimental psychology. Animal behavior processes*, *3*(1), 77–104. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/845545

Holmes, A., & Yang, R. (2003). Mice lacking the serotonin transporter exhibit 5-HT1A receptor-mediated abnormalities in tests for anxiety-like behavior. …. Retrieved from http://www.molecularpsychiatry.uk-wuerzburg.de/fileadmin/uk/molecularpsychiatry/PDFs/2003/holmesetal2003mice.pdf

Homberg, J. (2011). The stress-coping (mis) match hypothesis for nature x nurture interactions. *Brain Research*, *1432*, 114–121. doi:10.1016/j.brainres.2011.11.037

Homberg, J. R., & Lesch, K.-P. (2011). Looking on the bright side of serotonin transporter gene variation. *Biological psychiatry*, *69*(6), 513–9. doi:10.1016/j.biopsych.2010.09.024

Horwath, E., Wolk, S. I., Goldstein, R. B., Wickramaratne, P., Sobin, C., Adams, P., Lish, J. D., et al. (1995). Is the Comorbidity Between Social Phobia and Panic Disorder Due to Familial Cotransmission or Other Factors? *Archives of General Psychiatry*, *52*(7), 574–582. doi:10.1001/archpsyc.1995.03950190056008

Hyman, M. B., & Pedrick, C. (2006). *Anxiety Disorders*. Twenty-First Century Books (CT). Retrieved from http://www.amazon.co.uk/Anxiety-Disorders-Bruce-Pedrick-Cherry/dp/B006SRWPQ6

Indovina, I., Robbins, T. W., Núñez-Elizalde, A. O., Dunn, B. D., & Bishop, S. J. (2011). Fear-conditioning mechanisms associated with trait vulnerability to anxiety in humans. *Neuron*, *69*(3), 563–71. doi:10.1016/j.neuron.2010.12.034

Isbell, L. A. (2006). Snakes as agents of evolutionary change in primate brains. *Journal of human evolution*, *51*(1), 1–35. doi:10.1016/j.jhevol.2005.12.012

Iwata, J., LeDoux, J. E., Meeley, M. P., Arneric, S., & Reis, D. J. (1986). Intrinsic neurons in the amygdaloid field projected to by the medial geniculate body mediate emotional responses conditioned to acoustic stimuli. *Brain research*, *383*(1-2), 195–214. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3768689

Izquierdo, A., & Murray, E. A. (2004). Combined unilateral lesions of the amygdala and orbital prefrontal cortex impair affective processing in rhesus monkeys. *Journal of neurophysiology*, *91*(5), 2023–39. doi:10.1152/jn.00968.2003

Izquierdo, A., Suda, R. K., & Murray, E. A. (2005). Comparison of the effects of bilateral orbital prefrontal cortex lesions and amygdala lesions on emotional responses in rhesus monkeys. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *25*(37), 8534–42. doi:10.1523/JNEUROSCI.1232-05.2005

Jakovcevski, M., Schachner, M., & Morellini, F. (2008). Individual variability in the stress response of C57BL/6J male mice correlates with trait anxiety. *Genes, brain, and behavior*, *7*(2), 235–43. doi:10.1111/j.1601-183X.2007.00345.x

Jedema, H. P., Gianaros, P. J., Greer, P. J., Kerr, D. D., Liu, S., Higley, J. D., Suomi, S. J., et al. (2010). Cognitive impact of genetic variation of the serotonin transporter in primates is associated with differences in brain morphology rather than serotonin neurotransmission. *Molecular psychiatry*, *15*(5), 512–22, 446. doi:10.1038/mp.2009.90

John, O., & Srivastava, S. (1999). The Big Five trait taxonomy: History, measurement, and theoretical perspectives. *Handbook of personality: Theory and …*, (510). Retrieved from http://books.google.com/books?hl=en&lr=&id=b0yalwi1HDMC&oi=fnd&pg=PA102&dq=The+Big-Five+Trait+Taxonomy:+History,+Measurement,+and+Theoretical+Perspectives&ots=754CS6VrTq&sig=mLUQguBLFiu8Izk_WbS15OEnlTc

Johnson, D. R. (2009). Emotional attention set-shifting and its relationship to anxiety and emotion regulation. *Emotion (Washington, D.C.)*, *9*(5), 681–90. doi:10.1037/a0017095

Jorm, A. F., & Henderson, A. S. (2000). Association of smoking and personality with a polymorphism of the dopamine transporter gene: results from a community survey. *American journal of …*. Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/1096-8628(20000612)96:3<331::AID-AJMG19>3.0.CO;2-0/full

Jovanovic, T., Kazama, A., Bachevalier, J., & Davis, M. (2012). Impaired safety signal learning may be a biomarker of PTSD. *Neuropharmacology*, *62*(2), 695–704. doi:10.1016/j.neuropharm.2011.02.023

Kaidanovich-Beilin, O., Lipina, T., Vukobradovic, I., Roder, J., & Woodgett, J. R. (2011). Assessment of social interaction behaviors. *Journal of visualized experiments : JoVE*, (48), 1–6. doi:10.3791/2473

Kalin, N. H. (1993). The neurobiology of fear. *Scientific American*, (May). Retrieved from http://www.sciamdigital.com/gsp_qpdf.cfm?ISSUEID_CHAR=4E3B6963-3358-4EBE-9524-A7CFD3F328A&ARTICLEID_CHAR=E5A11D75-5D44-4022-A4C1-AAAD0B14781

Kalin, N. H., & Shelton, S. E. (1989). Defensive behaviors in infant rhesus monkeys: environmental cues and neurochemical regulation. *Science (New York, N.Y.)*, *243*(4899), 1718–21. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2564702

Kalin, N. H., & Shelton, S. E. (2003). Nonhuman primate models to study anxiety, emotion regulation, and psychopathology. *Annals of the New York Academy of Sciences*, *1008*, 189–200. Retrieved from http://onlinelibrary.wiley.com/doi/10.1196/annals.1301.021/full

Kalin, N. H., Shelton, S. E., & Davidson, R. J. (2004). The role of the central nucleus of the amygdala in mediating fear and anxiety in the primate. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *24*(24), 5506–15. doi:10.1523/JNEUROSCI.0292-04.2004

Kalin, N. H., Shelton, S. E., & Davidson, R. J. (2007). Role of the primate orbitofrontal cortex in mediating anxious temperament. *Biological psychiatry*, *62*(10), 1134–9. doi:10.1016/j.biopsych.2007.04.004

Kalin, N. H., Shelton, S. E., Davidson, R. J., & Kelley, A. E. (2001). The primate amygdala mediates acute fear but not the behavioral and physiological components of anxious temperament. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *21*(6), 2067–74. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11245690

Kalin, N. H., Shelton, S. E., Fox, A. S., Oakes, T. R., & Davidson, R. J. (2005). Brain regions associated with the expression and contextual regulation of anxiety in primates. *Biological psychiatry*, *58*(10), 796–804. doi:10.1016/j.biopsych.2005.05.021

Karadag, F., Oguzhanoglu, N., Ozdel, O., Atesci, F. C., & Amuk, T. (2005). Memory function in patients with obsessive compulsive disorder and the problem of confidence in their memories: a clinical study. *Croatian medical journal*, *46*(2), 282–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15849851

Kendler, K., & Myers, J. (2001). The genetic epidemiology of irrational fears and phobias in men. *Archives of General …*, *58*, 257–265. Retrieved from http://archpsyc.ama-assn.org/cgi/reprint/58/3/257.pdf

Kendler, K., & Neale, M. (1992). The genetic epidemiology of phobias in women: the interrelationship of agoraphobia, social phobia, situational phobia, and simple phobia. *Archives of General …*, *42*, 273–281. Retrieved from http://archpsyc.ama-assn.org/cgi/reprint/49/4/273.pdf

Kendler, K. S., Neale, M. C., Kessler, R. C., Heath, A. C., & Eaves, L. J. (1993). Panic disorder in women: a population-based twin study. *Psychological Medicine*, *23*(02), 397–406. Retrieved from http://journals.cambridge.org/abstract_S003329170002849X

Kennedy, B., Schwab, J., Morris, R., & Beldia, G. (2001). Assessment of state and trait anxiety in subjects with anxiety and depressive disorders. *Psychiatric Quarterly*, *72*(3), 263–276. Retrieved from http://www.springerlink.com/index/J417516631W2K410.pdf

Kessler, R., Berglund, P., & Demler, O. (2003). The epidemiology of major depressive disorder. *JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION*, *289*(23), 3095–3105. Retrieved from http://jama.ama-assn.org/content/289/23/3095.short

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of general psychiatry*, *62*(6), 593–602. doi:10.1001/archpsyc.62.6.593

Kim, M., & Whalen, P. (2009). The structural integrity of an amygdala–prefrontal pathway predicts trait anxiety. *The Journal of Neuroscience*, *29*(37), 11614–11618. doi:10.1523/JNEUROSCI.2335-09.2009

Kimura, A., & Stevenson, P. (2009). Overexpression of 5-HT2C receptors in forebrain leads to elevated anxiety and hypoactivity. *European Journal of …*. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1460-9568.2009.06831.x/full

King, F. A., Yarbrough, C. J., Anderson, D. C., Gordon, T. P., & Gould, K. G. (1988). Primates. *Science (New York, N.Y.)*, *240*(4858), 1475–82. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3287624

King, N., & Mietz, A. (1995). Psychopathology and cognition in adolescents experiencing severe test anxiety. *Journal of clinical child …*, 37–41. Retrieved from http://www.tandfonline.com/doi/abs/10.1207/s15374424jccp2401_6

Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., De Jong, I. C., et al. (1999). Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience and biobehavioral reviews*, *23*(7), 925–35. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10580307

Kotov, R., Gamez, W., Schmidt, F., & Watson, D. (2010). Linking "big" personality traits to anxiety, depressive, and substance use disorders: a meta-analysis. *Psychological bulletin*, *136*(5), 768–821. doi:10.1037/a0020327

Kringelbach, M. L., & Rolls, E. T. (2004). The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. *Progress in neurobiology*, *72*(5), 341–72. doi:10.1016/j.pneurobio.2004.03.006

LaBar, K., & Gatenby, J. (1998). Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. *NEURON- …*, *20*, 937–945. Retrieved from http://dionysus.psych.wisc.edu/lit/articles/LaBarK1998a.pdf

Lacroix, L., Spinelli, S., Heidbreder, C. A., & Feldon, J. (2000). Differential role of the medial and lateral prefrontal cortices in fear and anxiety. *Behavioral neuroscience*, *114*(6), 1119–30. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11142644

Landgraf, R., & Wigger, A. (2002). High vs low anxiety-related behavior rats: an animal model of extremes in trait anxiety. *Behavior genetics*, *32*(5), 301–14. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12405513

Lang, P. J., Davis, M., & Ohman, A. (2000). Fear and anxiety: animal models and human cognitive psychophysiology. *Journal of affective disorders*, *61*(3), 137–59. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11163418

Lang, S., Kroll, A., Lipinski, S. J., Wessa, M., Ridder, S., Christmann, C., Schad, L. R., et al. (2009). Context conditioning and extinction in humans: differential contribution of the hippocampus, amygdala and prefrontal cortex. *The European journal of neuroscience*, *29*(4), 823–32. doi:10.1111/j.1460-9568.2009.06624.x

Lau, J. Y. F., Eley, T. C., & Stevenson, J. (2006). Examining the state-trait anxiety relationship: a behavioural genetic approach. *Journal of abnormal child psychology*, *34*(1), 19–27. doi:10.1007/s10802-005-9006-7

LeDoux, J. (2000). Emotion circuits in the brain. *Annual review of neuroscience*, 155–184. Retrieved from http://www.annualreviews.org/doi/abs/10.1146/annurev.neuro.23.1.155

LeDoux, J. E. (1995). Emotion: clues from the brain. *Annual review of psychology*, *46*, 209–35. doi:10.1146/annurev.ps.46.020195.001233

LeDoux, J. E. (2000). The amygdala and emotion: a view through fear. In J. P. Aggleton (Ed.), *The Amygdala* (pp. 289–310). New York: Oxford University Press.

LeDoux, J. E., Iwata, J., Cicchetti, P., & Reis, D. J. (1988). Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *8*(7), 2517–29. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2854842

Ledoux, J. E., Romanski, M., & Xagoraris, A. (1990). The Lateral Amygdaloid in Fear Conditioning Nucleus : Sensory Interface Amygdala. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *10*(April), 1062–1069.

Legrand, L. N., McGue, M., & Iacono, W. G. (1999). A twin study of state and trait anxiety in childhood and adolescence. *Journal of child psychology and psychiatry, and allied disciplines*, *40*(6), 953–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10509889

Lesch, K., & Mössner, R. (1998). Genetically driven variation in serotonin uptake: is there a link to affective spectrum, neurodevelopmental, and neurodegenerative disorders? *Biological psychiatry*. Retrieved from http://ukpmc.ac.uk/abstract/MED/9693390

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., Benjamin, J., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, *274*(5292), 1527. Retrieved from http://www.sciencemag.org/content/274/5292/1527.short

Levine, S., Atha, K., & Wiener, S. G. (1993). Early experience effects on the development of fear in the squirrel monkey. *Behavioral and neural biology*, *60*(3), 225–33. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8297318

Linares, I., & Trzesniak, C. (2012). Neuroimaging in specific phobia disorder: a systematic review of the literature. *Revista Brasileira de …*, *34*(1), 101–111. Retrieved from http://www.scielo.br/scielo.php?pid=S1516-44462012000100017&script=sci_arttext&tlng=pt

Lissek, S., & Grillon, C. (2010). Overgeneralization of Conditioned Fear in the Anxiety Disorders. *Zeitschrift für Psychologie / Journal of Psychology*, *218*(2), 146–148. doi:10.1027/0044-3409/a000022

Lissek, S., Powers, A. S., McClure, E. B., Phelps, E. A., Woldehawariat, G., Grillon, C., & Pine, D. S. (2005). Classical fear conditioning in the anxiety disorders: a meta-analysis. *Behaviour research and therapy*, *43*(11), 1391–424. doi:10.1016/j.brat.2004.10.007

Lissek, S., Rabin, S., Heller, R. E., Lukenbaugh, D., Geraci, M., Pine, D. S., & Grillon, C. (2010). Overgeneralization of conditioned fear as a pathogenic marker of panic disorder. *The American journal of psychiatry*, *167*(1), 47–55. doi:10.1176/appi.ajp.2009.09030410

Lissek, S., Rabin, S. J., McDowell, D. J., Dvir, S., Bradford, D. E., Geraci, M., Pine, D. S., et al. (2009). Impaired discriminative fear-conditioning resulting from elevated fear responding to learned safety cues among individuals with panic disorder. *Behaviour research and therapy*, *47*(2), 111–8. doi:10.1016/j.brat.2008.10.017

Lister, R. (1987). The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology*, (t 987), 0–5. Retrieved from http://www.springerlink.com/index/n3148570r2632146.pdf

Liston, C., Miller, M. M., Goldwater, D. S., Radley, J. J., Rocher, A. B., Hof, P. R., Morrison, J. H., et al. (2006). Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *26*(30), 7870–4. doi:10.1523/JNEUROSCI.1184-06.2006

López-Aumatell, R., Blázquez, G., Gil, L., Aguilar, R., Cañete, T., Giménez-Llort, L., Tobeña, A., et al. (2009). The Roman High- and Low-Avoidance rat strains differ in fear-potentiated startle and classical aversive conditioning. *Psicothema*, *21*(1), 27–32. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19178852

Machado, C. J., & Bachevalier, J. (2008). Behavioral and hormonal reactivity to threat: effects of selective amygdala, hippocampal or orbital frontal lesions in monkeys. *Psychoneuroendocrinology*, *33*(7), 926–941. doi:10.1016/j.psyneuen.2008.04.012.Behavioral

Macmillan, A. N., & Creelman, C. D. (2005). *DETECTION THEORY A User's Guide* (Second Edi.). New Jersey: Lawrence Erlbaum Associations, Inc.

Maier, W., Lichtermann, D., Minges, J., Oehrlein, A., & Franke, P. (1993). A controlled family study in panic disorder. *Journal of Psychiatric Research*, *27*(null), 79–87. doi:10.1016/0022-3956(93)90019-X

Man, M. S., Clarke, H. F., & Roberts, A. C. (2009). The role of the orbitofrontal cortex and medial striatum in the regulation of prepotent responses to food rewards. *Cerebral cortex (New York, N.Y. : 1991)*, *19*(4), 899–906. doi:10.1093/cercor/bhn137

Man, M.-S., Mikheenko, Y., Braesicke, K., Cockcroft, G., & Roberts, A. C. (2011). Serotonin at the level of the amygdala and orbitofrontal cortex modulates distinct aspects of positive emotion in primates. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, 1–15. doi:10.1017/S1461145711000587

Mannuzza, S., & Schneier, F. (1995). Generalized social phobia: reliability and validity. *Archives of general …*, *52*, 230–237. Retrieved from http://archpsyc.ama-assn.org/cgi/reprint/52/3/230.pdf

Maren, S., & Quirk, G. J. (2004). Neuronal signalling of fear memory. *Nature reviews. Neuroscience*, *5*(11), 844–52. doi:10.1038/nrn1535

Marks, I. M., Nesse, R. M., & Arbor, A. (1994). Fear and Fitness : An Evolutionary Analysis of Anxiety Disorders. *Group*, *261*, 247–261.

Massana, J., López Risueño, J. A., Masana, G., Marcos, T., González, L., & Otero, A. (2001). Subtyping of panic disorder patients with bradycardia. *European psychiatry : the journal of the Association of European Psychiatrists*, *16*(2), 109–14. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11311175

Mathews, A., & MacLeod, C. (1985). Selective processing of threat cues in anxiety states. *Behaviour research and therapy*, *23*(5), 563–569. Retrieved from http://www.sciencedirect.com/science/article/pii/0005796785901044

Mathews, A., Richards, A., & Eysenck, M. (1989). Interpretation of homophones related to threat in anxiety states. *Journal of abnormal psychology*, *98*(1), 31–4. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2708637

Mauchnik, J., Ebner-Priemer, U. W., Bohus, M., & Schmahl, C. (2010). Classical Conditioning in Borderline Personality Disorder With and Without Posttraumatic Stress Disorder*. *Zeitschrift für Psychologie / Journal of Psychology*, *218*(2), 80–88. doi:10.1027/0044-3409/a000015

Maximino, C., Brito, T., & Gouveia Jr., A. (2010). Construct validity of behavioral models of anxiety: where experimental psychopathology meets ecology and evolution. *Psychology and Neuroscience*, *3*(1), 117–123. doi:10.3922/j.psns.2010.1.015

McFall, M. E., Murburg, M. M., Ko, G. N., & Veith, R. C. (1990). Autonomic responses to stress in Vietnam combat veterans with posttraumatic stress disorder. *Biological psychiatry*, *27*(10), 1165–75. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2340325

McKeon, P., & Murray, R. (1987). Familial aspects of obsessive-compulsive neurosis. *The British Journal of Psychiatry*, *151*, 528–534. Retrieved from http://bjp.rcpsych.org/content/151/4/528.short

McNally, R. J., Kaspi, S. P., Riemann, B. C., & Zeitlin, S. B. (1990). Selective processing of threat cues in posttraumatic stress disorder. *Journal of abnormal psychology*, *99*(4), 398–402. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2266215

McNeil, D. W., Vrana, S. R., Melamed, B. G., Cuthbert, B. N., & Lang, P. J. (1993). Emotional imagery in simple and social phobia: fear versus anxiety. *Journal of abnormal psychology*, *102*(2), 212–25. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8315134

Melke, J., & Landén, M. (2001). Serotonin transporter gene polymorphisms are associated with anxietyâ€related personality traits in women. *American journal of …*. Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/ajmg.1434/full

Mendlewicz, J., Papadimitriou, G., & Wilmotte, J. (1993). Family study of panic disorder: comparison with generalized anxiety disorder, major depression and normal subjects. *Psychiatric genetics*, *3*(2), 73–78. Retrieved from http://journals.lww.com/psychgenetics/Abstract/1993/22000/Family_study_of_panic_disorder__comparison_with.2.aspx

Meunier, M., & Bachevalier, J. (2002). Comparison of emotional responses in monkeys with rhinal cortex or amygdala lesions. *Emotion*, *2*, 147–161. Retrieved from http://psycnet.apa.org/journals/emo/2/2/147/

Meunier, M., Bachevalier, J., Murray, E. A., Málková, L., & Mishkin, M. (1999). Effects of aspiration versus neurotoxic lesions of the amygdala on emotional responses in monkeys. *The European journal of neuroscience*, *11*(12), 4403–18. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10594668

Mezzich, J. E. (2002). International surveys on the use of ICD-10 and related diagnostic systems. *Psychopathology*, *35*(2-3), 72–5. doi:65122

Michael, T., Blechert, J., Vriends, N., Margraf, J., & Wilhelm, F. H. (2007). Fear conditioning in panic disorder: Enhanced resistance to extinction. *Journal of abnormal psychology*, *116*(3), 612–7. doi:10.1037/0021-843X.116.3.612

Mikheenko, Y., Man, M.-S., Braesicke, K., Johns, M. E., Hill, G., Agustín-Pavón, C., & Roberts, A. C. (2010). Autonomic, behavioral, and neural analyses of mild conditioned negative affect in marmosets. *Behavioral neuroscience*, *124*(2), 192–203. doi:10.1037/a0018868

Milad, M. R., & Rauch, S. L. (2007). The role of the orbitofrontal cortex in anxiety disorders. *Annals of the New York Academy of Sciences*, *1121*, 546–61. doi:10.1196/annals.1401.006

Millan, M. J. (2003). The neurobiology and control of anxious states. *Progress in Neurobiology*, *70*(2), 83–244. doi:10.1016/S0301-0082(03)00087-X

Mineka, S, & Cook, M. (1993). Mechanisms involved in the observational conditioning of fear. *Journal of experimental psychology. General*, *122*(1), 23–38. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8440976

Mineka, Susan, & Oehlberg, K. (2008). The relevance of recent developments in classical conditioning to understanding the etiology and maintenance of anxiety disorders. *Acta psychologica*, *127*(3), 567–80. doi:10.1016/j.actpsy.2007.11.007

Mineka, Susan, & Zinbarg, R. (2006). A contemporary learning theory perspective on the etiology of anxiety disorders: it's not what you thought it was. *The American psychologist*, *61*(1), 10–26. doi:10.1037/0003-066X.61.1.10

Mitsuyasu, H., Hirata, N., & Sakai, Y. (2001). Association analysis of polymorphisms in the upstream region of the human dopamine D4 receptor gene (DRD4) with schizophrenia and personality traits. *Journal of human …*. Retrieved from http://www.springerlink.com/index/923XNDMJ4VJRWKRA.pdf

Miyake, A., Friedman, N. P., Emerson, M. J., Witzki, A. H., Howerter, A., & Wager, T. D. (2000). The unity and diversity of executive functions and their contributions to complex “Frontal Lobe” tasks: a latent variable analysis. *Cognitive psychology*, *41*(1), 49–100. doi:10.1006/cogp.1999.0734

Monchi, O., Petrides, M., Petre, V., Worsley, K., & Dagher, a. (2001). Wisconsin Card Sorting revisited: distinct neural circuits participating in different stages of the task identified by event-related functional magnetic resonance imaging. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *21*(19), 7733–41. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11567063

Monkul, E. S., Onur, E., Tural, U., Hatch, J. P., Alkın, T., Yücel, B., & Fidaner, H. (2010). History of suffocation, state-trait anxiety, and anxiety sensitivity in predicting 35% carbon dioxide-induced panic. *Psychiatry research*, *179*(2), 194–7. doi:10.1016/j.psychres.2009.06.015

Morgan, M. A., & LeDoux, J. E. (1999). Contribution of ventrolateral prefrontal cortex to the acquisition and extinction of conditioned fear in rats. *Neurobiology of learning and memory*, *72*(3), 244–51. doi:10.1006/nlme.1999.3907

Morgan, M. A., Romanski, L. M., & LeDoux, J. E. (1993). Extinction of emotional learning: contribution of medial prefrontal cortex. *Neuroscience letters*, *163*(1), 109–13. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8295722

Most, S. B., Chun, M. M., Johnson, M. R., & Kiehl, K. a. (2006). Attentional modulation of the amygdala varies with personality. *NeuroImage*, *31*(2), 934–44. doi:10.1016/j.neuroimage.2005.12.031

Muris, P., Merckelbach, H., Schmidt, H., & Tierney, S. (1999). Disgust sensitivity, trait anxiety and anxiety disorders symptoms in normal children. *Behaviour research and therapy*, *37*(10), 953–61. doi:10.1016/S0005-7967(99)00045-5

Murray, E. A. (2007). The amygdala, reward and emotion. *Trends in cognitive sciences*, *11*(11), 489–97. doi:10.1016/j.tics.2007.08.013

Nelson, E. E., Shelton, S. E., & Kalin, N. H. (2003). Individual differences in the responses of naïve rhesus monkeys to snakes. *Emotion*, *3*(1), 3–11. doi:10.1037/1528-3542.3.1.3

Newman, J. D., & Farley, M. J. (1995). An ethologically based, stimulus and gender-sensitive nonhuman primate model for anxiety. *Progress in neuro-psychopharmacology & biological psychiatry*, *19*(4), 677–85. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8588065

Nitschke, J. B., Sarinopoulos, I., Oathes, D. J., Johnstone, T., Whalen, P. J., Davidson, R. J., & Kalin, N. H. (2009). Anticipatory activation in the amygdala and anterior cingulate in generalized anxiety disorder and prediction of treatment response. *The American journal of psychiatry*, *166*(3), 302–10. doi:10.1176/appi.ajp.2008.07101682

Noble, E. P., Ozkaragoz, T. Z., Ritchie, T. L., Zhang, X., Belin, T. R., & Sparkes, R. S. (1998). D2 and D4 dopamine receptor polymorphisms and personality. *American journal of medical genetics*, *81*(3), 257–67.

Noyes, R., Crowe, R. R., Harris, E. L., Hamra, B. J., McChesney, C. M., & Chaudhry, D. R. (1986). Relationship between panic disorder and agoraphobia. A family study. *Archives of general psychiatry*, *43*(3), 227–32. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3954542

Noyes, R. J., & Clarkson, C. (1987). A family study of generalized anxiety disorder. *The American journal …*, *144*, 1019–1024. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3605423

Ochsner, K., & Bunge, S. (2002). Rethinking feelings: An fMRI study of the cognitive regulation of emotion. *Journal of Cognitive …*, 1215–1229. Retrieved from http://www.mitpressjournals.org/doi/abs/10.1162/089892902760807212

Ochsner, K. N., & Gross, J. J. (2008). Cognitive emotion regulation: insights from social, cognitive and affective neuroscience. *Current directions in psychological science*, *17*(2), 153–158.

Okuyama, Y., Ishiguro, H., & Nankai, M. (2000). Identification of a polymorphism in the promoter region of DRD4 associated with the human novelty seeking personality trait. *Molecular …*. Retrieved from http://ukpmc.ac.uk/abstract/MED/10673770

Onishi, B. K. A., & Xavier, G. F. (2010). Contextual, but not auditory, fear conditioning is disrupted by neurotoxic selective lesion of the basal nucleus of amygdala in rats. *Neurobiology of learning and memory*, *93*(2), 165–74. doi:10.1016/j.nlm.2009.09.007

Orr, S. P., Metzger, L. J., Lasko, N. B., Macklin, M. L., Peri, T., & Pitman, R. K. (2000). De novo conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. *Journal of Abnormal Psychology*, *109*(2), 290–298. doi:10.1037//0021-843X.109.2.290

Otto, T., & Poon, P. (2006). Dorsal hippocampal contributions to unimodal contextual conditioning. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *26*(24), 6603–9. doi:10.1523/JNEUROSCI.1056-06.2006

Paparrigopoulos, T., Melissaki, A., Efthymiou, A., Tsekou, H., Vadala, C., Kribeni, G., Pavlou, E., et al. (2006). Short-term psychological impact on family members of intensive care unit patients. *Journal of psychosomatic research*, *61*(5), 719–22. doi:10.1016/j.jpsychores.2006.05.013

Parks, C., & Robinson, P. (1998). Increased anxiety of mice lacking the serotonin1A receptor. *Proceedings of the …*. Retrieved from http://www.pnas.org/content/95/18/10734.short

Paterson, A., Sunohara, G., & Kennedy, J. (1999). Dopamine D4 receptor gene: novelty or nonsense? *Neuropsychopharmacology*. Retrieved from http://www.sciencedirect.com/science/article/pii/S0893133X98001158

Pauls, D. L., Alsobrook, J. P., Goodman, W., Rasmussen, S., & Leckman, J. F. (1995). A family study of obsessive-compulsive disorder. *American Journal of Psychiatry*, *152*, 76–84.

Pawlak, C. R., Ho, Y.-J., & Schwarting, R. K. W. (2008). Animal models of human psychopathology based on individual differences in novelty-seeking and anxiety. *Neuroscience and biobehavioral reviews*, *32*(8), 1544–68. doi:10.1016/j.neubiorev.2008.06.007

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*(3), 149–67. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2864480

Peri, T., Ben-Shakhar, G., Orr, S. P., & Shalev, A. Y. (2000). Psychophysiologic assessment of aversive conditioning in posttraumatic stress disorder. *Biological psychiatry*, *47*(6), 512–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10715357

Perna, G., Caldirola, D., Arancio, C., & Bellodi, L. (1997). Panic attacks: a twin study. *Psychiatry Research*, *66*(1), 69–71. doi:10.1016/S0165-1781(97)85177-3

Pezawas, L., & Meyer-Lindenberg, A. (2005). 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. *Nature …*, *8*(6), 828–834. doi:10.1038/nn1463

Phelps, E. A., & LeDoux, J. E. (2005). Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron*, *48*(2), 175–87. doi:10.1016/j.neuron.2005.09.025

Phillips, R. G., & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral neuroscience*, *106*(2), 274–85. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1590953

Pineles, S. L., Shipherd, J. C., Mostoufi, S. M., Abramovitz, S. M., & Yovel, I. (2009). Attentional biases in PTSD: More evidence for interference. *Behaviour research and therapy*, *47*(12), 1050–7. doi:10.1016/j.brat.2009.08.001

Pistorio, A. L., Vintch, B., & Wang, X. (2006). Acoustic analysis of vocal development in a New World primate, the common marmoset (Callithrix jacchus). *The Journal of the Acoustical Society of America*, *120*(3), 1655. doi:10.1121/1.2225899

Piñeyro, G., & Blier, P. (1999). Autoregulation of serotonin neurons: role in antidepressant drug action. *Pharmacological reviews*. Retrieved from http://pharmrev.aspetjournals.org/content/51/3/533.short

Plehn, K., & Peterson, R. A. (2002). Anxiety sensitivity as a predictor of the development of panic symptoms, panic attacks, and panic disorder: a prospective study. *Journal of anxiety disorders*, *16*(4), 455–74. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12213039

Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *European Journal of Pharmacology*, *463*(1-3), 3–33. doi:10.1016/S0014-2999(03)01272-X

Quirk, G J, & Gehlert, D. R. (2003). Inhibition of the Amygdala: Key to Pathological States? *Annals of the New York Academy of*, *985*, 263–272. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1749-6632.2003.tb07087.x/full

Quirk, Gregory J, Garcia, R., & González-Lima, F. (2006). Prefrontal mechanisms in extinction of conditioned fear. *Biological psychiatry*, *60*(4), 337–43. doi:10.1016/j.biopsych.2006.03.010

Rachman, S., & Silva, P. de. (1978). Abnormal and normal obsessions. *Behaviour research and therapy*. Retrieved from http://www.sciencedirect.com/science/article/pii/0005796778900220

Ramboz, S., & Oosting, R. (1998). Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. *Proceedings of the …*. Retrieved from http://www.pnas.org/content/95/24/14476.short

Ramboz, S., Saudou, F., Amara, D. A., Belzung, C., Segu, L., Misslin, R., Buhot, M. C., et al. (1996). 5-HT1B receptor knockout -behavioral consequences. *Behavioural brain research*, *73*(1-2), 305–12.

Rasmussen, S. (1993). Genetic studies of obsessive-compulsive disorder. *Annals of clinical psychiatry*. Retrieved from http://psycnet.apa.org/psycinfo/1994-21667-001

Ray, R. D., Ochsner, K. N., Cooper, J. C., Robertson, E. R., Gabrieli, J. D. E., & Gross, J. J. (2005). Individual differences in trait rumination and the neural systems supporting cognitive reappraisal. *Cognitive, affective & behavioral neuroscience*, *5*(2), 156–68. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16180622

Redfern, W. S., & Williams, A. (1995). A re-evaluation of the role of alpha 2-adrenoceptors in the anxiogenic effects of yohimbine, using the selective antagonist delequamine in the rat. *British journal of pharmacology*, *116*(3), 2081–9. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1908953&tool=pmcentrez&rendertype=abstract

Reekie, Y. L., Braesicke, K., Man, M. S., & Roberts, A. C. (2008). Uncoupling of behavioral and autonomic responses after lesions of the primate orbitofrontal cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(28), 9787–92. doi:10.1073/pnas.0800417105

Reiss, S. (1997). Trait anxiety: it's not what you think it is. *Journal of anxiety disorders*, *11*(2), 201–14. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9168342

Reiss, S., & Peterson, R. (1986). Anxiety sensitivity, anxiety frequency and the prediction of fearfulness. *Behaviour research and …*, *24*(1), 1–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3947307

Resstel, L. B. M., Joca, S. R. L., Guimarães, F. G., & Corrêa, F. M. a. (2006). Involvement of medial prefrontal cortex neurons in behavioral and cardiovascular responses to contextual fear conditioning. *Neuroscience*, *143*(2), 377–85. doi:10.1016/j.neuroscience.2006.08.002

Richards, A., French, C. C., Calder, A. J., Webb, B., Fox, R., & Young, A. W. (2002). Anxiety-related bias in the classification of emotionally ambiguous facial expressions. *Emotion*, *2*(3), 273–287. doi:10.1037//1528-3542.2.3.273

Risch, N., Herrell, R., & Lehner, T. (2009). Interaction Between the Serotonin Transporter Gene (5-HTTLPR), Stressful Life Events, and Risk of Depression. *JAMA: the journal of …*, *301*(23). Retrieved from http://jama.ama-assn.org/content/301/23/2462.short

Roberts, A. C., Reekie, Y., & Braesicke, K. (2007). Synergistic and regulatory effects of orbitofrontal cortex on amygdala-dependent appetitive behavior. *Annals of the New York Academy of Sciences*, *1121*, 297–319. doi:10.1196/annals.1401.019

Roberts, A. C., Tomic, D. L., Parkinson, C. H., Roeling, T. A., Cutter, D. J., Robbins, T. W., & Everitt, B. J. (2007). Forebrain connectivity of the prefrontal cortex in the marmoset monkey (Callithrix jacchus): An anterograde and retrograde tract-tracing study. *The Journal of comparative neurology*, *502*(1), 86–112. doi:10.1002/cne

Rodgers, R. J., Cao, B. J., Dalvi, A., & Holmes, A. (1997). Animal models of anxiety: an ethological perspective. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas médicas e biológicas / Sociedade Brasileira de Biofísica ... [et al.]*, *30*(3), 289–304. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9246227

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*(2), 297–303. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8577794

Roiser, J. P., Müller, U., Clark, L., & Sahakian, B. J. (2007). The effects of acute tryptophan depletion and serotonin transporter polymorphism on emotional processing in memory and attention. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, *10*(4), 449–61. doi:10.1017/S146114570600705X

Rozanski, A., Blumenthal, J. A., & Kaplan, J. (1999). Impact of Psychological Factors on the Pathogenesis of Cardiovascular Disease and Implications for Therapy. *Circulation*, *99*(16), 2192–2217. doi:10.1161/01.CIR.99.16.2192

Sabol, S., Nelson, M., & Fisher, C. (1999). A genetic association for cigarette smoking behavior. *Health …*. Retrieved from http://psycnet.apa.org/journals/hea/18/1/7/

Salzman, C. D., & Paton, J. J. (2007). Flexible neural representations of value in the primate brain. *Annals of the New …*, *354*, 336–354. doi:10.1196/annals.1401.034

Sandi, C., & Richter-Levin, G. (2009). From high anxiety trait to depression: a neurocognitive hypothesis. *Trends in neurosciences*, *32*(6), 312–20. doi:10.1016/j.tins.2009.02.004

Savitz, J., & Ramesar, R. (2004). Genetic variants implicated in personality: a review of the more promising candidates. *… Journal of Medical Genetics Part B: …*. Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/ajmg.b.20155/full

Scherrer, J., True, W., & Xian, H. (2000). Evidence for genetic influences common and specific to symptoms of generalized anxiety and panic. *Journal of affective …*. Retrieved from http://ukpmc.ac.uk/abstract/MED/10708813/reload=0;jsessionid=hmIs2H5hMtwjxaW2UmD8.4

Schinka, J. A., Busch, R. M., & Robichaux-Keene, N. (2004). A meta-analysis of the association between the serotonin transporter gene polymorphism (5-HTTLPR) and trait anxiety. *Molecular psychiatry*, *9*(2), 197–202. doi:10.1038/sj.mp.4001405

Schoenbaum, G., Roesch, M. R., & Stalnaker, T. A. (2006). Orbitofrontal cortex, decision-making and drug addiction. *Trends in neurosciences*, *29*(2), 116–24. doi:10.1016/j.tins.2005.12.006

Schreurs, B. G., Smith-Bell, C. A., & Burhans, L. B. (2011). Classical conditioning and conditioning-specific reflex modification of rabbit heart rate as a function of unconditioned stimulus location. *Behavioral neuroscience*, *125*(4), 604–12. doi:10.1037/a0024325

Schwarting, R., & Pawlak, C. (2004). Behavioral neuroscience in the rat: taking the individual into account. *Methods and Findings in Experimental and Clinical …*. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Behavioral+Neuroscience+in+the+Rat,+Taking+the+Individual+into+Account#0

Sen, S., Burmeister, M., & Ghosh, D. (2004). Meta-analysis of the association between a serotonin transporter promoter polymorphism (5-HTTLPR) and anxiety-related personality traits. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*, *127B*(1), 85–9. doi:10.1002/ajmg.b.20158

Shabel, S. J., & Janak, P. H. (2009). Substantial similarity in amygdala neuronal activity during conditioned appetitive and aversive emotional arousal. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(35), 15031–6. doi:10.1073/pnas.0905580106

Shephard, R. A., & Broadhurst, P. L. (1982). Hyponeophagia and arousal in rats: Effects of diazepam, 5-methoxy-N,N-dimethyltryptamine, d-amphetamine and food deprivation. *Psychopharmacology*, *78*(4), 368–372. doi:10.1007/BF00433744

Shephard, R., & Estall, L. (1984). Effects of chlordiazepoxide and of valproate on hyponeophagia in rats Evidence for a mutual antagonism between their anxiolytic properties. *Neuropharmacology*, *23*(6), 677–681. Retrieved from http://www.sciencedirect.com/science/article/pii/0028390884901503

Shepherd, J. K., Grewal, S. S., Fletcher, a, Bill, D. J., & Dourish, C. T. (1994). Behavioural and pharmacological characterisation of the elevated "zero-maze" as an animal model of anxiety. *Psychopharmacology*, *116*(1), 56–64. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7862931

Shibasaki, M., & Kawai, N. (2009). Rapid detection of snakes by Japanese monkeys (Macaca fuscata): an evolutionarily predisposed visual system. *Journal of comparative psychology (Washington, D.C. : 1983)*, *123*(2), 131–5. doi:10.1037/a0015095

Shin, L M, Whalen, P. J., Pitman, R. K., Bush, G., Macklin, M. L., Lasko, N. B., Orr, S. P., et al. (2001). An fMRI study of anterior cingulate function in posttraumatic stress disorder. *Biological psychiatry*, *50*(12), 932–42. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11750889

Shin, Lisa M, & Liberzon, I. (2010). The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *35*(1), 169–91. doi:10.1038/npp.2009.83

Shin, Lisa M, Rauch, S. L., & Pitman, R. K. (2006). Amygdala, medial prefrontal cortex, and hippocampal function in PTSD. *Annals of the New York Academy of Sciences*, *1071*, 67–79. doi:10.1196/annals.1364.007

Sierra-Mercado, D., Corcoran, K. a, Lebrón-Milad, K., & Quirk, G. J. (2006). Inactivation of the ventromedial prefrontal cortex reduces expression of conditioned fear and impairs subsequent recall of extinction. *The European journal of neuroscience*, *24*(6), 1751–8. doi:10.1111/j.1460-9568.2006.05014.x

Sinici, E., Yildiz, C., Tunay, S., Ozkan, H., & Altinmakas, M. (2004). Assessment of anxiety levels in patients with posttraumatic stress disorder. *Acta orthopaedica et traumatologica turcica*, *38*(2), 145–8.

Skre, I., Onstad, S., Torgersen, S., Lygren, S., & Kringlen, E. (1993). A twin study of DSM-III-R anxiety disorders. *Acta Psychiatrica Scandinavica*, *88*(2), 85–92. doi:10.1111/j.1600-0447.1993.tb03419.x

Smoller, J., & Acierno, J. (2001). Targeted genome screen of panic disorder and anxiety disorder proneness using homology to murine QTL regions*. *American journal of …*. Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/ajmg.1209/full

Somers, J. M., Goldner, E. M., Waraich, P., & Hsu, L. (2006). Prevalence and incidence studies of anxiety disorders: a systematic review of the literature. *Canadian journal of psychiatry. Revue canadienne de psychiatrie*, *51*(2), 100–13. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16989109

Somerville, L. H., Whalen, P. J., & Kelley, W. M. (2010). Human bed nucleus of the stria terminalis indexes hypervigilant threat monitoring. *Biological psychiatry*, *68*(5), 416–24. doi:10.1016/j.biopsych.2010.04.002

Sorg, B., & Whitney, P. (1992). The Effect of Trait Anxiety and Situational Stress on Working Memory Capacity. *Journal of Research in Personality*, *241*, 235–241. Retrieved from http://www.sciencedirect.com/science/article/pii/0092656692900412

Sotres-Bayon, F., & Quirk, G. J. (2010). Prefrontal control of fear: more than just extinction. *Current opinion in neurobiology*, *20*(2), 231–5. doi:10.1016/j.conb.2010.02.005

Spielberger, C. (1975). *ANXIETY STATE TRAIT PROCESS* (Vol. 1975). WASHINGTON, D.C., U.S.A.: HEMISPHERE PUBLISHING CORPORATION. Retrieved from http://apps.webofknowledge.com/full_record.do?product=UA&search_mode=GeneralSearch&qid=2&SID=Q15BakFJKBoDAJOIajn&page=1&doc=7

Spielberger, C. D. (1973). *State-Trait Anxiety Inventory for Children (STAIC)*. Mind Garden. Retrieved from https://shop.psych.acer.edu.au/acer-shop/group/990QCG

Spielberger, C. D. (1985). Assessment of state and trait anxiety: Conceptual and methodological issues. *Southern Psychologist*, *2*(4), 6–16.

Starr, K. R., Price, G. W., Watson, J. M., Atkinson, P. J., Arban, R., Melotto, S., Dawson, L. A., et al. (2007). SB-649915-B, a novel 5-HT1A/B autoreceptor antagonist and serotonin reuptake inhibitor, is anxiolytic and displays fast onset activity in the rat high light social interaction test. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *32*(10), 2163–72. Retrieved from http://dx.doi.org/10.1038/sj.npp.1301341

Stead, J. D. H., Clinton, S., Neal, C., Schneider, J., Jama, A., Miller, S., Vazquez, D. M., et al. (2006). Selective breeding for divergence in novelty-seeking traits: heritability and enrichment in spontaneous anxiety-related behaviors. *Behavior genetics*, *36*(5), 697–712. doi:10.1007/s10519-006-9058-7

Steimer, T, Escorihuela, R. M., Fernandez-Teruel, A., & Driscoll, P. (1998). Long-term behavioural and neuroendocrine changes in Roman high-(RHA/Verh) and low-(RLA-Verh) avoidance rats following neonatal handling. *International journal of …*, 54–63. Retrieved from http://www.sciencedirect.com/science/article/pii/S073657489800032X

Steimer, Thierry. (1997). Neuroendocrine correlates of emotional reactivity and coping in male rats from the Roman high (RHA/Verh)-and low (RLA/Verh)-avoidance lines. *Behavior genetics*, *27*(6). Retrieved from http://www.springerlink.com/index/gq85022gg2724018.pdf

Steimer, Thierry, & Driscoll, P. (2003). Divergent stress responses and coping styles in psychogenetically selected Roman high-(RHA) and low-(RLA) avoidance rats: behavioural, neuroendocrine and developmental aspects. *Stress (Amsterdam, Netherlands)*, *6*(2), 87–100. doi:10.1080/1025389031000111320

Stein, M. B., Simmons, A. N., Feinstein, J. S., & Paulus, M. P. (2007). Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. *The American journal of psychiatry*, *164*(2), 318–27. doi:10.1176/appi.ajp.164.2.318

Stein, M., & Chartier, M. (1998). A direct-interview family study of generalized social phobia. *American Journal of …*, *155*, 90–97. Retrieved from http://ajp.psychiatryonline.org/article.aspx?articleID=172661

Stevenson, M. F., & Poole, T. B. (1976). An ethogram of the common marmoset (Calithrix jacchus jacchus): general behavioural repertoire. *Animal behaviour*, *24*(2), 428–51. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/820223

Stiedl, O., Tovote, P., Ogren, S. O., & Meyer, M. (2004). Behavioral and autonomic dynamics during contextual fear conditioning in mice. *Autonomic neuroscience : basic & clinical*, *115*(1-2), 15–27. doi:10.1016/j.autneu.2004.07.006

Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *Journal of experimental psychology*, *18*, 643–662.

Sullivan, G. M., Apergis, J., Bush, D. E. a, Johnson, L. R., Hou, M., & Ledoux, J. E. (2004). Lesions in the bed nucleus of the stria terminalis disrupt corticosterone and freezing responses elicited by a contextual but not by a specific cue-conditioned fear stimulus. *Neuroscience*, *128*(1), 7–14. doi:10.1016/j.neuroscience.2004.06.015

Sylvers, P., Lilienfeld, S. O., & LaPrairie, J. L. (2011). Differences between trait fear and trait anxiety: implications for psychopathology. *Clinical psychology review*, *31*(1), 122–37. doi:10.1016/j.cpr.2010.08.004

Tabor, H., Risch, N., & Myers, R. (2002). Candidate-gene approaches for studying complex genetic traits: practical considerations. *Nature Reviews Genetics*. Retrieved from http://www.forum.nslij-genetics.org/ld/tabor02.pdf

Takeda, H., Tsuji, M., & Matsumiya, T. (1998). Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. *European journal of pharmacology*, *350*(1), 21–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9683010

Tanaka, Y., Ishitobi, Y., Maruyama, Y., Kawano, A., Ando, T., Okamoto, S., Kanehisa, M., et al. (2012). Salivary alpha-amylase and cortisol responsiveness following electrical stimulation stress in major depressive disorder patients. *Progress in neuro-psychopharmacology & biological psychiatry*, *36*(2), 220–4. doi:10.1016/j.pnpbp.2011.10.005

Telzer, E. H., Mogg, K., Bradley, B. P., Mai, X., Ernst, M., Pine, D. S., & Monk, C. S. (2008). Relationship between trait anxiety, prefrontal cortex, and attention bias to angry faces in children and adolescents. *Biological psychology*, *79*(2), 216–22. doi:10.1016/j.biopsycho.2008.05.004

Thayer, J., & Hansen, A. (2009). Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. *Annals of Behavioral …*, 141–153. doi:10.1007/s12160-009-9101-z

Topçuoğlu, V., Fistikci, N., Ekinci, Ö., Gimzal Gönentür, A., & Cömert Agouridas, B. (2009). Assessment of Executive Functions in Social Phobia Pati ents Using the Wisconsin Card Sorting Test. *Turkish Journal of Psychiatry*, *20*(4), 322–331. Retrieved from http://www.turkpsikiyatri.com/PDF/C20S4/en/02.pdf

Torgersen, S. (1983). Genetic Factors in Anxiety Disorders. *Archives of General Psychiatry*, *40*(10), 1085–1089. doi:10.1001/archpsyc.1983.01790090047007

Trullas, R., & Skolnick, P. (1993). Differences in fear motivated behaviors among inbred mouse strains. *Psychopharmacology*, 323–331. Retrieved from http://www.springerlink.com/index/Y47318628748XK11.pdf

Tsai, S. J., Hong, C. J., & Cheng, C. Y. (2002). Serotonin transporter genetic polymorphisms and harm avoidance in the Chinese. *Psychiatric genetics*. Retrieved from http://journals.lww.com/psychgenetics/Abstract/2002/09000/Serotonin_transporter_genetic_polymorphisms_and.8.aspx

Visu-Petra, L., Miclea, M., & Visu-Petra, G. (2012). Individual differences in anxiety and executive functioning : A multidimensional view. *International Journal of Psychology*, (August), 37–41. doi:10.1080/00207594.2012.656132

Vogt, J. L., Coe, C. L., & Levine, S. (1981). Behavioral and adrenocorticoid responsiveness of squirrel monkeys to a live snake: is flight necessarily stressful? *Behavioral and neural biology*, *32*(4), 391–405. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7197154

Vyas, A., Mitra, R., Shankaranarayana Rao, B. S., & Chattarji, S. (2002). Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *22*(15), 6810–8. doi:20026655

Waddell, J., Morris, R. W., & Bouton, M. E. (2006). Effects of bed nucleus of the stria terminalis lesions on conditioned anxiety: aversive conditioning with long-duration conditional stimuli and reinstatement of extinguished fear. *Behavioral neuroscience*, *120*(2), 324–36. doi:10.1037/0735-7044.120.2.324

Walker, D L, & Davis, M. (1997). Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *17*(23), 9375–83. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9364083

Walker, David L, & Davis, M. (2002). Light-enhanced startle: further pharmacological and behavioral characterization. *Psychopharmacology*, *159*(3), 304–10. doi:10.1007/s002130100913

Walker, David L, Toufexis, D. J., & Davis, M. (2003). Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. *European Journal of Pharmacology*, *463*(1-3), 199–216. doi:10.1016/S0014-2999(03)01282-2

Wall, P. M., Blanchard, R. J., Yang, M., & Blanchard, D. C. (2004). Differential effects of infralimbic vs. ventromedial orbital PFC lidocaine infusions in CD-1 mice on defensive responding in the mouse defense test battery and rat exposure test. *Brain research*, *1020*(1-2), 73–85. doi:10.1016/j.brainres.2004.06.008

Wallis, J. D., Dias, R., Robbins, T. W., & Roberts, A. C. (2001). Dissociable contributions of the orbitofrontal and lateral prefrontal cortex of the marmoset to performance on a detour reaching task. *The European journal of neuroscience*, *13*(9), 1797–808. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11359531

Walsh, D. M., Stratton, S. C., Harvey, F. J., Beresford, I. J., & Hagan, R. M. (1995). The anxiolytic-like activity of GR159897, a non-peptide NK2 receptor antagonist, in rodent and primate models of anxiety. *Psychopharmacology*, *121*(2), 186–91. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8545524

Weems, C. F., Pina, A. A., Costa, N. M., Watts, S. E., Taylor, L. K., & Cannon, M. F. (2007). Predisaster trait anxiety and negative affect predict posttraumatic stress in youths after hurricane Katrina. *Journal of consulting and clinical psychology*, *75*(1), 154–9. doi:10.1037/0022-006X.75.1.154

Wehner, J. (2001). Quantitative genetics and mouse behavior. *Annual review of …*. Retrieved from http://www.annualreviews.org/doi/pdf/10.1146/annurev.neuro.24.1.845

Wei, Q., Lu, X.-Y., Liu, L., Schafer, G., Shieh, K.-R., Burke, S., Robinson, T. E., et al. (2004). Glucocorticoid receptor overexpression in forebrain: a mouse model of increased emotional lability. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(32), 11851–6. doi:10.1073/pnas.0402208101

Williamson, D. E., Coleman, K., Bacanu, S.-A., Devlin, B. J., Rogers, J., Ryan, N. D., & Cameron, J. L. (2003). Heritability of fearful-anxious endophenotypes in infant rhesus macaques: a preliminary report. *Biological psychiatry*, *53*(4), 284–91. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12586447

Yamazaki, Y., & Watanabe, S. (2009). Marmosets as a next-generation model of comparative cognition. *Japanese Psychological Research*, *51*(3), 182–196. doi:10.1111/j.1468-5884.2009.00398.x

Yassa, M. A., Hazlett, R. L., Stark, C. E. L., & Hoehn-Saric, R. (2012). Functional MRI of the amygdala and bed nucleus of the stria terminalis during conditions of uncertainty in generalized anxiety disorder. *Journal of psychiatric research*, *46*(8), 1045–52. doi:10.1016/j.jpsychires.2012.04.013

Yasumatsu, H. (1995). [Assessment of anxiolytics (4)--Social interaction test]. *Nihon shinkei seishin yakurigaku zasshi = Japanese journal of psychopharmacology*, *15*(4), 295–304. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7584724

Yilmazer-Hanke, D. M., Wigger, A., Linke, R., Landgraf, R., & Schwegler, H. (2004). Two Wistar rat lines selectively bred for anxiety-related behavior show opposite reactions in elevated plus maze and fear-sensitized acoustic startle tests. *Behavior genetics*, *34*(3), 309–18. doi:10.1023/B:BEGE.0000017874.40934.64

Zelinski, E. L., Hong, N. S., Tyndall, A. V., Halsall, B., & McDonald, R. J. (2010). Prefrontal cortical contributions during discriminative fear conditioning, extinction, and spontaneous recovery in rats. *Experimental brain research. Experimentelle Hirnforschung. Expérimentation cérébrale*, *203*(2), 285–97. doi:10.1007/s00221-010-2228-0

Zhang, J. X., Harper, R. M., & Ni, H. F. (1986). Cryogenic blockade of the central nucleus of the amygdala attenuates aversively conditioned blood pressure and respiratory responses. *Brain research*, *386*(1-2), 136–45. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3779405

Zhuang, X., Gross, C., & Santarelli, L. (1999). Altered emotional states in knockout mice lacking 5-HT1A or 5-HT1B receptors. …. Retrieved from http://www.sciencedirect.com/science/article/pii/S0893133X99000470

Zinbarg, R. E., & Barlow, D. H. (1996). Structure of anxiety and the anxiety disorders: a hierarchical model. *Journal of abnormal psychology*, *105*(2), 181–93. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8722999

Öhman, A., & Mineka, S. (2001). Fears, phobias, and preparedness: Toward an evolved module of fear and fear learning. *Psychological Review*, *108*(3), 483–522. doi:10.1037//0033-295X.108.3.483