UNCONDITIONED ESCAPE BEHAVIOUR IN RATS: IMPLICATIONS FOR PRE-CLINICAL RESEARCH INTO PATHOLOGICAL EXTREME ANXIETY DISORDERS

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ABSTRACT

Pre-clinical models of anxiety act as paradigms to facilitate investigation into the biological bases of anxiety and assess the efficacy of anxiolytic compounds. Models that measure the flight/escape component of rodents’ defensive behaviours are potentially analogous to the symptoms of extreme anxiety conditions (e.g. panic disorder [PD] and post traumatic stress disorder [PTSD]) as opposed to generalised anxiety disorder (GAD). The unstable elevated exposed plus maze (UEEPM) is a novel model of extreme anxiety in rats that possesses elements of face and construct validity. The current thesis aimed to investigate the pharmacological substrates of escape in the UEEPM.

As pre-clinical models are susceptible to a range of methodological variables, experiments 1 and 2 examined the effects of circadian phase, test illumination and strain of subject in the UEEPM and a battery of anxiety models. Anxiety-related behaviour on all tests was independent of circadian phase and illumination. Clear strain differences in escape behaviour were observed, suggesting the use of strains optimal for observing panicolytic and panicogenic drug effects may benefit pre-clinical research into extreme anxiety disorders.

Experiments 3 and 5 assessed the predictive validity of the UEEPM by examining the behavioural profiles of compounds known to affect the symptoms of PD, PTSD and GAD. Drugs which potentiate the symptoms of PD and PTSD increased escape in the UEEPM. Treatment regimes effective for PD decreased escape, whereas those effective for GAD were without effect. Thus, the UEEPM displayed bi-directional predictive validity as a model of extreme, as opposed to generalised, anxiety disorders. Experiment 4 revealed the behavioural effects of the 5-HT$_{2c/2b}$ receptor agonist m-chlorophenylpiperazine (mCPP) in the UEEPM were attenuated by the 5-HT$_{2c}$ antagonist SB-242084. Thus, escape in the paradigm may be mediated, at least in part, by the 5-HT$_{2c}$ receptor and 5-HT$_{2c}$ antagonists may hold potential as therapeutic agents for panic-related disorders.
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This thesis includes five studies, each presented in a separate experimental chapter. All the experimental chapters have been published, with the exception of Chapter III which is submitted for publication, and are included in a format very similar to these papers. Chapters I and VII present a general overview of the research area and some proposals for further research. Each experimental chapter contains an introduction and discussion specifically relevant to its findings. Whilst this may lead to occasional repetition of information, for instance in the introductions of each chapter, it is hoped future readers of this work will be able to understand the aims, findings and implications of each experiment from the individual chapters, without the need for extensive cross referencing to other sections of the thesis.

Publications


Jones N, Duxon MS, King SM (2002) 5-HT$_{2c}$ receptor mediation of unconditioned escape behaviour in the unstable elevated exposed plus maze. Psychopharmacology 164: 214-220


Jones N, Duxon MS, King SM (submitted, Physiology and Behavior) Strain differences in unconditioned escape behaviour in laboratory-bred rats.

Abstracts


Jones N, Duxon MS, King SM (2002) The role of the 5-HT$_{2c}$ receptor in unconditioned escape responses in the unstable elevated exposed plus maze, a novel model of extreme anxiety. FENS Abstr. Vol 1, A154.12
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CHAPTER I

1. Fear and Anxiety

1.1. Clinical Anxiety Disorders

Anxiety disorders constitute a major world health problem with an estimated lifetime prevalence rate of approximately 25% (Kessler et al. 1994). It is estimated that they account for around 50% of non substance abuse-related psychiatric consultations each year (Manderscheid and Sonnenschein 1994). There are currently nine separate anxiety disorders described in the Diagnostic and Statistical Manual (DSM-IV, American Psychiatric Association 1994), each possessing their own clinical presentation, course and response to treatment. These comprise of panic disorder, panic disorder with agoraphobia, generalised anxiety disorder, post traumatic stress disorder, obsessive compulsive disorder, acute stress disorder, social phobia, agoraphobia and specific phobia. Patients suffering from these disorders may also exhibit symptoms of depression (Lindsay 1994). The anxiety disorders most relevant to the current thesis are panic disorder, post traumatic stress disorder and generalised anxiety disorder.

Generalised Anxiety Disorder

Generalised anxiety disorder (GAD) is characterised by chronic exaggerated and uncontrollable worries about life events and is estimated to affect 3-4 % of the population
in any given year (Brown et al. 94; Wolk et al. 96). DSM-IV criteria for GAD state that
the patient must experience excessive anxiety and worry about several events or activities
for at least 50% of a six month period. During this time, three or more of the following
must also be displayed; feeling restless, edgy or keyed up, tiring easily, difficulty in
concentrating, irritability, increased muscle tension and disturbed sleep. Cognitive
Behavioural Therapy (CBT) (Lindsay 1994) has proven effective for symptom relief in
GAD. Benzodiazepines (BDZ’s e.g. diazepam and chlordiazepoxide) are the most
commonly used effective pharmacological treatment (Nutt 1991; Argyropoulos and Nutt
1997; Pies 1998), although the serotonin (5-HT)₁A agonist buspirone has also displayed
efficacy in the treatment of GAD (Petracca et al. 1990; Strand et al. 1990, see table 1.1).

Panic Disorder

Panic disorder (PD) involves discrete episodes of more extreme anxiety accompanied by
a range of physiological symptoms and psychological feelings of terror and impending
harm (American Psychiatric Association 1994; Fyer et al. 1996). These ‘panic attacks’
are defined by the presence of at least four of the following symptoms; chest pain, chills
or hot flushes, choking sensation, derealisation or depersonalisation, dizziness, fear of
dying, fear of loss of control or becoming insane, heart pounding, nausea, sweating,
shortness of breath or trembling. Patients also commonly report an urgent desire to flee or
escape from the situation where an attack is occurring. An ongoing worry or concern over
the recurrence of attacks and an avoidance of the places or situations where attacks have
occurred is also commonplace.
Prevalence rates have been estimated at between 1.5% and 3.5% of the population (Klerman et al. 1991; Kessler et al. 1994; Londborg et al. 1998). CBT has proved effective in the treatment of PD (Chambless and Gillis 1993; Otto et al. 2000), as has chronic administration of the high potency benzodiazepines alprazolam and clonazepam (Ballenger et al. 1988; Tesar et al. 1991; Jonas and Cohon 1993; Rosenbaum 1997). Chronic treatment with the tricyclics imipramine and clomipramine is also effective for the treatment of PD (Cross National Collaborative Panic Study 1992; Modigh et al. 1992; Nair et al. 1996) although symptoms are often potentiated in the initial stages of therapy (Nutt and Lawson 1992; Westenberg 1996). However, long-term selective serotonin reuptake inhibitors (SSRI's e.g. fluoxetine, paroxetine) display the greatest efficacy in the treatment of PD and are the recommended first line pharmacological treatment (Ballenger et al. 1998; den Boer and Slaap 1998; Sheehan 1999; Pollack and Marzol 2000) although, as with the tricyclics, an exacerbation of symptoms is often observed before any clinical improvement (Johnson et al. 1995; Westenberg 1996). Conversely, drugs which have been shown to induce panic attacks and increase symptoms of anxiety and panic in PD patients include the serotonergic agonist m-chlorophenylpiperazine (mCPP), the α2-adrenergic antagonist yohimbine and the adenosine antagonist caffeine (Charney et al. 1985, 1987a, 1987b; Klein et al. 1991; Benjamin et al. 1999, see table 1.1).
Post Traumatic Stress Disorder

Post traumatic stress disorder (PTSD) is a debilitating condition that follows a traumatic event involving both ‘actual or threatened death or serious injury’ to the patient or to others and ‘feelings of intense fear, horror or helplessness’ (American Psychiatric Association 1994). As in PD, PTSD sufferers experience discrete episodes of anxiety, although they are specifically centred around reliving the traumatic event through intrusive thoughts and dreams, hallucinations, or flashbacks. Cues that symbolise or resemble the event cause marked mental distress to the patient and lead to physiological responses such as rapid heart beat and elevated blood pressure. Thus, the patient attempts to avoid thoughts, feelings, places or activities related to the event. The occurrence of at least two of the following symptoms are also required for DSM-IV diagnosis of PTSD; insomnia, irritability, poor concentration, hypervigilance and increased startle response.

Prevalence rates are estimated at between 1.3% and 10.4% (Davidson et al. 1991; Kessler et al. 1995). Psychological therapies, such as psychological debriefing, have proven largely unsuccessful in the treatment of PTSD (Rose et al. 2002). Pharmacological studies have revealed that whilst mCPP and yohimbine potentiate the symptoms of PTSD (Morgan et al. 1995; Bremner et al. 1997; Southwick et al. 1997), SSRI’s, when administered chronically, display potential as therapeutic agents (for review see Fichtner et al. 1997; Hidalgo and Davidson 2000, see table 1.1).
Table 1.1. Compounds shown to reduce (-) and increase (+) the symptoms of GAD, PD and PTSD

<table>
<thead>
<tr>
<th>Pharmacological Agent</th>
<th>GAD</th>
<th>PD</th>
<th>PTSD</th>
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<tr>
<td>Low potency BDZ’s (e.g. diazepam, CDP)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Buspirone</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>High potency BDZ’s (e.g. alprazolam, clonazepam)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tricyclics (e.g. imipramine and clomipramine) Acute</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricyclics (e.g. imipramine and clomipramine) Chronic</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSRI’s (e.g. fluoxetine, paroxetine) Acute</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSRI’s (e.g. fluoxetine, paroxetine) Chronic</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>mCPP</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Yohimbine</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Caffeine</td>
<td>+</td>
<td></td>
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</table>

1.2. The Relationship Between Fear and Pathological Anxiety

Fear is an emotion caused by the presence or imminence of danger. Therefore, it is a normal reaction to a threatening situation (Marks 1987; Öhman 1992). However, dysfunction of this normal fear reaction could be the cause of pathological anxiety states. It has been suggested that anxiety disorders are a result of excessive fear (Kalin 1993; Rosen and Schulkin 1998), fear expressed to a greater extent than a situation warrants, or the expression of fear in inappropriate situations (Marks 1987; Öhman 1992). More specifically, LeDoux (1996) proposes that specific defects in the fear response can be seen in distinct anxiety syndromes. For example, phobias are fears taken to the extreme, obsessive-compulsive disorder often involves an extreme fear of something with patients engaging in compulsive rituals to avoid the fear object once it is encountered. Panic disorder patients often experience the overwhelming fear that death is near and PTSD
sufferers can be sent into an intense distress by a stimulus associated with a previously fearful situation.

Anxiety refers to feelings of apprehension or uncertainty resulting from anticipation of a threatening event or situation. Distinctions have been made between 'normal' and 'pathological' anxiety states. For example, Belzung and Griebel (2001) define 'fear' as the behavioural and physiological response of a subject to a real threat that may impair its homeostasis, and 'normal anxiety' as the same response, but to a potential threat. Anxiety becomes 'pathological anxiety' when the response is excessive or maladaptive. Rodgers et al. (1997) describe 'anxiety' as the expectation or anticipation of a known or unidentifiable imminent danger and 'pathological anxiety' as excessive behavioural, physiological and psychological reactions to this danger. Similarly, Clément et al. (2002) refer to adaptive responses to various physiological, psychological and sociological stressors as 'normal anxiety', and inappropriate responses as leading to anxiety disorders. Whilst these definitions differ slightly in concept, they all agree on the notion that whilst fear and anxiety serve a useful purpose, dysfunction of these emotional states can interfere with normal activity and have a negative impact on life.

The Aetiology of Pathological Anxiety

It has been proposed that the dysfunction responsible for the manifestation of pathological anxiety states is a result of sensitisation or hyperexcitability in the neural substrates mediating normal fear reactions. Rosen and Schulkin (1998) contend that the
normal fear reaction is an adaptive response to aversive situations and is characterised by an ‘apprehensive expectation’ that subsides once the danger has passed. For example, fear may be induced by public speaking, yet this emotion fades once the presentation is underway or completed. However, if this circuitry which mediates normal attention, perception, and behaviour to detect and respond to aversion is activated repeatedly, or to a sufficient extent, it becomes hyperexcitable and more primed for response. This neural sensitisation, via processes possibly resembling kindling or long term potentiation (LTP), results in the lowering of thresholds for activation of the fear response (for example by mere cues regarding public speaking) and the exaggeration of the fear response itself. Eventually, activation of the fear circuits becomes independent and autonomous from the triggering stimuli and out of the conscious control of the individual. This manifests in the chronic ‘hypervigilant’ state observed in patients suffering from pathological anxiety disorders (Charmezy and Deutch 1996; Rosen and Schulkin 1998).

The role of spontaneous activation of the neural fear system in the aetiology of pathological anxiety is also suggested by Graeff and colleagues (Graeff 1990; Deakin and Graeff 1991; Graeff et al. 1993). The authors propose there are two circuits controlling adaptive responses to aversive events. The first mediates reactions to unconditioned innately fear-inducing stimuli, such as pain and natural predators, and is responsible for active defensive responses such as freezing, flight or defensive aggression. Panic disorder is the result of this system being activated in the absence of the fear inducing stimuli. The second system mediates the ‘anticipatory’ physiological and hormonal preparations for defensive responses elicited by stimuli which have previously been predictive of an
aversive event. Dysfunction in the activation of this system manifests clinically in the state of 'chronically exaggerated and uncontrollable worry' which is observed in GAD.

1.3. The Neural Fear Circuitry

The neural circuitry mediating the fear response is a highly integrated and interconnected system capable of detecting danger and eliciting a range of reactions including increased arousal, analgesia, autonomic and hormonal changes and behavioural reflexes such as immobility, a shifting of attention towards potential threats and the 'fight or flight' response (Ratner 1975; Bolles and Fanselow 1980; Deakin and Graeff 1991). The circuitry is located in the limbic and midbrain regions and its primary structures are, on a rostro-caudal axis, the amygdala (AM), medial hypothalamus (MH) and the periaqueductal grey (PAG) and adjacent superior colliculus (SC) (Graeff 1990; Panksepp 1990; Deakin and Graeff 1991; Graeff et al. 1993; Brandão et al. 1999).

The AM receives highly processed input from the surrounding temporal cortex, thus creating ready avenues for various perceptions and cognitions to influence the fear system (Panksepp 1990). Thus, it is suggested as a sensory interface which detects threatening visual, olfactory and auditory elements of environmental stimuli and activates the amygdalo-hypothalamico-PAG system (Deakin and Graeff 1991; Fanselow 1991; Graeff et al. 1993). Once the system is activated the MH modulates the autonomic and neuroendocrine reactions preparing the organism to cope with the potential threat, such as increases in heart rate, respiratory rate and blood pressure and activation of the
hypothalamic-pituitary-adrenal (HPA) axis (Kalin 1993; Shekhar 1993; Shekhar et al. 1993). The PAG is seen as the final common pathway of the defence response (Bandler 1988). It is the most caudal area in the defence circuitry capable of eliciting the full range of defensive behaviours and is therefore viewed as a co-ordinator of the various defensive activities, selecting and eliciting the appropriate reaction (Graeff 1990; Fanselow 1991).

The SC is situated directly dorsal to, and possesses extensive connections with, the PAG (Redgrave et al. 1987; 1988). It is proposed that the SC is involved in the communication of visual elements of threatening situations to the PAG which then organises the relevant defensive behaviours (Redgrave and Dean 1991).

1.4. Cross-Species Similarity in the Neural Fear Circuitry

Study of the neural mechanisms of fear and pathological anxiety has been greatly facilitated by the fact that the circuitry detailed above is remarkably homogeneous in humans and lower animals. It is estimated that the system has an evolutionary history of at least some several hundred million years to the time of the split between avian and proto-mammalian species (Panksepp 1990). Certainly, as described below, the responses elicited by artificial stimulation or lesions of the AM, MH, PAG and SC are very consistent across mammals.
Amygdala

The AM receives sensory information into its lateral (LN) and basolateral (BN) nuclei which then project to the central nucleus (CN) which, in turn, projects to the hypothalamic and midbrain regions of the fear circuit (Davis 1997). Stimulation and lesion of all these nuclei have been shown to similarly affect fear-related responses in animals including rats, non-human primates, rabbits and cats.

Electrical stimulation of the cat LN and CN results in a fear response consisting of cringing and cowering followed by rapid escape and jumping behaviour, whereas stimulation of the LN and BN elicits defensive aggressive responses including the baring of teeth and hissing (Ursin and Kaada 1960). Correspondingly, lesions of the LN and CN reduce flight and escape responses in cats following approach by a human experimenter (Ursin 1965a) and administration of an aversive electric shock (Ursin 1965b). Physiological fear reactions such as dramatic increases in respiratory rate are also observed after electrical stimulation of the cat CN (Harper et al. 1984).

In rats, electrical stimulation of the CN induces increases in arterial pressure and heart rate (Iwata et al. 1987). Chemical activation of the amygdaloid complex results in a pressor response and tachycardia, along with immobility and body shaking (Ohta et al. 1991) and chemical stimulation of the BN elicits increased heart rate and blood pressure (Sanders and Shekhar 1991; Iwata et al. 1987). Lesions of the CN reduce the behavioural, physiological and hormonal defensive responses to an aversive footshock (Roozendaal et
and the startle response to a loud tone previously paired with a footshock (Walker and Davis 1997). Ablation of the rat BN results in attenuation of the conditioned freezing response to aversive footshock (Maren et al. 1996) and the acoustic startle reflex (Sananes and Davis 1992; Walker and Davis 1997). Furthermore, lesions of the BN and LN significantly reduce flight and escape responses in wild rats when they are approached by a human experimenter (Kemble et al. 1990).

Physiological fear responses are observed after electrical stimulation of the rabbit CN (Applegate et al. 1983). In rhesus monkeys, neurotoxic lesions of the AM attenuate the freezing response elicited by an encounter with an innately fear-inducing stimulus (a snake) and the defensive aggressive behaviours, such as lunging and striking, elicited by presentation of a conspecific and an unfamiliar human face (Meunier et al. 1999; Kalin et al. 2001). Human patients with damage to the AM also display impaired recognition of both facial (Adolphs et al. 1994) and auditory (Scott et al. 1997) expressions of fear.

**Medial Hypothalamus**

Electrical and chemical stimulation of the rat MH results in rapid flight-directed behaviour, jumping and attempts to escape from the test arena (Lammers et al. 1988; Silveira and Graeff 1992; Silveira et al. 1995). Correspondingly, lesions within the rat MH result in a dramatic reduction in escape behaviours and freezing following exposure to a natural predator (Canteras et al. 1997). Studies in rabbits have revealed that electrical stimulation of the MH produces immobility and orienting behaviour which gives way to
running and vigorous escape attempts as the stimulation intensity is increased (Duan et al. 1986). The cardio-respiratory components of the defence response (pressor response, tachycardia, hyperventilation, decrease in visceral bloodflow and increase in hindlimb bloodflow) are also produced by stimulation of the rabbit MH (McCabe et al. 1994; Markgraf et al. 1991). Furthermore, numerous experiments have also shown that stimulation of the feline MH results in both flight and defensive attack behaviours (e.g. Di Scala et al. 1984; Fuchs et al. 1985; Brandão et al. 1986; Stoddard et al. 1986; Luo et al. 1998).

**Periaqueductal Grey**

Electrical stimulation of the rat PAG results in defensive behavioural responses (Olds and Olds 1963; Schmitt et al. 1981), which progress from arousal and freezing through to abrupt escape as the stimulation intensity increases (Brandão et al 1982, Brandão et al 1985). Likewise, PAG lesions decrease defensive behaviours in laboratory-bred (Liebman et al. 1970) and feral subjects (Blanchard et al. 1981). More specifically, the PAG consists of dorsomedial, dorsolateral, lateral, ventromedial and ventrolateral regions (Bandler and Shipley 1994) and evidence suggests the dorsolateral PAG (dPAG) and ventrolateral PAG (vPAG) mediate different aspects of defensive behaviour (Lovick 1993). Electrical and chemical stimulation of the dPAG results in activity bursts, jumping and vigorous escape attempts whereas stimulation of the vPAG results in immobility and freezing (Bandler and Depaulis 1991; Fanselow 1991). Correspondingly, rats with lesions to the dPAG display a complete lack of escape behaviour when presented with electric
shocks, whereas rats with vPAG lesions display activity bursts but little freezing (Fanselow 1991).

Likewise, activation of the cat PAG also results in behavioural components of the defensive response which appear to be mediated by distinct sub-regions (for review see Bandler and Depaulis 1991). Chemical stimulation of the PAG in areas lateral to the aqueduct result in a 'fight / flight' reaction characterised by rapid running around the test cage, multiple jumps and escape attempts (Zhang et al. 1990) and threat displays including piloerection, howling and hissing (Bandler and Carrive 1988). Conversely, a suppression of exploratory behaviour, immobility and dramatic reductions in spontaneous behaviours including grooming, stretching and vocalisation are observed following chemical stimulation of the ventrolateral PAG (Zhang et al. 1990). The same distinction between active and passive defence responses elicited by electrical stimulation of the dPAG and vPAG respectively is also observed in rabbits (Markgraf et al. 1991; McCabe et al. 1994).

Although limited in number, studies detailing the effects of PAG stimulation in humans reveal similar behavioural and physiological symptoms indicative of a fearful state. Nashold et al. (1969) reported the effects of electrical stimulation of the human midbrain in patients suffering from chronic pain. Stimulation via electrodes in the PAG (0.5 – 5.0 mm lateral to the aqueduct) produced increases in pulse rate, diffuse medial pain in the face and 'around the heart' or 'deep in the chest' and intense anxiety described as 'fearful', 'frightful' and 'terrible'. Patients also reported being 'scared to death', feeling
the 'urge to void' and experiencing 'a fear which was so unpleasant they were not willing to tolerate repeated stimulations'. In similar studies patients have described 'fear sensations' and 'burning sensations of the entire body', with one subject reporting they felt like 'someone is now chasing me, I am trying to escape from them' (Amano et al. 1978). Likewise, Sano et al. (1972) described stimulated patients as being 'obsessed by extreme horror'. Interestingly, Young (1989) reports that whilst patients electrically stimulated in the dPAG report sensations of anxiety and fear, stimulation in the ventral or lateral PAG evokes a generalised feeling of well-being at threshold levels and oscillopsia and diplopia at higher intensities.

**Superior Colliculus**

The SC is an evolutionary ancient subcortical visual area which lies directly dorsal to the PAG and appears to be involved in both orienting to, and escaping from, certain visual stimuli (Dean et al. 1986; 1989). Electrical stimulation of the rat SC produces behavioural responses resembling either approach or avoidance (Dean et al. 1986; Sahibzada et al. 1986; King et al. 1996). Whilst the former involves head and body movements towards the area of the visual field being stimulated, the latter consists of head and body movements away from the stimulated visual field, along with 'cringing', 'flinching', 'shying away' and vigorous running and escape behaviours. Chemical stimulation of the rat SC produces all the above responses as well as periods of freezing where the animal displays an exaggerated startle response to mild auditory, visual or tactile stimulation (Redgrave et al. 1981; Dean et al. 1988). Studies in the hamster have
revealed freezing, movements away from the stimulated site and running escape behaviour following electrical stimulation of the SC (Northmore et al. 1988).

Lesion studies further implicate the SC in defensive responses. Goodale and Murison (1975) observed that rats with SC lesions failed to display the typical reactions of freezing, retreat or escape when presented with novel visual or auditory stimuli in an open arena. Tree shrews with SC lesions fail to flinch or avoid an experimenter’s hand travelling quickly towards them on a collision trajectory (Casagrande and Diamond 1974) and gerbils with SC ablation show no response to looming overhead visual threats (Ellard and Goodale 1988). Furthermore, monkeys with damage to the main cortical visual pathway (i.e. to the striate cortex), but with intact SC, display avoidance responses to a visual stimulus on a collision course to their head when it is presented in their ‘blind field’ (King and Cowey 1992).

1.5. Cross-Species Similarity in Fear-Related Behaviour

As well as cross-species correspondence between the brain areas mediating fear, once the fear circuitry is activated the behavioural responses to fear-evoking stimuli are also remarkably consistent. Although some of the antecedents of fear such as multi-sensory cues from relevant predators are species-specific (Russell 1979), others such as suddenly looming stimuli or environmental hazards like fire, height and instability are universally threatening (Russell 1979; King et al. 1992; Blanchard 1997). To maintain homeostasis, once these sources of danger are encountered, all animals will freeze or attempt to hide
from, escape, or otherwise avoid the aversive stimuli. For example, Gray (1987) proposes a phylogenetically stable response to innate fear stimuli and signals of punishment which consists of an inhibition of ongoing behaviour and increased arousal and attention to environmental stimuli. Active avoidance of looming visual stimuli which signal the approach of an object or impending collision is also seen across different classes of vertebrates and invertebrates. Fiddler crabs, frogs and chicks all flee following presentation of an accelerated magnification of a dark form in their field of view (Schiff 1965). Humans and non-human primates will respond with defensive head and body movements when a stimulus is moved towards their heads (King and Cowey 1992; King et al. 1992), gerbils will actively flee from a black shape travelling towards them from above (Ellard 1993) and chickens (Gallus gallus) display instances of ‘running in a crouched posture’ when presented with overhead computer-generated animations of raptor-shaped images of sufficient size (Evans et al. 1993).

This homogeneity is not confined to the more primitive, lower order aspects of defensive responses. Attempts to characterise the more complex elements of mammalian defensive behavioural strategies has revealed further commonalities across species. The distance-dependant-defence-hierarchy (Gallup 1974; Ratner 1977) proposes that animals' defensive behaviour consists of a dynamic range of responses which are selected according to the salience and proximity of the threat stimuli. For instance, prey species are continuously vigilant to the possibility of predation and will take immediate action to distance themselves from a predator once it is detected. If the predator continues to approach rapidly the prey exhibits various escape strategies such as freezing and protean
behaviour. However, once the predator becomes very proximal the prey may display
defensive attack behaviours directed towards the predator. A series of studies conducted
by the Blanchards, and detailed below, have revealed that the defensive repertoire of rats,
mice and humans does indeed comprise of a number of behaviours which are selected
and elicited according to specific properties of the threat stimulus. Importantly, these
behaviours also appear similarly organised between species.

Defensive Behaviour in Rodents – The Defence Test Batteries

Part of the Fear/Defence Test Battery (F/DTB, for review see Blanchard 1997) consists of
an oval arena with a central wall, thus forming a continuous runway. The straight sections
of the runway can also be blocked off thus forming an inescapable alleyway. A rat is
placed in the apparatus prior to a human experimenter entering the runway and making
several approaches towards the subject. Data from the control groups of several
experiments (e.g. Blanchard et al. 1986a, 1986b, 1988, 1989, 1991, 1994) reveal that in
the escapable runway, flight from the experimenter is the predominant response and is
elicited at an experimenter - subject distance of approx. 3 m. In the inescapable runway,
freezing is the predominant response, however this gives way to defensive vocalisations
and jump attacks as the experimenter’s approach continues until contact is made. The
Mouse Defence Battery (MDTB, Griebel et al. 1996) is similar to the F/DTB except mice
are approached with a hand-held anaesthetised rat. A corresponding pattern of flight
when the runway is continuous, and freezing giving way to defensive aggressive
behaviours in the inescapable runway, are observed. When chased around the runway by
the threat stimulus, mice also exhibit a variety of risk assessment (RA) behaviours including orientations and movements towards the rat.

The Visible Burrow System (VBS, Blanchard and Blanchard 1989b) is a seminatural habitat consisting of a series of tunnels and burrows connected to an open area. When a predatory stimulus (a cat) is introduced into the open area rats immediately flee to the tunnels. This is followed by subjects typically orienting themselves to the tunnel via which they entered, and freezing. After this period, subjects produce ultrasonic distress calls and exhibit several hours of increased immobility. At around 7 – 10 hours post cat exposure, rats begin to approach the tunnel openings and display various RA behaviours, such as poking their heads out of the tunnels, scanning the open area and occasionally making brief forays across the open area.

These data from the F/DTB, MDTB and VBS suggest a defensive behavioural repertoire in rats and mice consisting of flight, freezing, risk assessment and defensive threat and attack (Blanchard 1997). The selection and implementation of these behaviours appears to be dependant on the specific nature and salience of the threat and the available behavioural opportunities (Rodgers 1997).

**Defensive Behaviour in Humans**

Anecdotal descriptions of human defence bear considerable similarity to the behaviours of subjects in the defence test batteries. Thus, a poorly identifiable threat can elicit “an
active immobility so profound that the frightened person can hardly speak or even breathe”, an identifiable threat from which an “avenue for flight or concealment is plausible” may result in the person “trying to flee or hide” and “actual contact with the threat is likely to elicit thrashing, biting, scratching, and other potentially damaging activities by the terrified person” (Blanchard and Blanchard 1989a).

More recently, Blanchard et al. (2001b) presented a sample of undergraduate students with twelve scenarios all detailing potential fear-evoking situations involving either a threatening individual, cues of potential threat from an individual or a threatening human-constructed device (a bomb). When asked to describe what their first response would be to these scenarios, 92% of women and 93% of men chose either to hide; freeze or become immobile; flee or try to escape; yell or scream; attack or struggle; or ‘check out’ or investigate. Whilst it is clear that further investigation into human defensive behaviours is necessary, these initial data suggest the basic mammalian defence strategies of freezing, flight, defensive threat, defensive attack and risk assessment are well represented in humans.

1.6. Summary

Pathological anxiety disorders are debilitating illnesses and comprise of a number of distinct syndromes with individual symptoms, time course and response to treatment. It is proposed that dysfunction in the neural circuitry mediating normal or adaptive responses to dangerous and fear-evoking stimuli may play a key role in the aetiology of these
pathological states. The main neural system mediating this fear response consists of the AM, MH, PAG and SC and is commonly located and organised across mammalian species. Furthermore, the behavioural reactions to fear evoking stimuli also appear comparable in humans and lower mammals. Given this anatomical and behavioural homogeneity it has been proposed that the controlled laboratory study of fear-related behaviour in animals may provide valuable insights into clinical pathological anxiety states:

“Commonality of functional patterns is the rule, what distinguishes the fear reactions in animals and man is not so much the ways in which fear is expressed as the different kinds of trigger stimuli that activate the appraisal mechanism of the defensive system. Humans fear things that a rat could never conceptualise, but the human and rat body respond much the same to their special triggers. The implications of this situation are enormous. For the purpose of understanding how fear is generated, it does not matter so much how we activate the system or whether we activate the system in a person or a rat. The system will respond in pretty much the same way using a limited set of given defence response strategies available to it. We can thus design experiments in rats (or other laboratory animals) for the purpose of understanding how the human fear system works.” (LeDoux 1996 p134).
2. Pre-Clinical Research into Anxiety Disorders

2.1. Pre-Clinical Models of Anxiety

It is estimated that over 30 pre-clinical models of anxiety have been developed which measure experimentally-induced fear- and anxiety-related behaviours in species including rodents, pigeons, tree-shrews and non-human primates (Green 1991; Griebel 1995; Barros and Tomaz 2002). All animal models measure responses to stimuli which can be interpreted as threats to subjects’ well-being, safety or survival (Handley 1991). Pre-clinical models act as paradigms from which to investigate the neuroanatomical and neurochemical bases of anxiety and are also used to screen for the safety and efficacy of novel anxiolytic compounds (Treit 1985; Lister 1990; Green and Hodges 1991; Rodgers and Cole 1994; Hendrie et al. 1996; Hascoët et al. 2001).

Validity of Pre-Clinical Models of Anxiety

As pre-clinical models attempt to reproduce some of the symptoms of anxiety disorders seen in humans, they must fulfil the criteria of predictive, face and construct validity (Green and Hodges 1991; Rodgers and Cole 1994; Belzung and Griebel 2001; Clèment et al. 2002). To achieve predictive validity, the model should be sensitive to clinically effective compounds. Thus, anxiolytic drugs, which decrease anxiety symptoms in humans, should decrease fear-related or anxiety-related behaviour in the test, anxiogenic drugs must have the opposite effect and compounds which do not affect anxiety in
humans should similarly not affect animals' behaviour in the paradigm. Face validity requires that the model produces reactions in animals that are similar to anxiety-related behaviours in humans. To achieve construct validity, the anxiety behaviour being measured and the condition being modelled must share a common aetiology, thereby potentially furthering our theoretical understanding of the clinical disorder.

2.2. Conditioned vs. Unconditioned Tests

Tests of anxiety have predominantly been separated into two categories on the basis of the response measured, either conditioned or unconditioned. The former rely on subjects' conditioned responses to stressful and often painful events (Griebel 1995). They typically require the training of subjects and employ food or water deprivation and/or electric shock as an aversive stimulus (Rodgers and Cole 1994). For example, in the Geller-Seifter conflict test (Geller and Seifter 1960) rats are trained to lever-press for food reward on two schedules. In the first 'non-conflict' schedule, responses are reinforced with food and in the second 'conflict schedule' responses are reinforced with food and electric shock. Following the training period, an increase in responding during the conflict schedule (i.e. an 'anticonflict effect') is interpreted as a decrease in anxiety.

Unconditioned models rely on spontaneous behaviour to stress stimuli which do not explicitly involve pain or discomfort (Griebel 1995). For example, in the social interaction test, pairs of rats are placed in a brightly lit, unfamiliar open arena. These conditions suppress social activity in comparison to familiar, dimly lit conditions, and
this suppression forms the index of anxiety (File and Hyde 1978, 1979). Of more relevance to the current thesis are unconditioned exploratory models which rely on the spontaneous or natural reactions of rodents to environmental situations which are fear-evoking under feral conditions and thus possess increased ethological validity (Rodgers and Cole 1994; Barros and Tomaz 2002). Although such relatively short and artificial test situations cannot fully model the dynamic interactions between an animal and threatening stimuli in its natural environment (Kavaliers and Choleris 2001), these models are potentially more ethologically appropriate and relevant in terms of adaptive function of behaviour (Rodgers and Randall 1987; Blanchard et al. 1993b, 1998a) and may, therefore, be more analogous to anxiety-related behaviour in humans (Rodgers and Cole 1994; Blanchard 1997). It is beyond the scope of this introduction to provide a comprehensive review of unconditioned animal models, however, three commonly used tests are detailed below.

The Elevated Plus Maze

An example of an unconditioned behavioural model of anxiety, and perhaps the most widely used paradigm, is the elevated plus maze (EPM). The EPM evolved from studies by Handley and Mithani (1984) who produced an X shaped maze comprising of two open arms and two enclosed arms, with the two open arms and the two closed arms facing each other. The test is based on rodents’ innate preference for enclosed, protected areas over unprotected areas versus their desire to explore novel environments. Thus, an anxiogenic effect is defined as an increase in time spent in the closed arms, hence increasing the
aversion of the anxiety-provoking open arms, whilst greater exploration of the open arms suggests a decrease in the natural aversion of the open arms, and an anxiolytic effect (Hogg 1996). Forced or voluntary passage onto the open arms is associated with elevated plasma corticosterone, increased freezing and production of fecal boli, and hormonal and behavioural changes that are indicative of increased anxiety (Pellow et al. 1985).

The Open Field and Light/Dark Box Tests

Other examples of unconditioned exploratory models include the open field arena and the light/dark box. The open field comprises of a circular arena surrounded by an outer wall. The test is based on rats’ desire to remain near the perimeter of a novel environment (thigmotaxis). Thus, anxiolytic effects are determined as an increase in the subjects’ exploration of the centre of the arena in the absence of any concomitant changes in overall locomotor activity (Treit and Fundytus 1988). The light/dark box (Crawley and Goodwin 1980) typically uses mice as subjects and consists of a closed dark compartment and an open light component. As with the EPM, the test is based on the natural conflict between subjects’ desire to explore unfamiliar environments versus their tendency to avoid open unprotected areas (Hascoët et al. 2001). Thus, time spent in, and the number of transitions between, the two compartments are recorded with an anxiolytic effect determined as an increase in exploration of the light section of the apparatus.
2.3. Validity of Unconditioned Behavioural Models

Despite their widespread use, the validity of existing unconditioned behavioural models such as the EPM, open field arena and light/dark box as simulations of the more extreme pathological anxiety states remains questionable. As discussed below, whilst these tests may model more generalised anxiety states such as GAD, they do not fulfil the predictive, face and construct validity required for use as pre-clinical models of panic-related disorders.

Predictive Validity

The predictive validity of existing behavioural models of anxiety has typically been demonstrated using drugs that display limited efficacy in the treatment of extreme anxiety disorders, such as BDZ anxiolytics (Rodgers and Cole 1994; Viana et al. 1994; Dawson and Tricklebank 1995). The extent of this reliance on validating paradigms to the BDZ ‘gold standard’ has even led to the suggestion that the tests may merely be modelling BDZ receptor function as opposed to anxiety (Rodgers and Cole 1994; Green and Hodges 1991). Certainly, whilst BDZ’s are an effective and commonly used treatment for GAD, at similar doses they are not efficacious for disorders such as PD and phobias (Nutt 1991; Ballenger 1991, 1994), thus suggesting heterogeneity in the neurochemical substrates, as well as the manifestations, of anxiety disorders (Graeff 1990; Deakin and Graeff 1991; Viana et al. 1994). Moreover, treatment regimes effective for PD, such as chronic administration of the SSRI fluoxetine have failed to reliably reduce anxiety-related
behaviour in the EPM (e.g. Durand et al. 1999; Griebel et al. 1999; Silva and Brandão 2000). Therefore, it has been suggested that unconditioned behavioural models such as the EPM and light/dark box may be specifically sensitive to drugs efficacious for GAD, as opposed to panic-related disorders (Rodgers et al. 1995).

**Face Validity**

The behaviours used as indices of anxiety in established animal models also bear more resemblance to the behavioural symptoms of GAD than PD. Many of the criteria for a panic attack such as ‘a fear of dying’, ‘depersonalisation or derealisation’, ‘nausea’ and ‘choking sensations’ involve subjective cognitive experiences impossible to model in rodents. However, during a panic attack patients typically experience an ‘urgent desire to flee or escape from the situation where an attack is occurring’ (American Psychiatric Association 1994). Whilst this is akin to the abrupt active escape or flight responses in animals elicited by the close proximity of a predator or a looming stimulus it does not appear analogous to the passive avoidance of unprotected or brightly lit areas of the EPM, open field and light/dark box. The decision whether or not to enter the relatively aversive exposed or bright lit areas of the test apparatus from the relative safety of the closed or dark areas more closely resembles ‘risk assessment’ behaviour and the ‘apprehensive expectation’ which characterises GAD (Rodgers 1997; Blanchard et al. 2001b).

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Construct Validity

Construct validity for behavioural models attempting to model pathological anxiety states is hard to attain as it implies homology between the animal paradigm and the condition being modelled. Therefore, it requires the aetiology of anxiety behaviour and the biological factors underlying anxiety are similar in animals and humans. Behavioural models such as the EPM and light/dark box may only achieve construct validity for a state of ‘normal anxiety’ (Treit 1985, Lister 1990, Belzung and Griebel 2001). Thus, the aversive properties of the exposed or brightly lit areas of the test apparatus cause similar responses in naïve subjects to those seen in humans faced with a naturally occurring threat. However, as anxiety disorders may be caused by dysfunction in the neural system mediating these responses, the tests fall short on the criteria of construct validity for ‘pathological anxiety’.

2.4. Flight-Based Behavioural Models of Extreme Anxiety

In light of these problems, attempts have been made to develop pre-clinical behavioural models which may be more specifically related to extreme anxiety disorders. Given the proposed relationship between flight and escape behaviour in animals and symptoms of panic in humans, potential models of panic have been developed which include, or focus on, measures of active avoidance and flight in rodents. Whilst this increases face validity, in order to achieve predictive validity these models must be sensitive to compounds that increase, decrease and have no effect on panic, as opposed to other anxiety disorders, in
humans. Examples of animal models measuring flight or escape behaviour, and the effects of such compounds in these models, are detailed below.

The Elevated T Maze

The elevated T maze (Viana et al. 1994) is based on the EPM and consists of three equally sized arms elevated 50cm from the floor. The one closed arm is surrounded by a 40cm wall and is positioned perpendicularly to the other two open, exposed arms. Each experimental session consists of two test situations. Initially rats are placed at the end of the closed arm and the latency to leave this section and enter the open arms is recorded over three trials. This is referred to as ‘inhibitory avoidance’. In the second test situation, the subject is placed at the end of one the exposed arms and the time taken to leave the open arm and enter the closed arm is referred to as ‘one way escape’. It has been proposed that whilst the ‘inhibitory avoidance’ task may measure behaviour more analogous to GAD, ‘one way escape’ may be more related to PD (Graeff et al. 1998).

Initial pharmacological studies revealed that diazepam (1.0 – 4.0 mg/kg) reduced inhibitory avoidance (i.e. it had an anxiolytic-like effect) but did not affect one way escape, thus suggesting some analogy to the clinical effectiveness of the compound in GAD as opposed to PD (Viana et al. 1994). Furthermore, the non-benzodiazepine anxiolytic buspirone, effective for the treatment of GAD, also impaired inhibitory avoidance (0.3 mg/kg) without affecting one way escape (Graeff et al. 1998). Yet, the behavioural profiles of compounds known to induce panic in humans in the elevated T-
maze call into question the proposal that one way escape models the symptoms of PD. The panicogenic agent yohimbine increased inhibitory avoidance at the top dose tested (3.0 mg/kg) (i.e. it had an anxiogenic effect) but had no effect on escape latencies. Caffeine (10 – 30 mg/kg) was without effect on either inhibitory avoidance or one way escape (Graeff et al. 1998). A significant main effect of mCPP (0.1 – 0.8 mg/kg) on inhibitory avoidance was observed which suggested an increase in the latency to exit the closed arm (Mora et al. 1997). However, mCPP also produced a non-significant dose-dependant increase in the time taken to escape the open arm, thus suggesting a panicolytic-like profile completely opposed to the clinical literature.

Closer examination of the control data from 14 separate studies cited in a review of the T-maze (Graeff et al. 1998) reveals that, on average, subjects take around 16 secs to travel the length of the exposed arm and enter the relative safety of the closed arm. As noted by Blanchard and colleagues (2001a) this speed of approx. 0.03 m/sec renders the behaviour more akin to ‘ambling’ than flight or escape. To address this issue, Teixeira et al. (2000) isolated the distal end of an open arm and placed subjects there for a period of 30 min. When re-exposed to the T-maze 24 hr later, control group one way escape latencies were reduced to 7.5 and 7.1 secs over two trials. Using this modified procedure, chronic imipramine administration (5.0 – 15.0 mg/kg) significantly increased the latency of one way escape, although a similar effect was also observed with an acute dose of 15.0 mg/kg. Overall, the effects of panicogenic and panicolytic agents in the elevated T maze are variable and further testing of the validity of one way escape as a model of PD is certainly required (Teixeira et al. 2000).
The Fear/Defence Test Battery

Although not a proposed model of panic, the F/DTB allows the measurement of a broad spectrum of defensive behaviours in wild rats and the available evidence suggests that drugs effective for anxiety, as opposed to panic, have no effect on flight or escape behaviours in the test. Blanchard et al. (1989) examined the profiles of the three low potency BDZ's, chlordiazepoxide (CDP 5.0 – 20.0 mg/kg), diazepam (1.0 – 5.0 mg/kg) and midazolam (1.0 – 10.0 mg/kg) in the F/DTB procedure described previously (see page 27). Upon initial approach by the experimenter in the escapable runway, the number of active avoidances of, and the distance escaped from, the experimenter were not altered by any of the BDZ's. When subjects were 'chased' by the experimenter around the runway, flight speed was not affected by CDP or diazepam. Although flight speed was reduced by 5.0 and 10.0 mg/kg midazolam, all subjects in these groups actively fled and maintained a consistent distance between themselves and the following experimenter. In contrast to the lack of effect on flight responses, the BDZ's significantly altered measures of defensive threat/attack. Thus, in the inescapable runway situation, all three compounds significantly reduced instances of defensive threat, boxing, biting and jump attacks.

Similarly, buspirone (5.0 mg/kg – 20.0 mg/kg) produced a reduction in defensive threat and attack in the inescapable runway but no reduction in flight measures in the escapable situation (Blanchard et al. 1988). Avoidance distance and flight speed were not altered, with flight distance and the number of subjects fleeing actually increasing following buspirone treatment. The predictive validity of the flight component of the F/DTB as a
model of panic anxiety cannot be established due to the lack of data regarding the profiles of panicolytic and panicogenic agents. However, the above studies certainly suggest that drugs effective for GAD as opposed to PD have specific effects on defensive threat and attack responses and minimal effect on flight or escape in wild subjects. It is noteworthy that similar effects of diazepam have also been observed in feral cats. When approached by an experimenter following an acute dose of the compound (1.0 mg/kg), subjects displayed a reduction in defensive behaviours such as hissing, growling and attack, but no changes in flight behaviour compared to saline treated animals (Langefeldt and Ursin 1971).

**The Mouse Defence Test Battery**

To date, the behavioural test which displays the most promising predictive validity as an animal model of panic is the flight component of the Mouse Defence Test Battery (Griebel et al. 1996). As described on page 28, the MDTB comprises of a scaled down version of the F/DTB and uses a hand held anaesthetised rat as the approaching threatening stimulus.

The available pharmacological data suggest that flight in the MDTB is increased by panicogenic agents, decreased by panicolytic agents and unaffected by drugs effective in the treatment of GAD as opposed to PD. An initial study into the effects of yohimbine in the MDTB used a larger runway and an approaching experimenter as the threat stimulus (Blanchard et al. 1993a). All doses tested (0.5 – 2.0 mg/kg) significantly increased the
number of active avoidances of the experimenter and avoidance distance was reduced at 0.5 mg/kg. Furthermore, these effects were specific to flight-related behaviours as none of the other defensive responses recorded in the runway were altered.

The behavioural profiles of acute and chronic imipramine and fluoxetine (5.0 – 15.0 mg/kg) also appear to mirror clinical findings, with a single dose increasing flight and repeated administration reducing flight in the MDTB (Griebel et al. 1995a). Thus, acute imipramine increased avoidance distance (10.0 and 15.0 mg/kg), flight distance from the threat stimulus (5.0 mg/kg) and flight speed when chased by the rat (5.0 – 15.0 mg/kg). In contrast, 21 days imipramine produced a dramatic reduction in avoidance distances at all doses and the number of active avoidances at 5.0 and 10.0 mg/kg. Similarly, whilst acute fluoxetine increased avoidance distance (5.0 and 10.0 mg/kg), the measure was reduced at all doses following repeated administration. Moreover, alprazolam reduced avoidance distances (0.5 – 3.0 mg/kg), the frequency of avoidances and flight speed (2.0 mg/kg) when administered for 10 days (Griebel et al. 1995c).

Compounds effective for the treatment of GAD, and not PD, do not reliably affect flight in the MDTB. Non sedative/myorelaxant doses of chlordiazepoxide (CDP) (< 10.0 mg/kg) reduced risk assessment behaviour and defensive attack but did not alter any measures of flight (Griebel et al. 1995b). Likewise, buspirone (1.25 – 5.0 mg/kg) reduced instances of biting but failed to reduce measures of flight/escape (Griebel et al. 1998). However, whilst the primary effects of an acute dose of diazepam in the MDTB were reductions in risk assessment (0.5 mg/kg – 3.0 mg/kg) and defensive attack (3.0
mg/kg), it should be noted that 3.0 mg/kg of the compound did significantly reduce avoidance distance (Griebel et al. 1998).

Although investigation into the MDTB profiles of additional drugs known to induce panic in humans (e.g. mCPP and caffeine) are needed for comparison with the observed effects of yohimbine, the differential effects of panico- and anxio-lytic treatment regimes certainly suggest the model possesses a good degree of predictive validity for PD. However, it is noteworthy that in terms of the ethological validity of the paradigm the use of an anaesthetised laboratory rat as a ‘predatory’ stimulus is questionable. Common natural predators of mice are feline (e.g. cats) or avian (e.g. hawks, owls) and domesticated rats (as used as the ‘predator’ stimulus in the MDTB) display very little muricide behaviour (Karli 1956). However, whilst this is a valid criticism of the model, the use of a rat has been shown to elicit more extreme flight responses than the gloved hand of an experimenter (Griebel et al. 1995b) and as this method reliably induces abrupt unconditioned escape responses in subjects, it could be argued that it adds to the face, if not the ethological, validity of the test.

Lack of Flight in Laboratory Rats

Importantly, of the two paradigms described above which use rats as subjects, the F/DTB, and not the elevated T maze, elicits the rapid escape response thought to resemble some of the behavioural symptoms of panic. A fundamental difference between the studies described using the F/DTB and those using the T maze is that the former use wild
subjects and the latter laboratory-bred animals. This is primarily due to observations that laboratory-bred rats display significantly reduced levels of flight/escape behaviour in comparison to their feral counterparts and are therefore potentially less suitable for pre-clinical research into panic-related disorders.

Blanchard and colleagues (1986b) compared the behaviours of wild-trapped rats, first generation offspring of wild-trapped rats and Long Evans laboratory-bred rats in the F/DTB. Following the initial approaches of the experimenter, active avoidance only occurred in approx. 57% of trials using laboratory rats as opposed to almost all trials using wild and lab-reared wild rats. The distance between the experimenter and the subject required to elicit avoidance was significantly higher in both sets of wild rats compared to the Long Evans rats. Similarly, if avoidance occurred, the distance fled and the escape speed were dramatically increased in both groups of wild rats compared to the laboratory-bred rats which had to be ‘virtually pushed around the alleyway’.

The reduced flight response of laboratory-bred rats tested in the F/DTB appears to be a function of selective breeding for ease of handling. Blanchard et al. (1994) tested groups of ‘wild-type’ and ‘domesticated’ rats from a colony maintained over 35 generations in the F/DTB. The two founder groups were initially selected from second generation wild-trapped rats on the basis of their defensive reaction to human handling. Active avoidance following experimenter approach occurred on very few trials using the domesticated group compared to almost 100% of trials using wild-type animals. Correspondingly, the
experimenter-subject distance preceding flight and the flight distance and speed were all
greater in wild compared to domesticated subjects.

2.5. Brain Stimulation-Based Models of Extreme Anxiety

Given the differences in the innate flight reactions observed in domesticated and wild
rats, and the relative unavailability of the latter, paradigms which elicit escape behaviour
via stimulation of the neural fear circuitry in laboratory bred rats have been developed. In
addition to increasing face validity by inducing more dramatic flight responses, such
models go further towards attaining elements of construct validity. The measurement of
behaviours elicited by activation of the fear circuitry in the absence of external triggering
stimuli is potentially more related to the spontaneous and uncontrollable activation of the
fear system suggested as fundamental in the aetiology of pathological anxiety (Deakin
and Graeff 1991; Graeff et al. 1993; Charney and Deutch 1996; Rosen and Schulkin
1998). Two such paradigms which use stimulation of the PAG and MH as simulations of
panic, and the effects of anxiety- and panic-modulating compounds on the flight
behaviour elicited, are discussed below.

Dorsal Periaqueductal Grey (dPAG) Stimulation

Based on observations that stimulation of the dorsal aspects of the PAG evoke flight and
escape responses in rats and symptoms of panic and extreme anxiety in humans, Jenck
and colleagues have developed an electrical dPAG stimulation model of panic in rats
(Jenck et al. 1995). Subjects are implanted with electrodes in the dPAG before being placed in a rectangular cage divided into two compartments by a 2cm high barrier. The thresholds of stimulation intensity required for displays of 'sudden running with vigorous and repeated attempts to escape from the cage' in each individual subject are calculated. Rats are then trained to self interrupt the stimulation by crossing the 2cm barrier into the opposite side of the cage and pressing a lever. The threshold for self-interruption behaviour then forms the index of flight behaviour.

The panicolytic high potency BDZ's alprazolam (0.32 – 3.2 mg/kg) and clonazepam (0.1 – 1.0 mg/kg) both increase the frequency threshold for self-interruption of dPAG stimulation, thus displaying antiaversive effects. Conversely, the adenosine antagonist caffeine (10.0 and 32.0 mg/kg) and the α₂-adrenergic antagonist yohimbine (10.0 mg/kg), that both induce panic attacks in PD patients, significantly decrease the frequency threshold and the latency to self-interrupt stimulation, thus displaying panicogenic-like effects and suggesting elements of predictive validity for the model (Jenck et al. 1995).

However, the profiles of other compounds in the dPAG stimulation model are less consistent with clinical observations. Acute doses of the serotonergic mCPP reliably elicit panic attacks in PD and PTSD patients and healthy volunteers. Yet, when tested in the dPAG stimulation model, mCPP (0.46 – 4.6 mg/kg) significantly and dose-dependently increased the thresholds for escape suggesting an anxiolytic-like profile. Indeed, at the highest dose tested (4.6 mg/kg) escape responses were completely abolished (Jenck et al. 1990). Furthermore, acute administration of fluoxetine which has been shown to
potentiate panic symptoms in PD patients has also proven antiaversive in the test (Jenck et al. 1998b).

More recently, Schenberg, Vargas and colleagues have proposed an unconditioned dPAG stimulation model of spontaneous panic attacks. dPAG stimulation thresholds are recorded for a range of behaviours including defecation, micturition, tense immobility, trotting, galloping and jumping. Following 21 days treatment with the panicolytic agent clomipramine, thresholds were increased for running, jumping (5.0 and 10.0 mg/kg) and galloping (5.0 mg/kg). Chronic administration of fluoxetine at 1.0 and 5.0 mg/kg also significantly increased thresholds for galloping (Vargas and Schenberg 2001). As the authors point out, these effects were seen over a 21 day treatment period similar to the time course of clinical therapeutic action, therefore suggesting the paradigm is sensitive to treatment regimes effective for PD. However, as with the conditioned dPAG model, acute fluoxetine also displayed an antiaversive profile, attenuating stimulation-evoked immobility and galloping at 1.0 and 5.0 mg/kg (Schenberg et al. 2002), a finding in conflict with clinical observations.

**Dorsomedial Hypothalamus (DMH) Inhibition**

Shekhar (1994) has proposed that tonic inhibition of GABAergic activity in the dorsomedial hypothalamus (DMH) in rats may be a potential model of panic disorder. Blocking GABA transmission in the DMH via injection the GABA\(_A\) antagonists bicuculline and picrotoxin and an inhibitor of GABA synthesis, isonicotonic acid
hydrazide (INH), elicits physiological and behavioural symptoms resembling those of a panic attack (Di Micco and Abshire 1987; Shekhar and Di Micco 1987; Wible 1989). These include sudden and dramatic increases in heart rate, respiratory rate and blood pressure and displays of running and jumping behaviour with many subjects actually jumping out of the experimental arena.

The physiological effects of GABA<sub>A</sub> blockade in the DMH are attenuated by systemic administration of imipramine and clonazepam (Shekhar 1994) which have shown efficacy in the treatment of PD. Thus, increases in heart rate evoked by bicuculline injection into the DMH were significantly reduced in subjects receiving imipramine (5 mg/kg and 15 mg/kg) for 7 days and clonazepam (5mg/kg) for 3 days. Increases in blood pressure were also blocked by 15 mg/kg imipramine. Conversely, infusions of sodium lactate, which have been shown to reliably induce panic attacks in PD patients (e.g. Hollander et al. 1989; Liebowitz et al. 1995; Otte et al. 2002), produced increases in heart rate, blood pressure and respiratory rate in rats with chronic GABA dysfunction in the DMH (Shekhar et al. 1996; Shekhar and Keim 2000).

It is important to note, however, that pharmacological modulation of GABA<sub>A</sub> in the DMH also elicits behavioural responses not indicative of flight or escape. Microinjection of bicuculline into the DMH produces an anxiogenic-like profile in the EPM, with a significant reduction in time spent on the open arms in comparison to vehicle-treated animals. Conversely, the GABA<sub>A</sub> agonist muscimol injected into the DMH produces an increase in open arm time and open arm entries (Shekhar 1993). When tested in the social
interaction paradigm, intradMH bicuculline produces an anxiogenic-like profile, although this is blocked by intraperitoneal administration of imipramine and clonazepam (Shekhar 1994). Furthermore, sodium lactate infusions following GABA blockade in the DMH produce anxiogenic effects in the EPM and the social interaction tests (Shekhar et al. 1996; Shekhar and Keim 2000). Thus, it is possible that manipulation of GABA_A receptors in the DMH produces wider effects on defensive behaviour than flight or escape (Blanchard et al. 2001a). Until the effects of clinically active panicolytic and panicogenic agents on 'escape-oriented locomotion' are assessed, the validity of the DMH inhibition paradigm as a model of panic remains in question.

2.6. Summary

Pre-clinical models of anxiety measure experimentally induced fear- and anxiety-related behaviours in animals in controlled laboratory conditions and are used as tools to investigate the neuroanatomical and neurological bases of anxiety disorders. Of these tests, those based on unconditioned behavioural responses to ethologically relevant aversive stimuli are thought to be potentially more closely related to human anxiety states. However, existing unconditioned models such as the EPM, light/dark box and open field arena possess limited face, predictive and construct validity as simulations of the more extreme anxiety disorders such as PD and PTSD. Attempts to increase face validity via the measurement of flight behaviours in rats thought to resemble the behavioural symptoms of panic-anxiety have been hampered by the reduced escape responses observed in laboratory-bred, as opposed to wild, subjects. Thus, models of
panic have been developed which measure flight and escape responses following stimulation of the rat neural defence circuitry. Yet, whilst the spontaneous and uncontrollable activation of the fear system may be more closely related to the dysfunctional neural activation thought to underlie pathological anxiety, the effects of panicolytic and panicogenic agents in these tests remains variable. Thus, at present, there is a lack of a widely used unconditioned ethological anxiety paradigm in rats which fulfils the required criteria for modelling extreme pathological anxiety states.
3. The Unstable Elevated Exposed Plus Maze – A Novel Behavioural Model of Extreme Anxiety

3.1. The Unstable Elevated Exposed Plus Maze (UEEPM)

The unstable elevated exposed plus maze (UEEPM) is a recently developed pre-clinical behavioural model of anxiety that elicits unconditioned flight/escape responses in laboratory-bred rats and is the focus of the present experiments. Initial studies suggest the UEEPM possesses both face validity and elements of construct validity for modelling extreme anxiety disorders such as PD and PTSD. The predictive validity of the model is, at present, unknown. In brief, the test is a modified version of the EPM and capitalises on many species innate aversion to instability, height and exposure. The apparatus consists of a plus-shaped maze elevated 50cm from the floor. However, the UEEPM has no protected areas with all the four maze arms exposed. The maze is also oscillated in the horizontal plane at a speed of 85 rpm.

3.2. Face Validity of the UEEPM

When exposed to the UEEPM, untreated experimentally naïve rats display unconditioned escape and escape-related behaviours resembling the highly avoidant state and ‘urge to flee’ seen in panic patients (King 1999a, b, c). In the first of a series of three experiments, King (1999a) observed that during testing some animals adopted a crouching position at the side of the apparatus with their heads directed towards the floor. This was frequently
followed by subjects actually jumping from the maze. Animals also appeared to search for potential escape routes by scanning over the edge of the maze, exploring the distal ends of the maze arms and rotating in small $90^\circ$ movements, as if to achieve a complete $360^\circ$ field of view. A subsequent study revealed an additional method of escape with a number of animals backing off the apparatus hindquarters first, often turning their heads and peering over their forequarters whilst doing so (King 1999c). This was frequently preceded by an attempt to back off from the UEEPM in this manner which was reversed immediately before the animals’ centre of gravity was shifted irreversibly over the arm.

Furthermore, factor analysis revealed that these behaviours observed in the UEEPM reflect a different form of anxiety from that modelled in the EPM (King 1999a). Hooded Lister rats were exposed to both tests and the behavioural data subjected to principal components analysis. Three factors emerged from the UEEPM testing. Jumping, preparing to jump, reaching the end of the maze arms, scanning and turning in $90^\circ$ movements all had high positive loadings on the first ‘escape-related’ factor. The ‘assessing the surroundings/evaluation of potential escape’ factor included the number of turns and the amount of time subjects spent in the centre of the maze, while the remaining factor represented the general ‘locomotor activity’ of subjects in the test. Analysis of EPM behaviour resulted in the emergence of two distinct factors, ‘anxiety’ which included the amount of activity in the open arms during the trial and ‘locomotor activity’. When the data for subjects’ behaviour on both tests were combined in a single analysis, all previously observed factors maintained their independence, thus suggesting the UEEPM and EPM measure two distinct facets of anxiety-like behaviour.
3.3. Construct Validity of the UEEPM

The escape behaviours observed in naïve animals in the above experiments more closely resemble the highly avoidant, flight-oriented symptoms observed in PD and PTSD than the symptoms of GAD. Furthermore, repetitive electrical stimulation of the rat SC prior to UEEPM exposure results in dramatic and long-lasting increases in these escape-behaviours possibly mirroring the long-term increase in sensitivity in the human fear system thought to be responsible for the chronically hyper-aroused fight/flight state seen in pathological anxiety. Moreover, this sensitised state selectively manifests in increased escape in the UEEPM and not increases in anxiety-like behaviour in other anxiety tests (King 1999c).

Hooded Lister rats with electrodes bilaterally implanted in the SC were placed in an open field arena. Over a number of trials subjects were stimulated with ascending frequencies and current until explosive escape behaviour occurred. The behaviour of subjects in the UEEPM was then analysed 20, 48 and 76 days post-stimulation. On all three trials, animals from the stimulated group jumped from the UEEPM significantly more frequently than the unstimulated control group. Correspondingly, the time spent on the maze before escaping was less for the stimulated groups on post-stimulation trials. Stimulated animals also scanned and prepared to jump more than control animals across the three test sessions. Importantly, when the same subjects were tested in the EPM and light/dark box at the same post-stimulation intervals anxiety-related behaviour did not
differ. Thus the UEEPM appears to model a more extreme, panic-related type of anxiety than other unconditioned anxiety models in both experimentally naïve and sensitised rats.
4. Experimental Overview and General Methods

In terms of face validity, the UEEPM elicits unconditioned escape behaviours in laboratory-bred rats which resemble the behavioural symptoms of panic-related disorders. Construct validity is suggested by chronic increases in escape behaviour following repetitive activation of the midbrain defence circuitry, potentially analogous to the long-term increase in sensitivity in the neural defence circuitry fundamental to the aetiology of pathological anxiety. However, at present the neurochemical substrates of escape in the UEEPM remains unknown. Thus, the current series of experiments aimed to examine the pharmacological bases of UEEPM behaviour and assess the predictive validity of the paradigm.

Pre-clinical models of anxiety are notoriously sensitive to a range of methodological variables which can affect pharmacological studies (e.g. Andrews and File 1991; Johnston and File 1991; Rodgers and Cole 1993; Trullas and Skolnick 1993; Hogg 1996). Hence, before ethopharmacological analysis of the UEEPM was conducted, the effects of a number of these potential confounds, namely circadian phase of testing, level of test illumination and strain of subjects, on baseline UEEPM behaviour were examined.
4.1. Experimental Overview

Experiment 1 – Influence of Circadian Phase and Test Illumination on Pre-clinical Models of Anxiety.

It has been suggested that circadian phase of testing may influence the effects of compounds in anxiety models (Cao and Rodgers 1998). Thus, experiment 1 investigated the effect of both time of day of testing and level of test illumination on the behaviour of naïve rats exposed to the UEEPM. As the effects of circadian phase on baseline behaviour in animal models of anxiety had not previously been investigated, subjects were also exposed to a battery of existing paradigms.

Experiment 2 – Strain Differences in Escape-related and Anxiety-related Behaviour in Rats

Different strains of rat have been shown to exhibit different levels of anxiety-related behaviour in a variety of anxiety models (e.g. Valle 1970; Trullas and Skolnick 1993; King 1999a). Therefore, the behaviour of four rat strains exposed to the UEEPM was assessed with a view to determine strains optimal for detecting anxiolytic and anxiogenic-like effects in subsequent pharmacological studies. To examine whether any observed differences were specific to escape behaviour observed in the UEEPM, subjects were also exposed to a Runway Test which measured flight responses to an approaching stimulus.
Furthermore, to assess whether strain differences were generalisable to other subtypes of anxiety-related responses, subjects behaviour in the EPM was also analysed.

**Experiment 3 – Evidence for the Predictive Validity of the Unstable Elevated Exposed Plus Maze: Effects of Anxiogenic Agents.**

In order to investigate the predictive validity of the UEEPM, the effects of compounds known to induce panic in humans (mCPP, caffeine, yohimbine) were assessed, as were the profiles of compounds known to increase anxiety-related behaviour in existing animal models (FG 7142, PTZ).

**Experiment 4 – 5-HT\textsubscript{2C} Receptor Mediation of Unconditioned Escape Behaviour in the Unstable Elevated Exposed Plus Maze.**

As mCPP acts as an agonist at two subtypes of serotonin (5-HT) receptors, 5-HT\textsubscript{2C} and 5-HT\textsubscript{2B}, the fourth experiment investigated which specific 5-HT receptor mediates mCPP-induced behavioural effects in the UEEPM. Hence, the effects of mCPP alone and in combination with a selective 5-HT\textsubscript{2C} receptor antagonist, SB 242084, were examined.

In order to assess the bi-directional predictive validity of the UEEPM, the profiles of treatment regimes effective (chronic administration of fluoxetine) and ineffective (acute administration of fluoxetine and the BDZ chlordiazepoxide) for panic disorder were investigated in experiment 3. Prior to commencing the chronic fluoxetine dosing regime, the effects of repeated dosing and handling on UEEPM behaviour were quantified.

4.2. General Methods used in the Current Experiments

The Unstable Elevated Exposed Plus Maze

Apparatus

The UEEPM consisted of a plus-shaped maze elevated 50cm above the floor with four exposed arms measuring 35 cm x 12 cm and a centre square measuring 12 cm x 12 cm (figure 1.1). The floor of the plus maze was covered with matt black rubber and each arm had a 0.5 cm ledge to reduce slippage. A motor oscillated the apparatus in the horizontal plane at 85 revs/min with a movement amplitude 2cm each side of the central point.
Procedure

In each UEEPM trial subjects were initially placed in the centre square facing one of the maze arms. The maze arm each subject faced was randomised across trials. After a period of 3 secs the maze was oscillated and the subjects’ behaviour recorded on videotape over a period of 5 mins. If a subject fell from the apparatus they were placed back into the area from which they exited. If an animal actively escaped from the apparatus, via jumping or backing off, the trial was terminated. Escape was easily differentiated from falling as this was invariably preceded by foot slippage and scrabbling to stay on the apparatus. Each trial was then later analysed by an observer blind to treatment using a specifically
designed software program (Mazetime, Oxford UK). In order to reduce olfactory cues, the maze was cleaned between each trial with a solution consisting of 80% water and 20% ethanol. A matt black surround was placed around the UEEPM during testing, to eliminate visual cues as far as possible.

**Behavioural Measures**

The escape-related and locomotor/exploratory behaviours described in tables 1.2 and 1.3 were recorded in each UEEPM trial (adapted from King 1999a, b, c). An example of jumping is shown in figure 1.2.

**Table 1.2. Escape and escape-related behaviours recorded in the UEEPM**

<table>
<thead>
<tr>
<th>BEHAVIOUR</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jumping</td>
<td>An animal removing itself from the apparatus forequarters first</td>
</tr>
<tr>
<td>Preparing to jump</td>
<td>A foreshortening of the body, with the back legs drawn up under the hindquarters, and leaning the forequarters over the side of the apparatus</td>
</tr>
<tr>
<td>Scanning</td>
<td>Protruding the head over the exposed sides of the arms and scrutinising in any direction</td>
</tr>
<tr>
<td>End reaching</td>
<td>The number of times the animal reached the end of any maze arm</td>
</tr>
<tr>
<td>Backing off</td>
<td>An animal shifting off the apparatus hindquarters first and simultaneously peering over their forequarters</td>
</tr>
<tr>
<td>Attempting to back off</td>
<td>Similar to backing off except the behaviour is reversed immediately prior to the animals' centre of gravity shifting irreversibly over the exposed arm</td>
</tr>
<tr>
<td>Turning</td>
<td>Rotating in 90° movements in any area of the maze</td>
</tr>
<tr>
<td>Duration</td>
<td>The length of time spent on the UEEPM (max 300 secs)</td>
</tr>
</tbody>
</table>
### Table 1.3. Exploratory/locomotor behaviours recorded in the UEEPM

<table>
<thead>
<tr>
<th>BEHAVIOUR</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone crossing</td>
<td>The number of times subjects moved between the following three zones: outer</td>
</tr>
<tr>
<td></td>
<td>arms (the distal half of any of the maze arms), inner arms (the inner half</td>
</tr>
<tr>
<td></td>
<td>of any of the maze arms) and the centre square.</td>
</tr>
<tr>
<td>Ratio centre time</td>
<td>The time spent in the centre square relative to trial duration</td>
</tr>
<tr>
<td>Ratio end time</td>
<td>The time spent in the distal half of the maze arms relative to trial duration</td>
</tr>
</tbody>
</table>

### Analysis of UEEPM Behaviours

As each trial was terminated when a subject actively escaped or after 5 minutes had elapsed, experimental duration varied between animals. Hence, to aid comparison of results the data for all behaviours exhibited (except escaping and trial duration) were converted into average frequencies over 10 secs. Previous research has revealed that the majority of animals escaping the UEEPM do so within the first 120 secs of the trial (King 1999a). To avoid misleading comparisons being drawn between the frequencies of behaviours exhibited by animals which escaped at an early stage, only data from the first 120 secs of the trials were included in the analyses. As there were no data for subjects escaping within the first 15 secs of the trial, scores for these animals were excluded (except escape and duration). Finally, if all group means for a particular behaviour fell below 0.02 per 10 secs in any given experiment, data for this measure were excluded from subsequent analysis.
Figure 1.2. An example of jumping behaviour in the UEEPM. The subject moves to the end of the maze arm (photos 1-3) and prepares to jump (photo 4), before jumping from the maze onto the floor (photos 5-8).
Chapter II

Experiment 1: Influence of Circadian Phase and Test Illumination on Pre-Clinical Models of Anxiety

Overview

Pre-clinical models of anxiety, particularly the elevated plus-maze (EPM), have been shown to be sensitive to a variety of methodological variations. Recent research has implicated circadian phase of testing in influencing the behavioural profile of 5-HT$_{1A}$ ligands on the elevated plus-maze. The present study investigated the effects of testing animals during the dark and light phases and in light and subjective dark test conditions on baseline behaviour in animal models of anxiety. Singly housed male Sprague Dawley rats were exposed to a battery of unconditioned, exploratory tests (EPM, open field arena, holeboard) and a new model of extreme anxiety, the unstable elevated exposed plus-maze (UEEPM). Circadian phase of testing failed to consistently alter behaviour on any model. Level of test illumination had no effect on subjects’ response to the open field arena, holeboard or UEEPM. Dark testing increased locomotor activity on the EPM (total arm entries, closed arm entries and distance moved) without decreasing open arm avoidance. The construct of anxiety as measured by a number of different paradigms withstood major intra-laboratory manipulation of circadian phase of testing and illumination of apparatus. It is suggested that the effects of circadian rhythmicity may be confined to the behavioural profiles of serotonergic, particularly 5-HT$_{1A}$, ligands on the EPM.
Introduction

Pre-clinical models of anxiety have failed to provide consistent profiles for established and novel anxiolytic agents. For example the widely used elevated plus-maze test (EPM) has provided contrasting profiles for serotonergic (5-HT), adrenoceptor, and cholecystokinin (CCK) receptor ligands (for review see, Rodgers and Cole 1994). Methodological variables such as gender, age and strain of subjects have been shown to influence behaviour on animal models and may go some way to explaining inconsistencies. For instance, sex differences have been reported in the social interaction test and the Vögel conflict test (Johnston and File 1991), whilst female subjects have been found less anxious than males (Johnston and File 1991; Rodgers and Cole 1993) on the EPM. Trullas and Skolnick (1993) found that 70 % of variability in plus-maze behaviours between mouse strains could be attributed to genetic factors. Strain differences have been reported for locomotor and thigmotactic behaviour in the open field arena (Valle 1970) and for escape-related responses in a new model of extreme anxiety, the unstable elevated exposed plus-maze (UEEPM, King 1999a). Young rats have also shown less propensity to escape the UEEPM than adult rats (King 1999c). In addition, procedural variables such as prior handling (Andrews and File 1991), individual housing, prior exposure to a novel arena (Rodgers and Cole 1993) and illumination levels have been shown to affect baseline behaviour in pre-clinical models, hence creating further potentially confounding methodological variables.
High lighting levels have been shown to suppress locomotion and rearing and increase thigmotaxis in the open field arena (Valle 1970) and decrease exploration in the black/white test box (Costall et al. 1989). In contrast, Griebel et al. (1993) reported that rats exposed to the EPM under low illumination (centre square 100 lux, open arms 80 lux, closed arms 90 lux) entered open arms more, spent a greater proportion of the trial on the open arms and were generally more active than those tested under high illumination (centre square 230 lux, open arms 220 lux, closed arms 100 lux). Thus, bright light appeared to increase the aversiveness of the test situation. However different research groups have found both pigmented (Pellow et al. 1985) and albino (Falter et al. 1992; Becker and Grecksch 1996) rats' behaviour on the EPM is independent of light levels ranging from 9.5 lux to 900 lux. Interestingly, level of test illumination has been found to dramatically affect the profiles of serotonergic ligands on the EPM. An identical dose of 8-OH-DPAT has produced anxiolysis at high levels of illumination, yet anxiogenesis at low levels (Handley et al. 1993).

Recently, rodent circadian rhythmicity has been suggested as a possible source of variability in the EPM. Rodents display circadian rhythms of approximately 24 hr in a range of physiological and behavioural measures including body temperature (Weinert and Waterhouse 1999), corticosterone release (Windle et al. 1998), locomotor activity (Gorka et al. 1996) and response to pain (Konecka and Sroczynska 1998). Cao and Rodgers (1998) propose that circadian phase of testing may be responsible for the reported contrasting effects of the selective 5-HT$_{1A}$ antagonist LY 297996. The compound has produced anxiolysis in mice tested on the EPM during the mid-dark phase,
but been ineffective in mice tested during the mid-light phase. Results reported in abstract form show WAY 100635, a selective 5-HT\textsubscript{1a} antagonist, produces significantly different effects on extracellular levels of 5-HT depending on circadian phase of administration (Gurling et al. 1994). Microdialysis of the rat hippocampus found the compound had no effect on 5-HT levels during the light phase, yet levels rose to 350% of control values during the dark phase.

Although rats' behaviour on the EPM has been shown to vary at different times during the light phase (Griebel et al. 1993), to date no research has compared baseline behaviour in each of the circadian phases on a variety of animal models of anxiety. The present study aimed to assess the validity of circadian factors as potential methodological confounds. Experimentally naive, untreated rats were exposed to a battery of unconditioned behavioural tests (UEEPM, EPM, open field arena, holeboard) during their subjective light and dark phases. To determine whether any observed differences were a function of lighting conditions and thus independent of circadian phase, two levels of test illumination, subjective light and subjective dark, were employed. Prior to behavioural testing, a small pilot study was conducted to investigate any circadian variations in subjects' activity in a familiar environment (see Appendix).
Method

Subjects

Animals were 40 male Sprague Dawley rats (Harlan UK) aged 10 weeks and weighing 345-400 g at the start of testing. Half were housed under a normal 12 hour light cycle (lights on 07:00, half-light 07:00-08:00 and 18:00-19:00) and half under a reversed cycle (lights on 19:00, half-light 07:00-08:00 and 18:00-19:00). Light levels were 297 lux approx. (light), 48 lux approx. (half-light) and 9 lux approx. (dark). Rats were singly housed upon arrival at the laboratory and kept in a temperature controlled environment (21 ± 1°C) with relative humidity 62 ± 5 %. Subjects were acclimatised to individual housing and light cycles for 2 weeks prior to testing. Food and water were available ad libitum.

Apparatus and Procedure

Behavioural Tests

Subjects housed under each light cycle were randomly allocated to a corresponding or contrasting testing condition thus forming four groups; LL (housed and tested in the light), LD (housed in the light and tested in the dark), DD (housed and tested in the dark) and DL (housed in the dark and tested in the light). Animals in the two contrasting groups (LD and DL) were exposed to the test apparatus immediately following transportation.
from the keeping room and hence were not dark / light adapted to the level of test illumination. The home cages of the DD group were covered with red filters for transportation to the testing room thus keeping lighting conditions constant. Light testing was conducted under diffuse white light at the same level of luminance as light cycle conditions in the keeping room (297 lux approx.). Each animal was exposed to a battery of tests with trials lasting 5 min. The order of test exposure was UEEPM, EPM, open field arena then holeboard and was consistent across experimental groups. There was a 48 hr gap between tests for all subjects. Analysis was conducted during trials by a specialised program (Ethovision Basic, Tracksys UK) except UEEPM data which was analysed via videotape and a specifically designed program (Mazetime, Oxford UK). Subjects’ location on all tests was determined by the placement of their centre of gravity. Testing occurred between 11:30 and 14:30 and apparatus was cleaned (20 % ethanol solution) between trials. A black surround was placed around all apparatus to minimise external visual cues as far as possible.

**Unstable Elevated Exposed Plus-Maze**

The apparatus and procedure for UEEPM testing was as previously described on pages 59-62 of this thesis.

**Elevated Plus-Maze**

The EPM consisted of two open (35 cm x 12 cm) and two enclosed arms (35 cm x 12 cm x 40 cm) and a centre square (12 cm x 12 cm, figure 2.1). The maze was elevated 50cm
above the floor. Open arms were surrounded by a 0.5 cm ledge and the entire floor was covered in black rubber. Each subject was placed in the centre square facing an open arm and a variety of anxiety and locomotor measures were recorded. Anxiety parameters consisted of the number of open arm entries relative to overall arm entries (ratio open entries) and the time spent by subjects on the open arms relative to overall trial duration (ratio open time). Locomotor measures analysed were the total number of entries into any of the maze arms (total entries), the number of entries into the closed arms (closed entries) and the total distance travelled (cm) by subjects over the entire trial (distance moved).

![Image of the elevated plus maze (EPM)](image)

Figure 2.1. The elevated plus maze (EPM)

**Open Field Arena**

The open field was circular (70 cm diameter) and surrounded by a 65 cm wall. For the purposes of analysis the arena was divided into two zones. The inner zone measured 35
cm in diameter, with the remaining annulus forming the outer zone. Animals were placed in the centre of the apparatus prior to the start of each trial. Locomotor / exploratory measures included distance moved (cm), the frequency and amount of time spent rearing (rears, reartime), the frequency of zone crossing and the ratio time spent in the inner zone relative to trial length.

**Holeboard**

The holeboard measured 60 cm x 60 cm surrounded by a wall 35 cm tall. Four 2.5 cm diameter holes 15 cm from each corner were located on the floor. Locomotion was assessed via distance moved (cm), rearing (frequency) and reartime (seconds spent rearing in trial). Exploration was analysed via frequency of head dipping into any of the holes.

**Statistical Analysis**

To assess the influence of circadian phase of testing and test illumination on behaviours, the scores for all measures were analysed using a two-factor between subjects ANOVA for independent samples. Given the lack of a widely used equivalent non-parametric procedure, any data not fulfilling the criteria for the ANOVA were transformed via the square root method (Howell 1997) thus achieving homogeneous variance. Significant interactions between housing and testing were analysed using Tukey’s HSD multiple pairwise comparisons. The frequency with which subjects jumped or backed off from the UEEPM was analysed using the Chi-Square Test of Association.
Results

Unstable Elevated Exposed Plus-Maze

Group means and standard errors for UEEPM behaviours are presented in Table 2.1.

Effect of Circadian Phase of Testing

No differences in the rate at which animals jumped (light phase 10 % [2 / 20], dark phase 10 % [2 / 20]) or backed off from the UEEPM (light phase 5 % [1 / 20], dark phase 10 % [2 / 20]) were observed between the two circadian phases. Trial duration was similarly unaffected by manipulation of circadian phase of testing (F [1,36] = 0.23, p = 0.64). None of the corresponding escape related behaviours were altered (scanning F [1,32] = 3.67, p = 0.06, end reaching F [1,32] = 0.24, p = 0.63, attempting to back off F [1,32] = 0.03, p = 0.86, retreating F [1,32] = 3.25, p = 0.08, turning F [1,32] = 0.83, p = 0.78). Locomotor / exploratory measures of ratio centre time (F [1,32] = 0.90, p = 0.35) and ratio end time (F [1,32] = 0.01, p = 0.99) were also independent of phase of testing. A significant housing X testing interaction was observed for zone crossing (F [1,32] = 5.58, p < 0.05). However Tukey’s HSD post hoc tests revealed no significant differences between any two groups.
The frequency with which animals escaped from the UEEPM did not differ as a function of level of test illumination. 15% (3/20) of subjects tested under light conditions jumped from the apparatus compared to 5% (1/20) of those tested under dark conditions. Instances of backing off were also unaffected; 5% (1/20) of animals tested under light conditions and 10% (2/20) tested under dark conditions backed off from the maze. In line with these results, trial length did not differ between lighting conditions (F[1,36] = 0.19, p = 0.67). Of the escape related behaviours scored, subjects tested under light conditions scanned more frequently than those tested under dark conditions (F[1,32] = 11.19, p < 0.05). However end reaching (F[1,32] = 0.21, p = 0.65), attempting to back off (F[1,32] = 0.46, p = 0.50), retreating (F[1,32] = 0.36, p = 0.55) and turning (F[1,32] = 2.36, p = 0.13) did not differ between the two lighting conditions. Locomotor/exploratory measures of zone crossing (F[1,32] = 1.21, p = 0.28), ratio centre time (F[1,32] = 1.06, p = 0.31) and ratio end time (F[1,32] = 0.01, p = 0.98) were independent of test illumination.

Table 2.1. Effect of test illumination and circadian phase on UEEPM behaviour

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Dark dark</th>
<th>Light light</th>
<th>Dark light</th>
<th>Light dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attempts back off</td>
<td>0.05 ± 0.04</td>
<td>0.03 ± 0.02</td>
<td>0.01 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Scanning</td>
<td>0.19 ± 0.07*</td>
<td>0.64 ± 0.09</td>
<td>0.62 ± 0.10</td>
<td>0.51 ± 0.07*</td>
</tr>
<tr>
<td>End reaching</td>
<td>0.13 ± 0.05</td>
<td>0.12 ± 0.04</td>
<td>0.24 ± 0.05</td>
<td>0.19 ± 0.04</td>
</tr>
<tr>
<td>Turning</td>
<td>1.15 ± 0.41</td>
<td>1.46 ± 0.14</td>
<td>1.73 ± 0.20</td>
<td>1.28 ± 0.10</td>
</tr>
<tr>
<td>Zone crossing</td>
<td>0.57 ± 0.14</td>
<td>0.85 ± 0.11</td>
<td>0.99 ± 0.11</td>
<td>1.01 ± 0.12</td>
</tr>
<tr>
<td>Ratio centre time</td>
<td>0.29 ± 0.09</td>
<td>0.32 ± 0.09</td>
<td>1.15 ± 0.85</td>
<td>0.30 ± 0.04</td>
</tr>
<tr>
<td>Ratio end time</td>
<td>0.21 ± 0.11</td>
<td>0.22 ± 0.09</td>
<td>0.28 ± 0.07</td>
<td>0.28 ± 0.05</td>
</tr>
<tr>
<td>Duration (secs)</td>
<td>215.2 ± 43.2</td>
<td>215.8 ± 43.1</td>
<td>270.2 ± 29.8</td>
<td>300.0 ± 0.00</td>
</tr>
</tbody>
</table>

Data are presented as mean values (± SEM). * p < .05, main effect of test illumination
**Elevated Plus-Maze**

Due to video recorder malfunction the DD group consisted of 8 subjects. Group means and standard errors for behavioural measures are presented in Table 2.2.

**Effect of Circadian Phase of Testing**

No main effects of circadian phase of testing were observed on any of the anxiety measures. Group means did not differ for ratio open entries (F [1,34] = 0.49, p = 0.82) or ratio open time (F [1,34] = 0.54, p = 0.81). The locomotor measures of distance moved (F [1,34] = 0.17, p = 0.67), total entries (F [1,34] = 0.58, p = 0.45) and closed entries (F [1,34] = 0.53, p = 0.47) were similarly unaffected.

**Effect of Test Illumination**

Anxiety measures were unaffected by illumination level: no differences in ratio open entries (F [1,34] = 2.16, p = 0.15) or ratio open time (F [1,34] = 0.57, p = 0.46) were observed. Significant main effects of test illumination were observed on all measures of locomotor activity. Subjects tested under dark conditions performed more arm entries (F [1,34] = 13.43, p < 0.01), closed arm entries (F [1,34] = 8.47, p < 0.01) and travelled further (F [1,34] = 8.81, p < 0.01) than those tested under light conditions.
Table 2.2. Effect of test illumination and circadian phase on EPM behaviour

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Dark dark</th>
<th>Light light</th>
<th>Dark light</th>
<th>Light dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total entries</td>
<td>6.75 ± 1.51**</td>
<td>3.10 ± 1.17</td>
<td>2.10 ± 0.69</td>
<td>8.00 ± 2.11**</td>
</tr>
<tr>
<td>Closed entries</td>
<td>4.38 ± 0.80**</td>
<td>2.40 ± 0.88</td>
<td>1.80 ± 0.63</td>
<td>5.10 ± 1.16**</td>
</tr>
<tr>
<td>Distance moved (cm)</td>
<td>697 ± 137</td>
<td>381 ± 58</td>
<td>348 ± 65</td>
<td>573 ± 90</td>
</tr>
<tr>
<td>Ratio open entries</td>
<td>0.28 ± 0.10</td>
<td>0.16 ± 0.07</td>
<td>0.16 ± 0.11</td>
<td>0.32 ± 0.10</td>
</tr>
<tr>
<td>Ratio open time</td>
<td>0.07 ± 0.03</td>
<td>0.03 ± 0.01</td>
<td>0.11 ± 0.10</td>
<td>0.19 ± 0.10</td>
</tr>
</tbody>
</table>

Data are presented as mean values (+ SEM). **p < .01, main effect of test illumination

Open Field Arena

Group means and standard errors for all behaviours exhibited in the open field arena are presented in Table 2.3.

Effect of Circadian Phase of Testing

Circadian phase of testing had no effect on the behaviours recorded (distance moved F[1,36] = 1.12, p = 0.29, rears F[1,36] = 2.03, p = 0.16, reartime F[1,36] = 0.46, p = 0.5, zone crossing F[1,36] = 0.38, p = 0.85, ratio centre time F[1,36] = 0.22, p = 0.88).

Effect of Test Illumination

Test illumination had no effect on subjects' locomotor and exploratory behaviour in the open field test; distance moved (F[1,36] = 2.66, p = 0.11), rears (F[1,36] = 0.53 p = 0.47), reartime (F[1,36] = 0.33, p = 0.57) and zone crossing (F[1,36] = 2.72, p = 0.11).
did not differ between groups. The relative amount of time subjects spent in the central area of the arena was also unaffected (F [1,36] = 2.65, p = 0.11).

Table 2.3. Effect of test illumination and circadian phase on open-field behaviour

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Dark dark</th>
<th>Light light</th>
<th>Dark light</th>
<th>Light dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance moved (cm)</td>
<td>1721 ± 133</td>
<td>1366 ± 115</td>
<td>1641 ± 98</td>
<td>1723 ± 204</td>
</tr>
<tr>
<td>Rears</td>
<td>6.60 ± 1.56</td>
<td>3.50 ± 1.46</td>
<td>4.50 ± 1.11</td>
<td>3.50 ± 1.57</td>
</tr>
<tr>
<td>Reartime</td>
<td>10.64 ± 3.07</td>
<td>7.36 ± 3.40</td>
<td>5.22 ± 1.22</td>
<td>4.94 ± 2.24</td>
</tr>
<tr>
<td>Zone crossing</td>
<td>4.00 ± 1.15</td>
<td>0.70 ± 0.37</td>
<td>3.60 ± 2.10</td>
<td>6.20 ± 2.62</td>
</tr>
<tr>
<td>Ratio centre time</td>
<td>0.01 ± 0.01</td>
<td>0.00 ± 0.00</td>
<td>0.01 ± 0.01</td>
<td>0.02 ± 0.02</td>
</tr>
</tbody>
</table>

Data are presented as mean values (+SEM).

Holeboard

Group means and standard errors for all behavioural measures exhibited in the holeboard are presented in Table 2.4.

Effect of Circadian Phase of Testing

Subjects tested during the dark circadian phase reared more than those tested during the light phase (F [1,36] = 3.08, p < 0.01). A significant housing x testing interaction (F [1,36] = 5.27, p < 0.03) was also observed for rearing. Follow up Tukey’s HSD tests revealed the DD group reared more than the LL (p < 0.01), DL (p < 0.05) and LD (p < 0.05) groups. Distance moved (F [1,36] = 0.76, p = 0.39), reartime (F [1,36] = 1.71, p = 0.2) and head dipping (F [1,36] = 1.55, p = 0.22) were not altered by manipulation of circadian phase.
**Effect of Test Illumination**

No main effects of test illumination were observed on the locomotor measures of distance moved ($F_{[1,36]} = 0.41, p = 0.84$), rears ($F_{[1,36]} = 3.08, p = 0.88$) and reartime ($F_{[1,36]} = 2.09, p = 0.16$) or the exploratory measure of head dipping ($F_{[1,36]} = 2.22, p = 0.15$).

**Table 2.4. Effect of test illumination and circadian phase on holeboard behaviour**

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Dark dark</th>
<th>Light light</th>
<th>Dark light</th>
<th>Light dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance moved (cm)</td>
<td>1252 ± 191</td>
<td>1135 ± 181</td>
<td>1148 ± 146</td>
<td>957 ± 189</td>
</tr>
<tr>
<td>Rears</td>
<td>8.60 ± 1.45***#</td>
<td>3.70 ± 1.16</td>
<td>4.10 ± 0.98**</td>
<td>3.10 ± 0.72</td>
</tr>
<tr>
<td>Reartime</td>
<td>10.66 ± 3.27</td>
<td>2.98 ± 0.88</td>
<td>7.74 ± 2.22</td>
<td>8.12 ± 3.84</td>
</tr>
<tr>
<td>Head dipping</td>
<td>6.60 ± 1.83</td>
<td>2.10 ± 0.59</td>
<td>5.30 ± 1.48</td>
<td>5.70 ± 2.22</td>
</tr>
</tbody>
</table>

Data are presented as mean values (± SEM). **p < .01, main effect of circadian phase of testing. # p < .05, DD group vs. LL, DL and LD groups

**Discussion**

**Effect of Circadian Phase of Testing**

None of the anxiety measures on the pre-clinical models used were sensitive to manipulation of circadian phase of testing. The ratios of open arm entries / open arm time on the EPM, the number of head dips on the holeboard and escape-related behaviour on the UEEPM did not significantly differ between dark and light circadian phases. Although time spent in the centre of the open field did not differ, the lack of an anxiogenic-like effect resulting from manipulation of circadian phase is proposed with
caution given the low levels of ratio centre time observed for all groups. Locomotor measures on all tests were consistent across both light cycles.

Despite rats being at directly opposing stages of a range of 24 hr bodily rhythms their reactions to the aversiveness of the test situations were unchanged. Thus, it appears that behavioural responses to spontaneously occurring stressful situations are independent of current physiological rhythms. It is suggested therefore, that reported circadian differences in behavioural profiles may be confined to the effects of specific pharmacological agents, particularly those acting at serotonin receptors, as opposed to any intrinsic properties of the tests themselves. This is certainly plausible given the reported inconsistencies in the profiles of serotonergic ligands and the involvement of serotonin in mammalian circadian regulation (for recent review see, Portas et al. 2000). In particular, Lu and Nagyama (1997) propose that 5-HT<sub>1A</sub> receptor tone is modulated by an endogenous circadian regulator. Recent research moreover, has failed to replicate the conflicting effects of 5-HT<sub>1A</sub> ligands administered during contrasting circadian phases in the Geller-Seifter conflict model (Gleason and Leander 1999). It is interesting to note that more recent work carried out in this laboratory has reported no differences in the behavioural profiles of the selective 5-HT<sub>2C</sub> receptor antagonist SB 242084 and the 5-HT<sub>2C/2B</sub> agonist m-Chlorophenylpiperazine (mCPP) on the UEEPM during light and dark circadian phases (King et al. 2000). The notion of a specific diurnal cycle sensitivity to anxiolytic drugs acting at the 5-HT<sub>1A</sub> receptor and also non-5-HT compounds (e.g. those acting at BDZ and CCK receptors) in unconditioned, ethological models of anxiety is worthy of further investigation yet beyond the scope of this discussion.
Effect of Test Illumination

Anxiety measures were independent of light / dark testing on all behavioural tests. Therefore, measures traditionally used as indices of anxiety in a number of paradigms (e.g. preference for the closed arms of the EPM, actively escaping the UEEPM and lack of exploration of the holeboard) withstood fairly major manipulations of test illumination. Again, low baselines of ratio centre time on the open field arena for all subjects rendered it difficult to observe an anxiogenic, if not anxiolytic, effect of test illumination on this test. It is worth noting that subjects were not permitted to adapt to the test conditions after transportation from the keeping room. The DL and LD groups were moved from a familiar environment and placed in anxiety provoking / novel situations under a dramatically different visual environment. However they appeared no more or less anxious than subjects tested in corresponding keeping and testing conditions.

The observation that scotopic conditions did not appear to alter the aversiveness of the open arms on the EPM is not in agreement with Griebel and colleagues (1993) who observed a reduction in the ratio of closed : open entries under low luminance. The conflict appears counter-intuitive given their cited luminance levels provided mean lux approx. of 90 (low) and 183 (high) compared to our greater disparity of 9 lux approx. (low) and 297 lux approx. (high) and use of dim red as opposed to white light. The reasons for this are unclear, although differences in strain (Wistar vs. Sprague Dawleys) and housing (group vs. singly housed) are apparent. The subjects used in this study were singly housed at 70 days of age, thus older than potentially critical periods during which
social isolation has been shown to increase anxiety in pre-clinical tests (Da Silva et al. 1996; Wongwitdecha and Marsden 1996) and defensive aggression in adulthood (Potegal and Einon 1989). Therefore it is unlikely that individual housing influenced the present results.

Only locomotor activity on the elevated plus-maze paradigm was sensitive to levels of test illumination. General maze activity was increased by dark testing conditions; animals travelled further, entered maze arms more frequently and performed more closed arm entries (a measure previously identified as a pure index of locomotor activity, Cruz et al. 1994). Although previous work has found no effect of illumination on EPM locomotor behaviour, various methodological discrepancies exist between the present and previous studies. The lowest illumination level employed in this experiment (9 lux approx.) was slightly less than the lowest level cited in any of the previous studies (9.5 lux, Falter et al. 1992). The rat visual system possesses a dark-adapted threshold for light detection of between -5.2 and -5.8 log cd / m² regardless of pigmentation (Herreros et al. 1992). Although the present dark lighting (equivalent to 0.4 log cd / m² approx.) exceeded this, it is possible the combination of lower light intensity, a red filter and subjects not being dark-adapted, provided a qualitatively different experimental environment. The rat retinal mechanism has two peaks of sensitivity at approximately 510 nm and in the ultra violet at 370 nm (Jacobs et al. 1991). Given the current light wavelength of > 600 nm, visual cues would be substantially less than under dim white light even if the animals were allowed to adapt to the dark conditions. Other minor procedural differences may also have contributed to the discrepancies. For example, this laboratory determined location on the
maze by the subject’s centre of gravity whereas Becker and Grecksch (1996) classed arm entry as all 4 feet being present in the arm, we placed animals on the maze facing an open not a closed arm and trial length was 5 min not 7 min.

Conclusions

Animal models of anxiety have produced well documented inter-laboratory inconsistencies in the profiles of novel and established anxiolytics. However, the present intra-laboratory comparisons suggest that, at least using Sprague Dawley rats, the open field arena, holeboard and unstable elevated exposed plus-maze are robust enough to cope with major changes in illumination and circadian phase of testing. The observation that general EPM activity was sensitive to light levels lends some support to the notion that this test is particularly confounded by methodological variation (Hogg 1996). Given subjects’ natural propensity to avoid the open arms, an increase in total arm entries may provide a more useful baseline from which to measure the effects of anxiogenic compounds which might be expected to decrease open arm entries (Rodgers and Johnson 1995). The levels of anxiety exhibited by rats in a range of exploratory-based pre-clinical models of anxiety appear independent of subjective lighting conditions during habitually active or inactive periods. The housing of subjects under reversed light cycles and testing under dark conditions may merely increase ethological as opposed to any experimental validity.
CHAPTER III

Experiment 2 – Strain Differences in Unconditioned Escape Behaviour in Laboratory-Bred Rats

Overview

Pre-clinical anxiety paradigms which measure unconditioned escape/flight behaviours are thought to model the more extreme, panic-related, pathological anxiety states. However, the lack of escape responses seen in laboratory-bred, as opposed to wild, rats has led to the suggestion that the species may be unsuitable for use in such tests. The current experiment examined whether this reduced flight response was apparent across different strains of laboratory-bred rats. Male Brown Norway, August, Fischer and Hooded Lister rats were exposed to two tests eliciting escape responses, the unstable elevated exposed plus maze (UEEPM) and a runway test measuring avoidance of a suddenly looming visual stimulus. To determine if any differences were specific to flight/escape responses or generalisable to other anxiety-related behaviours, subjects were also exposed to the elevated plus maze (EPM) test. Significant inter-strain differences were observed, with Brown Norway, and to a lesser extent, August subjects displaying higher levels of anxiety-related behaviour in all three tests compared to Fischer and Hooded Lister animals. The results suggest that unconditioned flight/escape behaviour can be elicited in certain strains of laboratory-bred rats and the selection of subjects according to baseline levels of escape behaviour may benefit pre-clinical research into panic-related disorders.
Introduction

Tests which measure fear-related behaviours in rodents are widely used to facilitate investigation into human anxiety conditions. As well as acting as tools to examine the biological bases of fear- and anxiety-related behaviour they are also used as screens to assess the efficacy of novel anxiolytic compounds (Green and Hodges 1991; Rodgers and Cole 1994). However, discrepancies between the profiles of drugs in such models are commonly reported and this lack of consistency may be a function of variation in test procedures both within and between laboratories (Hogg 1996). Methodological variables such as housing, gender, age, prior handling and method of transportation to the testing room have all been shown to affect the behaviour of subjects in pre-clinical models of anxiety (e.g. Andrews and File 1991; Johnston and File 1991; Rodgers and Cole 1993; Morato and Brandão 1996; King 1999c; Ramos et al 2002). In particular, baseline levels of anxiety-related behaviour in a range of unconditioned paradigms appear to be dependant on the strain of animal used.

Inter-strain differences in the behaviour of untreated, experimentally naïve rats and mice in tests employing passive avoidance of relatively aversive stimuli as indices of anxiety have been observed. For instance, in the elevated plus maze (EPM) test, Lewis and Wistar-Kyoto rats display less activity in the open arms of the apparatus than spontaneously hypertensive (SHR) rats (Durand et al. 1999; Pollier et al. 2000; Ramos et al. 2002) and Dark Agouti rats avoid the open arms more than Sprague Dawley subjects (Mechan et al. 2002). In the open field arena, Wistar-Kyoto and Long Evans rats enter the
more aversive central areas less than SHR and BD-IX subjects respectively (Valle 1970; Durand et al. 1999; Ramos et al. 2002). Fawn Hooded animals display reduced levels of social interaction with an unfamiliar conspecific in comparison to Wistar and Sprague Dawley rats (Kantor et al. 2000) and Lewis rats demonstrate less activity in the white area of the black/white box test than SHR subjects (Ramos et al. 2002). Moreover, EPM, open field and social interaction test behaviour also differs in rats of the same strain obtained from different suppliers (Rex et al. 1996; Bert et al. 2001). In a comparison of eight mouse strains, Griebel et al. (2000) reported that BALB/c mice displayed higher levels of anxiety-related behaviour in the light/dark box than other strains, and C57BL/6, DBA/2 and NZB animals were more avoidant of the exposed arms in the EPM. Similarly, Trullas and Skolnick (1993) exposed 16 inbred mouse strains to the EPM and found that approx. 70% of the variability in open arm activity could be attributed to genetic factors.

There is, however, less research assessing strain differences in anxiety tests measuring the active flight or escape element of rodents' defensive behavioural repertoire which is thought to be more related to the behavioural symptoms of extreme anxiety disorders such as panic disorder (PD, Blanchard et al. 2001a), than the generalised anxiety states modelled by tests such as the EPM (Rodgers et al. 1995). To date, examinations of individual differences in flight behaviour in rats have focused on comparisons between wild and domesticated subjects when exposed to a natural predator. Thus, Blanchard and colleagues (1896b; 1994) found that when confronted with an approaching experimenter in the Fear/Defence Test Battery (F/DTB), both laboratory-bred Long Evans rats, and wild-trapped rats bred over 35 generations for ease of handling, displayed dramatic
reductions in active avoidance and escape behaviours compared to feral subjects. Indeed, whilst wild rats chased by an experimenter reliably exhibited full blown flight and maintained a consistent distance between themselves and the potential threat, Long Evans rats had to be ‘virtually pushed around the alleyway’ (Blanchard et al. 1986b). This attenuation of the innate flight response, along with the practical problems of using wild animals in the laboratory, has led to suggestions that rats may be an unsuitable species for pre-clinical research into panic (Blanchard et al. 2001a).

However, as significant inter-strain variations in other aspects of fear-related behaviour are apparent, it is plausible the lack of a flight response may not be common to all domesticated rats, leaving certain strains more suitable for the controlled measurement of escape behaviour. This certainly appears to be the case in laboratory mice. Griebel and colleagues (1997c) tested a number of strains in the Mouse Defense Test Battery (MDTB) which measures the behavioural response of mice to an approaching hand-held rat. Significant differences in flight from the threat stimulus were observed, with over a 200% increase in the propensity to turn and escape from the rat in the most reactive (DBA/2), compared to the least reactive (CBA), strain. Moreover, when chased by an experimenter in a continuous runway, FBI creams (a strain of wild-trapped mice bred in the laboratory for over 52 generations by staff specially trained in the husbandry of wild animals) display a flight response which not only exceeds that of laboratory-bred albino mice, but is also greater than that seen in wild wood and house mice (Hendrie et al. 2001).
Furthermore, direct evidence of inter-strain variations in rats’ escape behaviour has been obtained in this laboratory using a recently developed behavioural model of extreme anxiety which elicits escape from an aversive and unstable test situation (the unstable elevated exposed plus maze [UEEPM], King 1999a, b, c; Jones and King 2001). Thus, King (1999a) observed that experimentally naive Dark Agouti and Sprague Dawley rats displayed over a two-fold increase in their propensity to escape from the UEEPM than Hooded Lister rats.

Therefore, the aim of the current study was to further characterise baseline levels of unconditioned flight and escape behaviour in four strains of rats obtained from a single commercial supplier (Harlan, UK). The behaviour of male Brown Norway, Fischer, August and Hooded Lister rats was compared in two tests measuring escape responses, namely the UEEPM and a novel runway test which assessed subjects’ active avoidance of a suddenly looming visual stimulus presented on a collision trajectory. In order to determine if any strain differences were specific to escape responses, or generalisable to other aspects of rats’ defensive behaviour, subjects were also tested in the EPM.

Method

Subjects

Animals were male Hooded Lister, Fischer, August and Brown Norway rats (Harlan UK) aged 10 weeks and weighing between 152g and 292g at the start of testing. Subjects were
singly housed under a normal 12 hour light/dark cycle (lights on 07:00) in a temperature
controlled environment (21 ± 1°C) with relative humidity 62 ± 5 %. Food and water were
available ad libitum. All in vivo studies were conducted in accordance with the United
Kingdom Animals (Scientific Procedures) Act (1986) and conformed to
GlaxoSmithKline ethical standards.

Apparatus and Procedure

Behavioural Testing

Subjects were divided into groups on the basis of strain, thus forming the following four
groups (all n=10); BN (Brown Norway), AG (August), FC (Fischer) and HL (Hooded
Lister). Each subject was exposed to the three behavioural tests. The order of test
exposure for all animals was UEEPM, EPM then Runway Test and there was a 48 hr gap
between tests. Testing occurred between 10:00 and 17:00 and all apparatus was cleaned
(20 % ethanol solution) between trials. All trials were videotaped, with UEEPM and
EPM data analysed via a specifically designed program (Mazetime, Oxford UK) and
Runway Test data analysed manually by the experimenter.

Unstable Elevated Exposed Plus-Maze

The apparatus and procedure for UEEPM testing was as previously described on pages
59-62 of this thesis.
Runway Test

Based on observations that many animals, including frogs, gerbils, non-human primates and humans, employ active avoidance behaviours as a defensive response to suddenly looming visual stimuli (Schiff 1965, King and Cowey 1992; King et al. 1992, Ellard 1993), the runway test (figure 3.1) measured subjects’ responses to a motorised stimulus travelling towards them on a collision trajectory. The apparatus was based on the Fear/Defence Test Battery (Blanchard et al. 1986a) and its derivative the Mouse Defence Test Battery (Griebel et al. 1996) and consisted of an oval runway measuring 250 cm x 60 cm which was surrounded by walls 60 cm high. A central wall 190 cm long ran down the middle of the apparatus forming a continuous runway with a constant width of 30 cm. The runway was raised 25 cm from the ground. The runway was made of wood and the inside was painted matt black with white lines every 10 cm on the floor of the apparatus.

A motorised stimulus was used in the Runway Test. This consisted of a radio controlled car ('Octane', Nikko) which was stripped of its plastic body, thus forming a black rectangular shape (25 cm long, 15 cm wide, 6 cm high). The front of the stimulus was covered in white foam. The test session consisted of a free exploration period to assess baselines of locomotor / exploratory behaviour and a series of five identical trials measuring avoidance of the moving stimulus. Each test session lasted approximately 9 min.
Free Exploration Period

Subjects were transported to the testing room, removed from their home cages and placed at the end of one of the straight sections of the runway, facing the curved end. The experimenter then left the room and the subject was allowed to freely explore for a period of 5 mins. The number of times subjects crossed a line on the floor of the apparatus formed an index of locomotor / exploratory behaviour.
Avoidance Testing

After the 5 mins free exploration period had elapsed, the experimenter returned to the testing room and placed a square black cardboard cover (20 cm x 20 cm x 20 cm) over the subject. With the animal still underneath it, the cover was gently manoeuvred to the end of the straight section where the subject had been initially introduced to the runway. The motorised stimulus was then placed at the opposite end of the straight section, facing the subject. The experimenter then crouched down by the side of the apparatus, below the outer wall, and using a pole placed into a hook on the top of the cover, removed it from over the subject. Approximately 2 secs later the stimulus was driven down the straight section of the runway towards the subject at a speed of approximately 2.0 m/sec. If the subject actively avoided the stimulus by moving away from it in the opposite direction, the stimulus was stopped. If no avoidance had occurred by the time the stimulus was 10 cm away from the subject, the power was turned off so the stimulus came to a halt before contact with the animal was made. After the trial the cover was again placed over the subject and the above procedure repeated until 5 trials had been completed.

Avoidance measures comprised of the frequency of active avoidances (expressed as the percentage of trials in which each subject actively avoided the stimulus), avoidance distance (the mean distance between the subject and the stimulus required to elicit avoidance behaviour) and escape distance (the mean distance travelled away from the stimulus after avoidance occurred). If a subject did not avoid the stimulus, the avoidance and escape distances for that trial were recorded as zero.
**Elevated Plus-Maze**

The apparatus and procedure for the EPM was as described on page 70 of this thesis. Briefly, anxiety parameters consisted of the number of open arm entries relative to overall arm entries (ratio open entries) and the time spent by subjects on the open arms relative to overall trial duration (ratio open time). Locomotor measures analysed were the total number of entries into any of the maze arms (total entries) and the number of entries into the closed arms (closed entries).

**Statistical Analysis**

Scores for each measure were analysed using a one-way ANOVA for independent samples. Any significant main effects were further analysed using a series of Tukey’s HSD tests comparing individual groups. Non-parametric data were analysed using a Kruskal-Wallis ANOVA, with any significant effects further examined using a series of Mann-Whitney U-tests comparing individual groups. The frequency with which subjects escaped from the UEEP M was analysed using a two tailed Fisher’s exact test.
Results

Unstable Elevated Exposed Plus Maze

One subject from the BN group fell from the apparatus and could not be easily retrieved. Hence, data for this animal were excluded from the analysis and the BN group consisted of 9 subjects. All subjects escaping from the UEEPM did so via jumping. Strain differences were observed in the propensity to escape the UEEPM. BN rats exhibited the highest escape rates (100%), followed by AG (60%), FC (20%) and HL (10%). Frequency analyses revealed that the BN group exhibited significantly higher escape than the HL (Fisher exact, two-tailed, p < 0.001) and FC (Fisher exact, two-tailed, p < 0.001) groups and a trend towards higher escape than AG (Fisher exact, two-tailed, p = 0.09) animals. The AG group displayed a trend towards more frequent escape than the HL group (Fisher exact, two-tailed, p = 0.06). No differences were found in escape rates of the FC animals compared to the AG (Fisher exact, two tailed, p = 0.17) and HL (Fisher exact, two-tailed, p = 1.00) groups (figure 3.2a). Correspondingly a main effect of strain on trial duration was observed (H = 18.15, p < 0.001) with the BN group spending less time on the apparatus than the AG, FC and HL groups (figure 3.2b).

All the BN animals escaped from the UEEPM within the first 15 secs of the trial. As there were no behavioural data for these animals, further analysis compared scores from the AG, FC and HL groups only. The frequency with which subjects prepared to jump from the apparatus differed between groups (F [2,21] = 3.66, p < 0.05) with Tukey’s
HSD tests revealing an increase in the AG compared to the HL group (figure 3.3a). Main effects on scanning (F[2,21] = 9.50, p < 0.05) and turning (H = 13.06, p < 0.001) were observed with both behaviours increased in the AG group compared to the HL and FC groups and HL animals turning more than FC animals (figures 3.3b and 3.3c). End reaching did not significantly differ across experimental groups (F[2,21] = 1.50, p = 0.25, figure 3.3d). There were no significant differences in the locomotor / exploratory behaviours of zone crossing (F[2,21] = 2.98, p = 0.08), ratio end time (F[2,21] = 2.01, p = 0.16) and ratio centre time (F[2,21] = 1.79, p = 0.09) (table 3.1).

Figures 3.2a to 3.2b. Effect of strain on escape and trial duration in the UEEPM. Data are presented as % of group escaping (% escape) and group medians and interoctile ranges (duration). n = 10 in each group except BN, n = 9. Significantly different from group indicated: * p < .05, ** p < .01, *** p <.001 by Chi Square Test of Association (% escape) or Mann-Whitney U-test (duration).
Figures 3.3a to 3.3d. Effect of strain on escape-related behaviours in the UEEPM. HL = Hooded Lister, FC = Fischer, AG = August. Data are presented as group means (± SEM) or medians and interoctile ranges. n = 10 in each group except BN, n = 9. Significantly different from group indicated: * p < .05, ** p < .01 by Tukey’s HSD test or Mann-Whitney U-test.
Table 3.1. Effects of strain on UEEPM exploratory/locomotor behaviour

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Hooded Lister</th>
<th>Fischer</th>
<th>August</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone cross / 10 secs</td>
<td>0.70 ± 0.17</td>
<td>0.22 ± 0.08</td>
<td>0.50 ± 0.11</td>
</tr>
<tr>
<td>Ratio end time</td>
<td>0.12 ± 0.06</td>
<td>0.00 ± 0.00</td>
<td>0.09 ± 0.05</td>
</tr>
<tr>
<td>Ratio centre time</td>
<td>0.49 ± 0.06</td>
<td>0.71 ± 0.13</td>
<td>0.46 ± 0.12</td>
</tr>
</tbody>
</table>

Data are presented as group means ± SEM.

Runway Test

Due to video recorder malfunction the BN group consisted of 9 subjects. Due to experimenter error, in 9 out of the total of 195 trials the stimulus was stopped too soon. In a further 37 trials, the subject moved away from the stimulus before it began its approach. Chi-square analysis confirmed that these movements away from the stationary stimulus were significantly greater in the BN (42.2% of trials) compared to the HL (2% of trials, chi [1] = 23.71, p < 0.001), FC (16% of trials, chi [1] = 8.12, p < 0.01) and AG (18% of trials, chi [1] = 7.10, p < 0.01) groups and less in the HL group compared to the AG (chi [1] = 7.11, p < 0.01) and FC (chi [1] = 6.15, p < 0.05) groups. Data for these trials were excluded from subsequent analyses.

During the initial free exploration period the number of line crossings differed between groups (F [3,38] = 14.22, p < 0.001) with FC subjects displaying less locomotor activity than the other three strains (figure 3.4a). A significant difference was observed in the percentage of trials in which active avoidance of the stimulus occurred (H = 26.17, p < 0.001). The HL group avoided the stimulus significantly less than all other groups. BN
Figures 3.4a to 3.4d. Effects of strain on locomotor/exploratory and avoidance related behaviours in the Runway test. HL = Hooded Lister, FC = Fischer, AG = August, BN = Brown Norway. Data are presented as group means ± SEM or medians and interoctile ranges. n = 10 in each group except BN n = 9. Significantly different from group indicated: * p < .05, ** p < .01, *** p < .001 by Tukey’s HSD test or Mann-Whitney U-test.
subjects avoided the stimulus more frequently than FC and AG animals and the AG group displayed more avoidance than the FC group (figure 3.4b). Similarly, a main effect on avoidance distance ($H = 23.78, p < 0.001$) was a function of an increase in the BN, AG and FC groups compared to the HL group. Avoidance distance was also greater in the BN compared to the FC group (figure 3.4c). Upon avoidance of the stimulus, the distance travelled by subjects also differed ($H = 28.59, p < 0.001$). Escape distance was greater in BN, AG and FC animals compared to HL subjects, and the BN group escaped further than the FC group (figure 3.4d).

**Elevated Plus Maze**

One animal from the AG group and one animal from the HL group jumped from an open arm of the EPM to the floor. Data for these subjects were excluded, hence both groups consisted of 9 subjects. The experimental groups differed in the amount of time spent in the open arms of the EPM relative to trial length ($H = 15.07, p < 0.01$). Follow up tests revealed that HL animals spent more time in the open arms than all other groups (figure 3.5a). Ratio open entries did not differ between strains ($F [3,37] = 1.99, p = 0.13$, figure 3.5b). Main effects on the exploratory / locomotor measures of total entries ($H = 21.59, p < 0.001$) and closed entries ($H = 21.36, p < 0.001$) were observed. HL subjects performed more arm entries and entries into the closed arms than the other three groups. BN animals exhibited more total entries and closed entries compared to AG subjects (figures 3.5c and 3.5d).
Figures 3.5a to 3.5d. Effects of strain on anxiety-related and locomotor/exploratory behaviours in the EPM. HL = Hooded Lister, FC = Fischer, AG = August, BN = Brown Norway. Data are presented as group means ± SEM or medians and interoctile ranges. FC and BN groups n = 10, HL and AG groups n = 9. Significantly different from group indicated: * p < .05, ** p < .01, *** p < .001 by Mann-Whitney U-test.
Discussion

Significant inter-strain differences in baseline levels of anxiety behaviour, as measured by three separate behavioural tests, were observed. Brown Norway and August rats exhibited higher levels of escape and escape-related behaviour in the UEEPM and runway test and higher levels of open arm avoidance in the EPM than Hooded Lister and Fischer subjects. The findings from each test will be summarised in turn and the implications of the findings for pre-clinical research into extreme anxiety states will be discussed.

Strain Differences in UEEPM Behaviour

Brown Norway rats exhibited extremely high levels of escape behaviour in the UEEPM compared to the other strains, with all subjects jumping from the apparatus and escape rates significantly higher than the Hooded Lister and Fischer groups. Furthermore, trial duration was lower than the other experimental groups, with all animals jumping within 15 seconds. The August strain displayed relatively high levels of escape with instances of jumping, preparing to jump, scanning and turning significantly higher than in Hooded Lister and Fischer animals. These latter two strains showed the lowest scores on all escape-related measures except end reaching. Moreover, these differences were specific to escape-related behaviour, as no inter-strain variation in general locomotion and exploration was observed. These findings are in line with a previous report of inter-strain differences in the UEEPM. King (1999a) observed that Hooded Lister rats displayed
fewer instances of jumping and preparing to jump than Dark Agouti and Sprague Dawley subjects.

**Strain Differences in Runway Test Behaviour**

The inter-strain variations in unconditioned flight behaviour were not specific to the responses elicited in the UEEPM. Brown Norway rats also displayed a significantly higher propensity to avoid the rapidly approaching motorised stimulus in the runway than the other three strains. Correspondingly, the distance required to elicit escape and the distance moved away from the stimulus were also greater in Brown Norways compared to Hooded Listers and Fischers. As in the UEEPM, August rats displayed the second highest levels of escape-related behaviour, avoiding the stimulus more frequently than Fischer and Hooded Lister subjects. Fischer rats displayed moderate levels of escape in the runway, albeit at a lesser avoidance distance than Brown Norway and August animals. The reduction in locomotor activity observed in the pre-test period, may account for the substantially shorter escape distance observed in this strain. Hooded Lister rats showed a complete lack of an escape response to the looming stimulus, with no instances of active avoidance.

**Strain Differences in EPM Behaviour**

In line with previous observations (Durand et al. 1999; Pollier et al. 2000; Meehan et al. 2002; Ramos et al. 2002), significant inter-strain differences in baseline anxiety-related
behaviour in the EPM were apparent. The Brown Norway, August and Fischer strains all displayed a greater aversion to the open areas of the maze than Hooded Lister subjects, with less time spent in the open arms relative to trial length. The three former strains were also generally less active than Hooded Lister rats in the EPM, with reductions in total arm entries and closed arm entries.

**Implications for Pre-Clinical Research into Panic**

The most striking finding of the current studies was that of the four groups of animals used, Brown Norway, and to a lesser extent August, rats displayed consistently high levels of unconditioned escape responses. This readily-activated flight response suggests the use of these strains may be of considerable benefit to pre-clinical research into extreme, panic-related anxiety disorders. To date, attempts to use laboratory rats in flight-based behavioural models of panic have been unsuccessful. As well as the near total lack of reactivity to an approaching experimenter seen in the F/DTB (Blanchard et al. 1986b), laboratory rats show little evidence of flight in the elevated T maze (e.g. Viana et al. 1994; Graeff et al. 1996; Mora et al. 1997). Although the authors suggest the measure of ‘one-way escape’ (the latency to leave the relatively aversive open arm and enter the relatively safe closed arm) is an index of ‘panic’ in this model, the typical speed of this behaviour in control animals (approx. 0.03 m/sec) is far from rapid fleeing and has perhaps been more correctly referred to by some as ‘ambling’ (Blanchard et al. 2001a).
In contrast, in the current runway experiment, Brown Norway rats fled from the approaching stimulus in over 90% of trials. This profile bears little resemblance to Long Evans rats in the F/DTB which had to be ‘virtually pushed around the alleyway’ by the experimenter (Blanchard et al. 1986b), a response more similar to the behaviour of Hooded Lister rats in this experiment. Although flight speed was not a measured variable in the present experiment, examination of data from the Brown Norway group reveals all subjects took less than 0.75 secs to travel their individual escape distances. This speed of at least 0.5 m/sec is over 16 times faster than Wistar rats travelling from the open to the closed arm of the elevated T maze and certainly much more akin to ‘full blown’ flight. Therefore, the proposal that laboratory-bred rats are unsuitable for pre-clinical research into panic (Blanchard et al. 2001a) maybe somewhat of an overgeneralization.

Indeed, the lack of flight behaviour observed in previous studies may have merely been a function of using subjects possessing generally low levels of defensive behaviour. The subjects tested in the F/DTB and elevated T maze were Long Evans and Wistar rats respectively. Previous comparisons in anxiety models measuring passive, as opposed to active, avoidance of threatening stimuli, reveal the latter strain exhibits relatively low baseline levels of anxiety-related behaviour. For instance, in the social interaction test and the EPM, Wistar rats are less ‘anxious’ than Fawn Hooded and Fischer animals respectively (Kantor et al. 2000; Bert et al. 2001) and in the open field they show an increased propensity to enter the unprotected central areas compared to Fischer animals (Rex et al. 1996). Importantly, in both the current experiment and the study by King (1999a), animals which displayed high (Brown Norway and Dark Agouti respectively)
and low (Hooded Lister) levels of escape in the UEEPM also displayed corresponding high and low levels of open arm aversion in the EPM, suggesting basal levels of defensive behaviour may be generalisable to various subtypes of anxiety-related behaviour measured in different paradigms.

The selection of experimental animals according to baselines of flight behaviour would be of considerable benefit to pharmacological studies in paradigms modelling panic-related disorders, such as the UEEPM. It is acknowledged that strain variations in behaviour have a potentially confounding effect on the profiles of compounds in pre-clinical paradigms (Hogg 1996), with low baselines of anxiety-related behaviour possibly obscuring anxiolytic properties of drugs and high baselines rendering it hard to detect anxiogenic effects (Rodgers and Cole 1994). For example, in the EPM, the benzodiazepine anxiolytic diazepam has proven ineffective in rats displaying low basal levels of open arm activity (Wistar) but dose-dependently anxiolytic in animals with a higher baseline (Fischer, Bert et al. 2001). Likewise, the anxiolytic effects of diazepam in the EPM and open field are more pronounced in BALB/cByJ than C57BL/6J mice, with control animals of the former strain showing significantly higher levels of anxiety-related behaviour (Lepicard et al. 2000). Therefore, animals showing high levels of flight reactions to threatening stimuli in pre-clinical models of panic, such as Brown Norway or August rats, may be more suitable for observing the effects of panicolytic agents, whereas subjects with low flight responses may be optimal for observing panicogenic effects.
The reasons for the dramatically increased reactivity to threat observed in selected strains of laboratory rat are unclear at present. In terms of their propensity to escape threat, Brown Norway rats seem more related to their feral counterparts than other laboratory strains such as Hooded Listers. To the best of our knowledge, the former are less commonly used in the laboratory, particularly in behavioural studies, than the latter. Given the selective breeding of wild rats on the basis of ease of handling results in significant reductions in flight behaviour (Blanchard et al. 1994), it can be speculated that the lower demand and less intensive breeding of Brown Norway animals has left some of the defensive behaviours of their wild ancestors more intact. Certainly, in the current experiment Brown Norway subjects were far harder to handle than the other strains and frequently directed bites towards the experimenter's hand, a behaviour indicative of the defensive attack response seen in wild, and not domesticated, subjects (Blanchard et al. 1986b). Brown Norway subjects also typically escaped from the UEEPM within a few seconds of being placed on the apparatus. Therefore, it is possible this flight behaviour may have been directed away from the experimenter as well as away from the aversive conditions of the test. More detailed investigation into the full spectrum of the defensive behaviour in Brown Norway rats is required, yet these initial data suggest these animals may be a commercially available strain that exhibit defensive responses similar to their feral counterparts.
Conclusions

The current experiments aimed to assess strain differences in unconditioned escape- and anxiety-related behaviour in laboratory-bred rats obtained from the same supplier. Of the four strains tested, Brown Norway rats displayed the highest levels of escape in the UEEPM and runway tests and the most aversion to the open arms in the EPM, whilst Hooded Lister rats displayed the lowest levels of anxiety-related behaviour. The use of strains such as Brown Norways may go some way to overcoming the lack of flight responses previously observed in pre-clinical models of panic-related anxiety using laboratory-bred, as opposed to wild, rats. The selection of subjects according to basal levels of escape may also optimise test situations for the detection of panicolytic and panicogenic drug effects.
CHAPTER IV

Experiment 3 – Evidence for the Predictive Validity of the Unstable Elevated Exposed Plus Maze: Effects of Anxiogenic Agents

Overview

The unstable elevated exposed plus maze (UEEPM) has been proposed as a novel model of anxiety which elicits unconditioned escape-related behaviour in rats thought to mimic the persistent ‘fight/flight’ state exhibited by patients suffering from extreme anxiety disorders. This study investigated the predictive validity of the UEEPM by examining the behaviour of rats exposed to the test following administration of drugs known to induce panic and anxiety in panic disorder and post traumatic stress disorder patients, namely m-chlorophenylpiperazine (mCPP), caffeine and yohimbine. The sensitivity of the UEEPM to two further putative anxiogenic agents; the benzodiazepine partial inverse agonist FG 7142 and pentylenetetrazole (PTZ) was also assessed. Male Hooded Lister rats received a single dose of mCPP (0.5 - 2.0 mg/kg; ip), caffeine (3.0 mg/kg - 30.0 mg/kg; ip), yohimbine (1.25 mg/kg - 5.0 mg/kg; ip), FG 7142 (3.0 mg/kg - 30.0 mg/kg; ip) or PTZ (3.0 mg/kg - 30.0 mg/kg; ip) before being exposed to the UEEPM for a period of 5 min. Subjects’ behaviour was analysed to determine the effects of each compound on unconditioned escape. mCPP (1.0 and 2.0 mg/kg), caffeine (30 mg/kg), FG 7142 (3.0 and 30.0 mg/kg) and PTZ (30.0 mg/kg) significantly increased animals’ propensity to escape from the UEEPM i.e. they had a clear anxiogenic effect, whilst yohimbine had no effect on escape. The UEEPM is sensitive to the behavioural effects of anxiogenic agents.
Furthermore, pharmacological similarities exist between symptoms of panic and anxiety in patients and escape from the UEEPM in rats. The UEEPM may therefore represent a paradigm to facilitate investigation into the neurochemical basis of extreme anxiety disorders.
Introduction

The unstable elevated exposed plus maze (UEEPM, King 1999a,b,c; Jones and King 2001) is a recently established pre-clinical model of extreme anxiety in rats. The test capitalises on the innate aversion of many species to instability, height and exposure. Rats exposed to the UEEPM typically exhibit escape and escape-related behaviour thought to be analogous to the highly avoidant, persistent ‘fight/flight’ state exhibited by patients suffering from the more extreme pathological anxiety disorders such as panic disorder (PD) and post traumatic stress disorder (PTSD). A positive correlation has been reported between the propensity to escape the UEEPM and well-validated anxiety measures on an established pre-clinical model of anxiety, the elevated plus maze (EPM). Furthermore, principal components analyses have revealed that escape in the UEEPM may reflect a different type of anxiety from that observed in the EPM (King 1999a), a test thought to model less severe conditions such as generalised anxiety disorder (GAD Rodgers et al. 1995). Moreover, repetitive electrical stimulation of the rodent midbrain defence system produces lasting increases in escape and escape-related behaviour in the UEEPM for up to 3 months, potentially analogous to the chronically hyper-aroused state observed clinically in many pathological anxiety disorders (King 1999b).

The primary aim of this study was to assess the predictive validity of the UEEPM by analysing the behaviour of rats on the apparatus following acute administration of compounds known to be anxiogenic in patients suffering from extreme anxiety disorders, namely m-chlorophenylpiperazine (mCPP), yohimbine and caffeine. A further aim was to
examine the sensitivity of the model to the effects of two reference compounds which have increased anxiety-related behaviour in a range of pre-clinical models, namely FG 7142 and pentylenetetrazole (PTZ).

*M*-chlorophenylpiperazine (mCPP), a $5\text{-HT}_{2B/2C}$ receptor agonist, elicits symptoms of panic and anxiety in clinical populations and healthy subjects. Acute mCPP induces panic attacks in PD patients (Charney et al. 1987b; Klein et al. 1991; Benjamin et al. 1999) and increases anxiety, panic and PTSD-specific symptoms in PTSD patients (Southwick et al. 1997). Challenge studies have found that mCPP elicits panic attacks (Charney et al. 1987b; Sevy et al. 1994), increases self reported symptoms of panic and anxiety (Charney et al. 1987b; Sevy et al. 1994; Benjamin et al. 1996) and induces physical symptoms of panic (Charney et al. 1987b; Kahn et al. 1990) in healthy volunteers.

The $\alpha_2$-adrenergic receptor antagonist yohimbine, which activates noradrenergic neurons via blockade of the presynaptic $\alpha_2$-adrenergic autoreceptor, induces panic attacks and increases anxiety in PD patients (Charney et al. 1987a). Panic attacks, increases in anxiety, panic and PTSD-specific symptoms (Bremner et al. 1997; Southwick et al. 1997) and enhanced acoustic startle response (Morgan et al. 1995), have been reported in PTSD patients following yohimbine challenge. In healthy volunteers yohimbine elicits feelings of anxiety and increases acoustic startle reflex (Morgan et al. 1993) yet does not induce panic attacks (Gurguis and Uhde 1990) or increase anxiety to the extent observed in PD patients (Charney et al. 1992).

Two further reference anxiogenic compounds were used in the present study; the benzodiazepine partial inverse agonist FG 7142 and the convulsive agent pentylenetetrazole (PTZ). Although neither compound has been extensively studied in humans, both produce anxiogenesis in a number of animal models. FG 7142 administered peripherally in rats (Cruz et al. 1994; Cole et al. 1995) and mice (Rodgers et al. 1995) and into the dorsal periaqueductal grey (dPAG) in rats (Russo et al. 1993) has anxiogenic effects in the EPM. The compound reduces social interaction in rats (File et al. 1985) and exploration of the holeboard in both rats (Meng and Drugan 1993) and mice (Takeda et al. 1998). Adamec (1991, 1993) has also documented chronic increases in feline defensive behaviour towards rats following acute FG 7142. PTZ displays an anxiogenic like profile in the EPM in rats (Wada and Fukuda 1991; Cruz et al. 1994; Cole et al. 1995; Wallis and Lal 1998) and mice (Rodgers et al. 1995) and in a mouse light / dark arena (De Angelis and Furlan 2000). Recently, acute PTZ has been shown to decrease the latency with which rats switch off aversive stimulation of the dPAG (Jung et al. 2001).
In the studies described herein, a series of five consecutive experiments were run with subjects receiving an acute dose of either mCPP, caffeine, yohimbine, FG 7142 or PTZ prior to UEEPM exposure. The behaviour of subjects on the UEEPM was then analysed to examine the effects of each compound on escape and escape-related responses.

**Method**

**Animals**

Male Hooded Lister rats (Harlan UK), aged 10 weeks and weighing 202 – 318g at the start of testing, were singly housed under a normal 12 hour light/dark cycle (lights on 07:00) in a temperature controlled environment (21 ± 1°C) with relative humidity 51 ± 5%. Food and water were available *ad libitum*. All *in vivo* studies were conducted in accordance with the United Kingdom Animal (Scientific Procedures) Act 1986 and conformed to GlaxoSmithKline ethical standards.

**Apparatus and Procedure**

The apparatus and procedure for UEEPM testing was as described on pages 59-62 of this thesis.
**Drugs**

FG 7142 (Tocris, UK) and yohimbine hydrochloride (Tocris, UK) were dissolved in 1% methyl cellulose in 0.9% saline. Caffeine (Sigma, UK), *meta*-Chlorophenylpiperazine hydrochloride (mCPP; Sigma, UK), and pentylenetetrazole (PTZ; Sigma, UK) were dissolved in 0.9% saline. Corresponding vehicles served for control injections. All drug solutions were prepared on test days and administered (2 ml/kg; ip) 30 min before testing (except PTZ; 15 min). The experiments were run over a 10 day period, with each compound tested over 2 consecutive days. The order of testing was FG 7142, mCPP, PTZ, caffeine then yohimbine. A randomised dosing schedule was employed across the 2 day period for each compound.

**Statistical Analysis**

Scores for each measure were analysed using a one-way ANOVA for independent samples. Any significant main effects were further analysed using a series of Dunnet's t tests comparing each dose with vehicle. Non-parametric data were analysed using a Kruskal-Wallis ANOVA, with any significant effects further examined using Mann-Whitney U-tests comparing each dose to vehicle. The frequency with which subjects jumped or backed off from the UEEP was analysed using the Chi-Square Test of Association.
Results

The majority of animals escaping the apparatus did so via backing off apart from one animal from the 10.0 mg/kg FG 7142 group and two animals receiving yohimbine (one at 1.25 mg/kg and one at 5.0 mg/kg) which jumped. These data were therefore combined with those animals backing off and are henceforth referred to simply as escape.

Effects of mCPP on UEEPM Behaviour

Escape rates and trial duration are presented in figures 4.1a and 4.1b. Group means for all other measures are presented in table 4.1. mCPP increased escape from the UEEPM at all doses compared to control, reaching statistical significance at 1.0 mg/kg (chi [1] = 6.14, p < 0.05) and 2.0 mg/kg (chi [1], = 9.60, p < 0.01). A concomitant main effect on trial duration was observed (H = 12.33, p < 0.01), with significant decreases apparent at 1.0 mg/kg and 2.0 mg/kg. Of the escape related behaviours, scanning was significantly altered by mCPP (F [3,52] = 20.85, p < 0.001) with reductions at all doses, along with turning (F [3,52] = 3.33, p < 0.05) which was significantly reduced by the top dose of 2.0 mg/kg. The number of times subjects reached the end of the maze arms was also affected (F [3,52] = 3.46, p < 0.05), with a reduction at 2.0 mg/kg. The frequency with which subjects attempted to back off the apparatus was unaffected by mCPP (H = 1.19, p = 0.36). Of the locomotor measures, zone crossing was significantly altered by mCPP (H = 16.85, p < 0.001), with a reduction at all doses compared to vehicle. A main effect of mCPP on ratio end time was also observed (H = 19.50, p < 0.001) and follow up tests
revealed a reduction at all doses. Ratio centre time did not differ between groups (F [3,52] = 2.14, p = 0.11).

Figures 4.1a and 4.1b. Effects of acute mCPP (0.5 mg/kg – 2.0 mg/kg), ip 30 mins pretest, on escape and trial duration in the UEEPM. Data are presented as % of groups escaping and group medians and interoctile ranges. n = 15 in each group, veh = vehicle. Significantly different from vehicle: ** p < .01 by Chi Square Test of Association (% escape) and Mann-Whitney U-Test (duration).

Table 4.1. Effects of acute mCPP on UEEPM behaviour.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Vehicle</th>
<th>0.5 mg/kg</th>
<th>1.0 mg/kg</th>
<th>2.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scanning/10 secs</td>
<td>0.74 ± 0.07</td>
<td>0.39 ± 0.07**</td>
<td>0.25 ± 0.06**</td>
<td>0.14 ± 0.04**</td>
</tr>
<tr>
<td>End reaching/10 secs</td>
<td>0.12 ± 0.02</td>
<td>0.07 ± 0.02</td>
<td>0.06 ± 0.04</td>
<td>0.02 ± 0.02**</td>
</tr>
<tr>
<td>Attempt back off/10 secs</td>
<td>0.04 ± 0.02</td>
<td>0.05 ± 0.03</td>
<td>0.11 ± 0.03</td>
<td>0.10 ± 0.04</td>
</tr>
<tr>
<td>Turning/10 secs</td>
<td>1.43 ± 0.10</td>
<td>1.06 ± 0.12</td>
<td>0.98 ± 0.18</td>
<td>0.85 ± 0.15*</td>
</tr>
<tr>
<td>Zone crossing/10 secs</td>
<td>0.56 ± 0.07</td>
<td>0.37 ± 0.05*</td>
<td>0.18 ± 0.03***</td>
<td>0.26 ± 0.07**</td>
</tr>
<tr>
<td>Ratio centre time</td>
<td>0.27 ± 0.07</td>
<td>0.40 ± 0.08</td>
<td>0.19 ± 0.09</td>
<td>0.47 ± 0.09</td>
</tr>
<tr>
<td>Ratio end time</td>
<td>0.37 ± 0.08</td>
<td>0.17 ± 0.08*</td>
<td>0.19 ± 0.09*</td>
<td>0.04 ± 0.04***</td>
</tr>
</tbody>
</table>

Data are presented as mean values ± SEM. n = 15 per group. Significantly different from vehicle: * p < .05, ** p < .01, *** p < .001 by Dunnet’s t-test or Mann-Whitney U-Test.
Effects of Caffeine on UEEPM Behaviour

Escape rates and trial duration are presented in figures 4.2a and 4.2b. Group means for all other measures are presented in table 4.2. Caffeine significantly increased escape from the UEEPM at the highest dose of 30.0 mg/kg only (chi $[1] = 3.95$, $p < 0.05$). Scanning (F $[3,56] = 6.52$, $p < 0.001$) and turning (F $[3,56] = 6.52$, $p < 0.05$) were significantly affected with follow up tests revealing increases in scanning at all doses and turning at 10.0 mg/kg. A main effect of caffeine on end reaching (H = 8.18, $p < 0.05$) led to follow up tests revealing an increase at 10.0 mg/kg compared to vehicle. A significant effect on trial duration was observed (H = 15.71, $p < 0.01$), however further analysis revealed no dose was significantly different from vehicle. Means for attempting to back off did not differ between groups (F $[3,56] = 0.20$, $p = 0.89$). Caffeine had no effect on subjects' locomotor and exploratory behaviours on the UEEPM. Neither zone crossing (H = 4.61, $p = 0.20$), ratio end time (F $[3, 56] = 1.09$, $p = 0.36$) or ratio centre time (F $[3,56] = 1.99$, $p = 0.13$) were affected across the dose range.

Table 4.2. Effects of acute caffeine on UEEPM behaviour.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Vehicle</th>
<th>3.0 mg/kg</th>
<th>10.0 mg/kg</th>
<th>30.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scanning/10 secs</td>
<td>0.67 ± 0.09</td>
<td>1.05 ± 0.10**</td>
<td>1.19 ± 0.09**</td>
<td>1.02 ± 0.06*</td>
</tr>
<tr>
<td>End reaching/10 secs</td>
<td>0.25 ± 0.04</td>
<td>0.26 ± 0.05</td>
<td>0.38 ± 0.03*</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>Attempt back off/10 secs</td>
<td>0.02 ± 0.01</td>
<td>0.03 ± 0.02</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Turning/10 secs</td>
<td>1.27 ± 0.06</td>
<td>1.55 ± 0.13</td>
<td>1.66 ± 0.09*</td>
<td>1.47 ± 0.07</td>
</tr>
<tr>
<td>Zone crossing/10 secs</td>
<td>1.00 ± 0.15</td>
<td>1.13 ± 0.20</td>
<td>1.43 ± 0.18</td>
<td>0.87 ± 0.11</td>
</tr>
<tr>
<td>Ratio centre time</td>
<td>0.25 ± 0.06</td>
<td>0.21 ± 0.05</td>
<td>0.10 ± 0.02</td>
<td>0.22 ± 0.05</td>
</tr>
<tr>
<td>Ratio end time</td>
<td>0.40 ± 0.07</td>
<td>0.50 ± 0.06</td>
<td>0.54 ± 0.05</td>
<td>0.45 ± 0.05</td>
</tr>
</tbody>
</table>

Data are presented as mean values ± SEM. n = 15 per group. Significantly different from vehicle: * $p < .05$, ** $p < .01$ by Dunnet's t-test or Mann-Whitney U-Test.
Figures 4.2a and 4.2b. Effects of acute caffeine (3.0 mg/kg – 30.0 mg/kg), ip 30 mins pretest, on escape and trial duration in the UEEPM. Data are presented as % of groups escaping and group medians and interoctile ranges. n = 15 in each group, veh = vehicle. Significantly different from vehicle: * p < .05 by Chi Square Test of Association.

Effects of PTZ on UEEPM Behaviour

Escape rates and trial duration are presented in figures 4.3a and 4.3b. Group means for all other measures are presented in table 4.3. PTZ increased the frequency with which animals escaped the UEEPM at all doses, reaching significance at 30.0 mg/kg (chi [1] = 9.60, p < 0.01). A corresponding effect on trial duration was observed (H = 11.07, p < 0.05) with a reduction at 30.0 mg/kg. The number of times subjects reached the end of maze arms was altered by PTZ (F [3,56] = 6.81, p < 0.001), with significant reductions observed at 10.0 mg/kg and 30.0 mg/kg. Attempting to back off (F [3,56] = .35, p =
0.79), scanning (F [3,56] = 1.56, p = 0.21) and turning (F [3,56] = 0.70, p = 0.56) were unaffected by PTZ. PTZ had a significant effect on subjects' locomotor and exploratory behaviours at the top two doses tested. Main effects on zone crossing (H = 10.24, p < 0.05) and ratio end time (F [3,56] = 3.68, p < 0.05) were apparent, with zone crossing reduced at 10.0 mg/kg and 30.0 mg/kg and ratio end time reduced at 30.0 mg/kg. Ratio centre time was unaffected by PTZ (F [3,56] = 1.62, p = 0.20).

![Graph a and b](image)

Figures 4.3a and 4.3b. Effects of acute PTZ (3.0 mg/kg – 30.0 mg/kg), ip 15 mins pretest, on escape and trial duration in the UEEPM. Data are presented as % of groups escaping and group medians and interoctile ranges. n = 15 in each group, veh = vehicle. Significantly different from vehicle: ** p < .01 by Chi Square Test of Association (% escape) and Mann-Whitney U-Test (duration).
Table 4.3. Effects of acute PTZ on UEEPM behaviour.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Vehicle</th>
<th>3.0 mg/kg</th>
<th>10.0 mg/kg</th>
<th>30.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scanning/10 secs</td>
<td>0.66 ± 0.08</td>
<td>0.76 ± 0.09</td>
<td>0.79 ± 0.10</td>
<td>0.55 ± 0.07</td>
</tr>
<tr>
<td>End reaching/10 secs</td>
<td>0.22 ± 0.03</td>
<td>0.19 ± 0.03</td>
<td>0.12 ± 0.03*</td>
<td>0.07 ± 0.02**</td>
</tr>
<tr>
<td>Attempt back off/10 secs</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.32 ± 0.02</td>
</tr>
<tr>
<td>Turning/10 secs</td>
<td>1.37 ± 0.12</td>
<td>1.48 ± 0.18</td>
<td>1.32 ± 0.09</td>
<td>1.22 ± 0.09</td>
</tr>
<tr>
<td>Zone crossing/10 secs</td>
<td>0.80 ± 0.11</td>
<td>0.74 ± 0.11</td>
<td>0.48 ± 0.08*</td>
<td>0.38 ± 0.05**</td>
</tr>
<tr>
<td>Ratio centre time</td>
<td>0.13 ± 0.03</td>
<td>0.19 ± 0.06</td>
<td>0.25 ± 0.06</td>
<td>0.28 ± 0.06</td>
</tr>
<tr>
<td>Ratio end time</td>
<td>0.59 ± 0.06</td>
<td>0.47 ± 0.07</td>
<td>0.41 ± 0.07</td>
<td>0.30 ± 0.05**</td>
</tr>
</tbody>
</table>

Data are presented as mean values ± SEM. n = 15 per group. Significantly different from vehicle: * p < .05, ** p < .01 by Dunnet's t-test or Mann-Whitney U-Test.

Effects of FG 7142 on UEEPM Behaviour

Escape rates and trial duration are presented in figures 4.4a and 4.4b. Group means for all other measures are presented in table 4.4. FG 7142 increased the propensity to escape the UEEPM compared to vehicle, reaching significance at 3.0 mg/kg (chi [1] = 4.62, p < 0.05) and 30.0 mg/kg (chi [1] = 6.00, p<0.05). Dunnet's t-tests following a significant effect of FG 7142 on scanning (F [3,55] = 3.83, p < 0.05) revealed an increase at 3.0 mg/kg. FG 7142 had no effects on trial duration (H = 5.92, p = 0.12) end reaching (F [3,55] = 2.44, p = 0.07) or attempting to back off (H = 5.93, p = 0.12). A main effect of FG 7142 on turning was observed (F [3,55] = 3.20, p < 0.05), however follow up analysis revealed no dose group was significantly different from vehicle. FG 7142 did not alter exploratory and locomotor behaviours. Scores for ratio end time (F [3,55] = 0.34, p = 0.79), and ratio centre time (F [3,55] = 0.62, p = 0.60) were consistent across experimental groups and although a main effect on zone crossing was apparent (F [3,55] = 2.79, p < 0.05), no follow up tests were significant.
Figures 4.4a and 4.4b. Effects of acute FG 7142 (3.0 mg/kg – 30.0 mg/kg), ip 30 mins pretest, on escape and trial duration in the UEEPM. Data are presented as % of groups escaping and group medians and interoctile ranges. n = 15 except in each group except 10.0 mg/kg = 14, veh = vehicle. Significantly different from vehicle: * p < .05, p < .01 by Chi Square Test of Association (% escape) and Mann-Whitney U-Test (duration).

Table 4.4. Effects of acute FG 7142 on UEEPM behaviour.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Vehicle</th>
<th>3.0 mg/kg</th>
<th>10.0 mg/kg</th>
<th>30.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scanning/10 secs</td>
<td>0.60 ± 0.07</td>
<td>0.85 ± 0.09*</td>
<td>0.77 ± 0.08</td>
<td>0.55 ± 0.06</td>
</tr>
<tr>
<td>End reaching/10 secs</td>
<td>0.18 ± 0.04</td>
<td>0.17 ± 0.03</td>
<td>0.08 ± 0.02</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>Attempt back off/10 secs</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.05 ± 0.02</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>Turning/10 secs</td>
<td>1.36 ± 0.07</td>
<td>1.53 ± 0.07</td>
<td>1.36 ± 0.06</td>
<td>1.22 ± 0.08</td>
</tr>
<tr>
<td>Zone crossing/10 secs</td>
<td>0.67 ± 0.12</td>
<td>0.76 ± 0.11</td>
<td>0.51 ± 0.10</td>
<td>0.36 ± 0.07</td>
</tr>
<tr>
<td>Ratio centre time</td>
<td>0.14 ± 0.04</td>
<td>0.20 ± 0.04</td>
<td>0.25 ± 0.07</td>
<td>0.22 ± 0.06</td>
</tr>
<tr>
<td>Ratio end time</td>
<td>0.50 ± 0.08</td>
<td>0.45 ± 0.07</td>
<td>0.40 ± 0.11</td>
<td>0.51 ± 0.08</td>
</tr>
</tbody>
</table>

Data are presented as mean values ± SEM. n = 15 per group except 10 mg/kg n = 14. Significantly different from vehicle: * p < .05 by Dunnet's t-test.
Effects of Yohimbine on UEEPM Behaviour

Escape rates and trial duration are presented in figures 4.5a and 4.5b. Group means for all other measures are presented in table 4.5. No dose of yohimbine altered escape rates compared to vehicle subjects. Similarly, trial duration did not differ between groups (H = 4.45, p = 0.21). Of the escape related behaviours, main effects of yohimbine on turning (F [3,56] = 17.72, p < 0.001), scanning (F [3,56] = 4.90, p < 0.01), and end reaching (F [3,56] = 3.18, p < 0.05) were observed. Follow up tests revealed a reduction in turning at all doses, a reduction in scanning at 2.5 mg/kg, and a reduction in end reaching at 5.0 mg/kg. Instances of attempting to back off were unaffected by yohimbine (H = 7.61, p = 0.06). No locomotor / exploratory measures were affected. Group means for zone crossing (F [3,56] = 2.08, p = 0.11), ratio end time (F [3,56] = 2.52, p = 0.07) and ratio centre time (F [3,56], p = 0.57) did not differ between the experimental groups.

Table 4.5. Effects of acute yohimbine on UEEPM behaviour.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Vehicle</th>
<th>1.25 mg/kg</th>
<th>2.5 mg/kg</th>
<th>5.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scanning/10 secs</td>
<td>0.64 ± 0.05</td>
<td>0.48 ± 0.07</td>
<td>0.32 ± 0.05**</td>
<td>0.48 ± 0.06</td>
</tr>
<tr>
<td>End reaching/10 secs</td>
<td>0.16 ± 0.03</td>
<td>0.11 ± 0.02</td>
<td>0.08 ± 0.02</td>
<td>0.06 ± 0.02*</td>
</tr>
<tr>
<td>Attempt back off/10 secs</td>
<td>0.01 ± 0.01</td>
<td>0.04 ± 0.02</td>
<td>0.03 ± 0.03</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Turning/10 secs</td>
<td>1.59 ± 0.11</td>
<td>1.11 ± 0.10**</td>
<td>0.71 ± 0.08**</td>
<td>0.80 ± 0.08**</td>
</tr>
<tr>
<td>Zone crossing/10 secs</td>
<td>0.30 ± 0.05</td>
<td>0.20 ± 0.05</td>
<td>0.15 ± 0.03</td>
<td>0.20 ± 0.04</td>
</tr>
<tr>
<td>Ratio centre time</td>
<td>0.22 ± 0.04</td>
<td>0.24 ± 0.70</td>
<td>0.23 ± 0.08</td>
<td>0.34 ± 0.08</td>
</tr>
<tr>
<td>Ratio end time</td>
<td>0.43 ± 0.07</td>
<td>0.46 ± 0.09</td>
<td>0.50 ± 0.09</td>
<td>0.21 ± 0.07</td>
</tr>
</tbody>
</table>

Data are presented as mean values ± SEM. n = 15 per group. Significantly different from vehicle: * p < .05, ** p < .01 by Dunnet's t-test.
Discussion

The unstable elevated exposed plus maze (UEEPM), a novel behavioural model of extreme anxiety, proved sensitive to the effects of anxiogenic compounds. The principal aim of this study was to assess the predictive validity of the UEEPM for the identification of anxiogenic agents. mCPP (1.0 and 2.0 mg/kg) and caffeine (30.0 mg/kg), which elicit panic clinically, significantly increased animals’ propensity to escape from the UEEPM i.e. they had a clear anxiogenic effect. The model also proved sensitive to the effects of two reference anxiogenic compounds, FG 7142 (3.0 and 30.0 mg/kg) and PTZ (30.0
mg/kg). Both drugs significantly increased escape from the UEEPM. Yohimbine had no effect on escape. Each of these findings will be discussed in turn.

**Effects of mCPP on UEEPM Behaviour**

The most striking finding of this study was that mCPP dose dependently increased escape from the UEEPM *despite* very marked hypolocomotor effects. With the exception of attempting to back off and ratio centre time, all locomotor and escape-related behaviours were reduced by mCPP, whereas actual escape was increased. Indeed, at the top dose of 2.0 mg/kg which produced the highest escape rates and shortest trial duration, zone crossing, ratio end time, end reaching, scanning and turning were all significantly less than vehicle. The number of times animals moved between the zones of the maze was significantly reduced across the dose range, even at the lowest dose of 0.5 mg/kg, which did not significantly increase actual escape. Given this profile, the decreases in scanning, turning and end reaching can be interpreted as a function of an overall behavioural suppression as opposed to any ‘anxiolytic’ action of the compound.

These results are in accordance with previous research showing mCPP-induced increases in anxiety-related behaviour in a number of animal models (e.g. social interaction: Kennett et al. 1989; marble burying: Njung'e and Handley 1991; elevated plus maze: Gibson et al. 1994; 'zero-maze': Shepherd et al. 1994 and light/dark box: Bilkei-Gorzo et al. 1998). Furthermore, increases in anxiety despite overall locomotor suppression are also documented (e.g. social interaction test: Kennedy et al. 1993; elevated plus-maze:
Fone et al. 1996; canopy stretched attend posture test: Grewal et al. 1997). Correspondingly, Meert et al. (1997) have found that mCPP treated animals appear to be generally aroused, as evidenced by a dose-related increase in plasma prolactin and ACTH levels, in spite of a decrement in locomotor performance.

The present results however, are not in agreement with the reported profile of mCPP in the elevated T-maze. Mora et al. (1997) reported that mCPP marginally inhibited unconditioned escape (as measured by time to leave the end of the open arm) and facilitated inhibitory avoidance (as measured by the time to leave the closed arm). Yet it is noteworthy that both these measures involve increases in movement latency. It is possible that the non-specific behavioural inhibitory effect of mCPP is reflected in the massive increases observed in the time to leave the relatively safe environment of the closed arm, whereas the anxiogenic effects of mCPP partially overcame the hypolocomotor effects of the compound when the animals were placed on the relatively more aversive open arm, resulting in the much smaller, non significant increase in time to leave. Rather than proposing that mCPP was having an anxiolytic effect on unconditioned escape, which these authors contend, it could be argued that it was actually promoting escape in spite of the increasing dose dependent hypolocomotor effects, rendering the findings more consistent with the present results.

Research suggests that the anxiogenic effects of mCPP in rats are predominantly mediated by 5-HT_{2C} receptors (Kennett et al. 1989; Rodgers et al. 1992; Gibson et al. 1994), and to a lesser extent by 5-HT_{1B} receptors (Gommans et al. 1998) and 5-HT_{2A}
receptors (Fiorella et al. 1995). SB-242084-A, a selective $5\text{-HT}_{2C}$ antagonist, potently inhibits mCPP-induced hypolocomotion in rats, a model of $5\text{-HT}_{2C}$ receptor function, and displays an anxiolytic profile when administered alone in the social interaction test (Kennett et al. 1997). Therefore, further investigation into the role of the $5\text{-HT}_{2C}$ receptor in UEEPM behaviour, via the administration of a selective antagonist in combination with mCPP, would be of considerable interest.

Effects of Caffeine on UEEPM Behaviour

Caffeine had a selectively anxiogenic effect on the behaviour of rats exposed to the UEEPM. Escape was significantly increased at the top dose of 30 mg/kg, scanning was increased across the dose range, and turning and end reaching increased at 10.0 mg/kg. Unlike mCPP, caffeine exerted no effects on the locomotor/exploratory behaviour of subjects on the UEEPM. This increase in anxiety-related behaviour corresponds to previous observations in the rat (Pellow et al. 1985) and mouse (Lister 1987; Lapin et al. 1993; Jain et al. 1995; Yacoubi et al. 2000) EPM, the mouse light/dark test (Lapin and Politi 1998; Yacoubi et al. 2000) and in the latency to self interrupt aversive stimulation of the rat dPAG (Jenck et al. 1995).

Evidence suggests that these increases in escape and escape-related behaviour may be mediated by caffeine’s effect at the adenosine, and more specifically, the adenosine $A_{2a}$ receptor. Aminophylline, another adenosine antagonist, has proven anxiogenic, and the adenosine uptake inhibitor papaverine proven anxiolytic, in rats in the EPM (Zangrossi et
al. 1992). Mice lacking in the $A_{2a}$ receptor gene have displayed increases in anxiety-related behaviour compared to wild type mice (Ledent et al. 1997). Furthermore, Deckert and colleagues (1998) screened patients with panic disorder and matched controls and found a significant association between $A_{2a}$ receptor gene polymorphism and panic disorder, suggesting the $A_{2a}$ receptor gene, or locus in linkage disequilibrium with it, confers susceptibility to the disease. These findings should be noted with some caution however, given the adenosine $A_{2a}$ antagonists ZM241385 and SCH58261 have recently proven ineffective in the murine plus-maze (Yacoubi et al. 2000). It would therefore be of interest to examine the behavioural profiles of these compounds in the UEEPM.

**Effects of Yohimbine on UEEPM Behaviour**

Despite extensive clinical literature reporting anxio- and panicogenic effects of yohimbine in extreme anxiety patients, the compound failed to increase escape from the UEEPM. However, pre-clinical literature on the effects of yohimbine in behavioural tests has been relatively inconsistent and may go some way to explaining the current findings. For example, although the compound has proven anxiogenic in the EPM (Pellow et al. 1985; Wada and Fukuda 1991; Cole et al. 1995), the fear defence test battery (Blanchard et al. 1993b) and the light/dark box (Bilkei-Gorzo et al. 1998), it has proven ineffective in the stretched attend posture test (Molewijk et al. 1995b), both anxiogenic and anxiolytic in ultrasound induced defence behaviour (Beckett et al. 1996) and selectively anxiolytic in conditioned ultrasonic distress vocalisations in a threatening situation (Molewijk et al. 1995a).
It is certainly worthy of consideration that, compared with mCPP, yohimbine has much less effect on panic and anxiety in healthy volunteers than in clinical populations. For instance, Bremner et al. (1997) found that whilst yohimbine induced panic attacks in 60% of PTSD subjects, no healthy volunteers were similarly affected. Likewise, Southwick et al. (1997) report 42% of PTSD patients experiencing a panic attack after yohimbine challenge compared to only 7% of healthy volunteers. Studies with PD patients and healthy subjects report similar trends (Gurguis and Uhde 1990; Charney et al. 1997a,b). In comparison, various authors have reported panic attacks in approx. 16% to 33% of healthy subjects after acute mCPP (Charney et al. 1987b; Kahn et al. 1990; Sevy et al. 1994; Silverstone and Cowen 1994).

The majority of the pre-clinical research into the behavioural effects of yohimbine has examined the effect of the compound on experimentally naïve animals exposed to a potentially fearful situation. The lack of a reliable anxiogenic profile in animal tests may parallel this lack of effect in healthy humans. Indeed, Park et al. (2001) found that rats exposed to a cat and housed with different cohorts every day for a total of 25 days exhibited a significantly increased sensitivity to the behavioural effects of yohimbine in the open field arena compared to non-stressed subjects. Given repetitive electrical stimulation of the rodent midbrain defence system produces long lasting increases in escape and escape-related behaviour in the UEEPM (King 1999b), the behavioural profile of yohimbine in animals displaying such a chronically hyper-aroused state is worthy of further investigation.
Another possible explanation for the lack of effect of yohimbine in the UEEPM is that escape behaviour in this paradigm is not mediated by the noradrenergic system. Southwick et al. (1997) suggest the existence of two neurobiological subgroups of PTSD patients, with a dysregulation of either the noradrenergic system or the serotonergic system – combat veterans tended to experience panic, anxiety and PTSD symptoms following mCPP or yohimbine challenge, but not both. It is pertinent to note that in the present study, whilst yohimbine failed to increase escape responses, mCPP did so dramatically. If such biological subtypes of panic exist within patients it is possible that the particular flight behaviour exhibited on the UEEPM may be more analogous to those under serotonergic, as opposed to noradrenergic, regulation.

**Effects of FG 7142 and PTZ on UEEPM Behaviour**

The UEEPM displayed sensitivity to the effects of FG 7142 and PTZ, with both compounds increasing escape. PTZ produced a profile similar to that of mCPP with increased escape despite locomotor decrements; concordant with previous findings in the rat (Wada and Fukuda 1991; Cruz et al. 1994; Cole et al. 1995) and mouse (Rodgers et al. 1995) EPM. FG 7142 selectively increased escape with no locomotor decrements; in line with previous findings in unconditioned models of anxiety (e.g. File et al. 1983; Meng and Drugan 1993; Russo et al. 1993; Cruz et al. 1994; Cole et al. 1995). It is worth noting that whilst no controlled clinical trials have examined the effects of FG 7142 in humans, symptoms intuitively resembling those of a panic attack such as “wavelike attacks of sheer, unfounded, and extreme anxiety”, “facial flushes, tremors, cold sweat and an
inability to concentrate” and “an impending fear of death or annihilation” were reported by two volunteers following oral FG 7142 (Dorow et al. 1983; Horowski and Dorow 2002). Therefore, similarities, although more tentative than with mCPP and caffeine, can be proposed between the effects of FG 7142 in humans and in rats exposed to the UEEPM.

Conclusion

This study aimed to examine the predictive validity of a new pre-clinical model of extreme anxiety, the UEEPM, which is thought to elicit behaviours in rats which model the persistent ‘fight/flight’ behaviour observed in extreme anxiety patients. The increases in flight behaviour seen in rats on the UEEPM parallel the increases in panic and anxiety symptoms observed in patients following mCPP and caffeine administration. Furthermore, FG 7142 and PTZ had anxiogenic effects on rats exposed to the UEEPM, corresponding to profiles reported in a number of established pre-clinical models.

Research is underway in this laboratory to assess the bi-directional pharmacological sensitivity of the UEEPM by investigating the behavioural profiles of drugs known to be clinically efficacious in the treatment of pathological anxiety states such as PD and PTSD. However, the current results suggest that the UEEPM may represent an unconditioned behavioural model which could facilitate further investigation into the neurochemical basis of extreme anxiety.
CHAPTER V

Experiment 4 – 5-HT$_{2C}$ Receptor Mediation of Unconditioned Escape Behaviour in the Unstable Elevated Exposed Plus Maze

Overview

$m$-Chlorophenylpiperazine (mCPP) induces panic in humans and dose dependently increases unconditioned escape behaviour in a novel pre-clinical model of extreme anxiety in rats, the unstable elevated exposed plus maze (UEEPM). Numerous studies indicate that the anxiogenic effects of mCPP may be mediated by its action at the 5-HT$_{2C}$ receptor. This study aimed to examine the involvement of the 5-HT$_{2C}$ receptor in the unconditioned fear responses observed in the UEEPM (after an acute dose of mCPP) by pre-treatment with the selective 5-HT$_{2C}$ receptor antagonist SB-242084. Male Hooded Lister rats received a single dose of SB-242084 (0.1-1.0 mg/kg ip) or vehicle 40 min pre-test followed by a single dose of mCPP (1.0 mg/kg ip) or saline 30 min before being exposed to the UEEPM for a period of 5 min. Subjects’ behaviour was analysed to determine the effects of SB-242084 on mCPP-induced increases in escape behaviour. mCPP alone increased animals’ propensity to escape from the UEEPM despite producing marked decreases in locomotor/exploratory behaviour. SB-242084 dose dependently inhibited the increases in escape and hypolocomotor effects induced by mCPP.

Conclusions: These results suggest that the escape-related behaviours exhibited by animals in the UEEPM are mediated, at least in part, by activation of the 5-HT$_{2C}$ receptor subtype.
Introduction

Rodents display a complex behavioural defensive repertoire when faced with threatening situations (Blanchard 1997). It is suggested that individual components of this repertoire may be specifically related to symptoms of discrete anxiety disorders in humans. Thus, flight and escape behaviour are thought to be more analogous to panic-related disorders and risk assessment behaviour more analogous to disorders involving anticipatory anxiety such as generalized anxiety disorder (GAD, Blanchard et al. 2001a). The unstable elevated exposed plus maze (UEEPM, King 1999a,b,c; Jones and King 2001; Jones et al. 2002a), a recently established model of extreme anxiety which capitalizes on many species' aversion to instability, height and exposure, elicits such unconditioned escape responses in rats.

Principal components analysis suggest that escape in the UEEPM may model a different type of anxiety to that observed in the elevated plus maze (EPM, King 1999a), a test that possibly models conditions such as GAD (Rodgers et al. 1995). Repetitive electrical stimulation of the rodent midbrain defense circuitry produces chronic increases in escape responses in the UEEPM (lasting up to three months), potentially analogous to the chronically hyper-aroused state observed clinically in disorders such as panic disorder (PD) and posttraumatic stress disorder (PTSD, King 1999b). Recently, the sensitivity of the UEEPM to compounds which cause panic in humans has also been reported (Jones et al. 2002a). Hence, the UEEPM may form a pre-clinical paradigm from which to examine the pharmacological bases of extreme pathological anxiety states.
The ability of the serotonergic compound m-chlorophenylpiperazine (mCPP) to induce panic attacks and symptoms of panic in PD and PTSD patients (Charney et al. 1987b; Klein et al. 1991; Southwick et al. 1997; Benjamin et al. 1999) and healthy volunteers (Charney et al. 1987b; Sevy et al. 1994; Benjamin et al. 1996) is well documented. Correspondingly, mCPP has been shown to significantly and dose dependently increase escape in the UEEPM (Jones et al. 2002a), in line with previously reported anxiogenic effects in the social interaction (Kennett et al. 1989), EPM (Rodgers et al. 1992; Gibson et al. 1994), elevated zero maze (Shepherd et al. 1994) and light/dark box (Bilkei-Gorzo et al. 1998) tests.

Despite the fact that mCPP is only a weakly selective 5-HT$_{2c}$ receptor agonist (over the 5-HT$_{1b}$ receptor for example), research suggests that mCPP-induced behavioural effects are often mediated by the 5-HT$_{2c}$ receptor. Hence, drug discrimination studies suggest the discriminative stimulus effect of mCPP are mediated by 5-HT$_{2c}$, and to a lesser extent 5-HT$_{1b}$ receptors (Gommans et al. 1998). Moreover, the anxiogenic profile of mCPP in the social interaction test is inhibited by the mixed 5-HT$_{2c/2a}$ antagonists mianserin, cyproheptadine and metergoline, but not by the 5-HT$_{2a}$ antagonists ketanserin or ritanserin (Kennett et al. 1989). Similarly, mCPP-induced increases in anxiety-like behaviour on the EPM are more potently attenuated by 1-(1-naphthyl)piperazine, which possesses higher affinity for 5-HT$_{2c}$ than 5-HT$_{2a}$ receptors, than by ketanserin (Gibson et al. 1994). Furthermore, mCPP-induced hypolocomotion has been used as a model of 5-HT$_{2c}$ receptor function in rats (Kennett et al. 1994; Kennett et al. 1997).
The development of ligands progressively more selective between the three 5-HT<sub>2</sub> receptor subtypes has further implicated the 5-HT<sub>2C</sub> receptor in the mediation of anxiety-like behaviour. For example, antagonists with higher affinity for the 5-HT<sub>2C/2B</sub> receptor than the 5-HT<sub>2A</sub> receptor display anxiolytic-like profiles in a number of animal models. Thus, SB-206553, SB-200646A and SB-221284 reduce anxiety in range of paradigms including the EPM, social interaction and Geller Seifter tests (Kennett et al. 1994, 1995, 1996; Griebel et al. 1997a; Bromidge et al. 1998). Both SB-206553 and SB-242084, the latter a selective 5-HT<sub>2C</sub> antagonist with over 100 fold selectivity for 5-HT<sub>2C</sub> over 5-HT<sub>2B</sub> and 5-HT<sub>2A</sub> receptors (Kennett et al. 1997), block the anxiogenic profile of an acute dose of the selective serotonin reuptake inhibitor (SSRI) citalopram in the rat social interaction test, whereas the 5-HT<sub>2A</sub> antagonist MDL 100907 does not (Dekeyne et al. 2001). Moreover, SB-242084 increases both time spent in social interaction and punished responding in rats with no effect on locomotor activity (Kennett et al. 1997; Millan et al. 2001).

The present study aimed to investigate 5-HT<sub>2C</sub> receptor involvement in the expression of the unconditioned fear responses observed in the UEEPM. Hence, rats were administered the selective 5-HT<sub>2C</sub> antagonist SB-242084 prior to a dose of mCPP which has previously been shown to increase escape before being exposed to the UEEPM (Jones et al. 2002a).
Method

Animals

Male Hooded Lister rats (Harlan UK), aged 10 weeks and weighing 292-364g at the start of testing, were singly housed under a normal 12 hour light/dark cycle in a temperature controlled environment (21 ± 1°C) with relative humidity 51 ± 5%. Food and water were available ad libitum. All in vivo studies were conducted in accordance with the United Kingdom Animal (Scientific Procedures) Act 1986 and conformed to GlaxoSmithKline ethical standards.

Apparatus

The apparatus and behavioural measures used in UEEPM testing were as previously described on pages 59-62 of this thesis.

Procedure

Subjects were randomly assigned to one of the following treatment conditions; (a) vehicle/saline [veh/saline], (b) vehicle/mCPP [veh/mCPP], (c) SB-242084 (0.1 mg/kg)/mCPP [SB 0.1/mCPP], (d) SB-242084 (0.3 mg/kg)/mCPP [SB 0.3/mCPP] or (e) SB-242084 (1.0 mg/kg)/mCPP [SB 1.0/mCPP]. The effects of SB-242084 alone were not assessed as previous research has shown the compound to be ineffective in the UEEPM
There were 15 subjects in each group except the SB 0.3/mCPP group which consisted of 14 subjects. mCPP was administered at 1.0 mg/kg in all groups as this dose has been shown to significantly increase escape in the UEEPM (Jones et al. 2002a). Vehicle or SB-242084 were injected 40 mins, and saline or mCPP were injected 30 mins prior to UEEPM exposure. Subjects were then exposed to the UEEPM.

**Drugs**

*Meta-Chlorophenylpiperazine hydrochloride (mCPP [Sigma, UK])* was dissolved in 0.9% saline. SB-242084 (GlaxoSmithKline, Harlow, UK) was dissolved in 0.9% saline containing 8% (w/v) hydroxypropyl-β-cyclodextrin (encapsin) and citric acid 25mM. Corresponding vehicles served for control injections. All drug solutions were prepared on test days and administered ip at a dose volume of 2 ml/kg.

**Statistical Analysis**

Scores for each measure were analysed using a one-way ANOVA for independent samples. Any significant main effects were further analysed using a series of Tukey's HSD tests comparing individual treatment groups. Non-parametric data were analysed using a Kruskal-Wallis ANOVA, with any significant effects further examined using Mann-Whitney U-tests. The frequency with which subjects jumped or backed off from the UEEPM was analysed using the Chi-Square Test of Association.
Results

All animals escaping the apparatus did so via backing off with the exception of one animal from the SB 0.1/mCPP group which jumped from the apparatus. Data were therefore combined and are referred to henceforth simply as escape.

Escape and Escape-Related Behaviours

Drug treatment significantly affected escape in the UEEPM (figure 5.1a). The veh/mCPP group displayed a greater propensity to escape the apparatus than the veh/saline group ($\chi^2 [1] = 5.40, p < 0.05$). This mCPP-induced increase in escape was dose-dependently blocked by SB-242084, with a significant reduction being achieved in the SB 0.3/mCPP ($\chi^2 [1] = 4.89, p < 0.05$) and SB 1.0/mCPP ($\chi^2 [1] = 7.78, p < 0.01$) groups compared to the veh/mCPP group. The escape rates of the SB 0.1/mCPP, SB 0.3/mCPP and SB 1.0/mCPP groups did not differ from the veh/saline group. Correspondingly, a main effect of trial duration was observed ($H = 12.09, p < 0.05$) (figure 5.1b). The veh/saline group stayed on the apparatus longer than the veh/mCPP group, however this difference only approached significance ($p = 0.06$). SB-242084 attenuated this decrease in trial duration across the dose range with a significant increase in the SB 1.0/mCPP group compared to the veh/mCPP group. As with escape rates, duration did not differ between the veh/saline group and the three groups receiving SB-242084 prior to mCPP.
Figures 5.1a – 5.1b Effects of acute administration of [1] vehicle (ip 40 mins pre-test) and saline (ip 30 mins pre-test); [2] vehicle (ip 40 mins pre-test) and mCPP (1.0 mg/kg ip 30 mins pre-test); [3] SB-242084 (0.1 mg/kg ip 40 mins pre-test) and mCPP (1.0 mg/kg ip 30 mins pre-test); [4] SB-242084 (0.3 mg/kg ip 40 mins pre-test) and mCPP (1.0 mg/kg ip 30 mins pre-test) and [5] SB-242084 (1.0 mg/kg ip 40 mins pre-test) and mCPP (1.0 mg/kg ip 30 mins pre-test) on UEEPM behaviour. Data are presented as % of group escaping and group medians and interoctile ranges n=15 in each group (except SB 0.3/mCPP n=14). Significantly different from treatment group indicated: * p < .05, ** p < .01, by Chi-Square test of Association (% escape) or Mann-Whitney U-Test (duration).

Of the escape-related behaviours, main treatment effects were observed for scanning (F [4,64] = 7.91, p < 0.001), turning (F [4,64] = 8.13, p < 0.001) and end reaching (H = 14.41, p < 0.01). Follow up analysis revealed highly significant decreases in scanning and turning in the veh/mCPP group compared to the veh/saline group. The decreases in scanning were attenuated by SB-242084 with the SB 0.3/mCPP and SB 1.0/mCPP groups, but not the SB 0.1/mCPP group, scanning more than veh/mCPP animals (figure 5.2a). All doses of SB-242084 given prior to mCPP significantly blocked the mCPP-induced decreases in turning with the SB 0.1/mCPP, SB 0.3/mCPP and SB 1.0/mCPP groups all turning more than veh/mCPP subjects (figure 5.2b). mCPP alone did not affect
Figures 5.2a – 5.2d. Effects of acute administration of [1] vehicle (ip 40 mins pre-test) and saline (ip 30 mins pre-test); [2] vehicle (ip 40 mins pre-test) and mCPP (1.0 mg/kg ip 30 mins pre-test); [3] SB-242084 (0.1 mg/kg ip 40 mins pre-test) and mCPP (1.0 mg/kg ip 30 mins pre-test); [4] SB-242084 (0.3 mg/kg ip 40 mins pre-test) and mCPP (1.0 mg/kg ip 30 mins pre-test) and [5] SB-242084 (1.0 mg/kg ip 40 mins pre-test) and mCPP (1.0 mg/kg ip 30 mins pre-test) on UEEPM behaviour. Data are presented as group means (+ SEM) or group medians and interquartile ranges. n=15 in each group (except SB 0.3/mCPP n=14). Significantly different from treatment group indicated: * p < .05, ** p < .01, *** p , < 0.001 by Tukey’s HSD test or Mann-Whitney U-Test.
the number of times animals reached the end of the maze arms. However, end reaching was reduced in the SB 0.1/mCPP and SB 0.3/mCPP groups in comparison to control animals and in the SB 0.1/mCPP group compared to the SB 1.0/mCPP group (figure 5.2c). The main effect of drug treatment on attempting to back off only approached significance ($H = 9.10, p = 0.06$), although the data suggests an increase in this measure in the veh/mCPP condition compared to other groups (figure 5.2d).

**Locomotor/Exploratory Behaviours**

Locomotor/exploratory measures also differed between experimental conditions. A significant main effect on zone crossing ($F [4,64] = 4.33, p < 0.01$) was a function of a decrease in the veh/mCPP group compared to the veh/saline group. This effect was blocked by all doses of SB-242084 prior to mCPP, reaching significance at the highest dose of 1.0 mg/kg (figure 5.3a). Post-hoc analysis following a significant main drug effect on ratio end time ($H = 14.14, p < 0.01$) revealed mCPP alone had no effect on end reaching but SB-242084 in combination with mCPP did. The SB 0.1/mCPP and SB 0.3/mCPP groups spent less time at the end of the maze arms than the control animals (figure 5.3b). Ratio centre time did not differ between groups ($F (4,64) = 0.66, p = 0.62$) (figure 5.3c).
Figures 5.3a – 5.3c. Effects of acute administration of [1] vehicle (ip 40 mins pre-test) and saline (ip 30 mins pre-test); [2] vehicle (ip 40 mins pre-test) and mCPP (1.0 mg/kg ip 30 mins pre-test); [3] SB-242084 (0.1 mg/kg ip 40 mins pre-test) and mCPP (1.0 mg/kg ip 30 mins pre-test); [4] SB-242084 (0.3 mg/kg ip 40 mins pre-test) and mCPP (1.0 mg/kg ip 30 mins pre-test) and [5] SB-242084 (1.0 mg/kg ip 40 mins pre-test) and mCPP (1.0 mg/kg ip 30 mins pre-test) on UEEPM behaviour. Data are presented as group means (± SEM) or group medians and interoctile ranges. n=15 in each group (except SB 0.3/mCPP n=14). Significantly different from treatment group indicated: * p < .05, ** p < .01 by Tukey's HSD test or Mann-Whitney U-Test.
Discussion

The selective 5-HT$_{2c}$ antagonist SB-242084 attenuated the acute mCPP-induced increases in escape behaviour at the two highest doses. The profile of mCPP alone and in combination with SB-242084 will be discussed along with the implications of these findings for the role of the 5-HT$_{2c}$ receptor in the unconditioned flight responses observed in the UEEPM.

The profile of mCPP is similar to that previously reported in this test (Jones et al. 2002a) with increases in escape despite a reduction in most active behaviours. Thus, the veh/mCPP group escaped more frequently and crossed zones less than the veh/saline group. Likewise, attempting to back off was increased and trial duration decreased by mCPP, although these differences fell just short of significance. The decreases in scanning and turning observed in subjects receiving mCPP only also correspond with previous findings and seem to reflect the much reported overall behavioural suppressant effect of the compound as opposed to an anxiolytic-like effect. Both scanning and turning have been shown to be increased by anxiogenic agents devoid of hypolocomotor effects which potentiate escape in the UEEPM, such as the adenosine receptor agonist caffeine and the benzodiazepine partial inverse agonist FG 7142 (Jones et al. 2002a), and by repetitive electrical stimulation of the rodent midbrain which produces chronic increases in escape (King 1999b). This anxiogenic-like profile with concomitant hypolocomotor effects is similar to that reported for mCPP in a variety of animal models (Kennedy et al. 1993; Fone et al. 1996; Grewal et al. 1997; Kantor et al. 2000).
Administration of SB-242084 (at pharmacodynamic doses previously shown to block 5-HT\textsubscript{2C} receptors [Kennett et al. 1997b]) prior to mCPP dose-dependently inhibited the behavioural effects of the agonist, reducing escape at 0.3 mg/kg and 1.0 mg/kg and increasing trial duration and zone crossing at 1.0 mg/kg. A dose-dependent trend towards reducing the frequency with which animals attempted to back off the apparatus following mCPP was also apparent. The mCPP-induced decreases in scanning were blocked by the middle and top doses of SB-242084, whilst decreases in turning were blocked across the dose range of the antagonist. One unexpected finding was that end reaching and ratio end time were reduced in the SB 0.1/mCPP and SB 0.3/mCPP groups compared to the veh/saline group. However, as only the top dose of SB-242084 significantly blocked the mCPP-induced decreases in zone crossing this effect may be due to the failure of the two lower doses of SB-242084 to completely attenuate the hypolocomotor effects of mCPP. The blockade of mCPP, by SB-242084, appears to be a true pharmacological effect rather than non-specific response competition as in a previous study (King et al. 2000) SB-242084, at the top dose used in the current study (1.0 mg/kg), failed to affect UEEPM behaviour.

The findings from the present experiment suggest that increases in escape behaviour in the UEEPM induced by mCPP are mediated by its activation of the 5-HT\textsubscript{2C} receptor. This supports previous findings showing that mixed 5-HT\textsubscript{2C/2A} receptor antagonists, but not selective 5-HT\textsubscript{2A} antagonists, attenuate mCPP-induced anxiogenesis in the social interaction (Kennett et al. 1989) and EPM (Gibson et al. 1994) tests. Likewise, the ability of the highest dose of SB-242084 to inhibit the reductions in zone crossings following
mCPP in the UEEPM corresponds to observations that SB-242084, and other compounds acting as antagonists at the 5-HT$_{2c}$ receptor, attenuate mCPP-induced hypolocomotion in rats (Kennett et al. 1994; Kennett et al. 1997; Kennett et al. 2000).

However, the exact role of the 5-HT$_{2c}$ receptor in specific subtypes of anxiety-related behaviour in animals remains unclear. Compounds acting as mixed and selective agonists at the receptor have displayed variable profiles in a range of pre-clinical paradigms. Whilst mCPP has proven anxiogenic in the social interaction test (Kennett et al. 1989), the EPM (Rodgers et al. 1992, Gibson et al. 1994), the light/dark box (Bilkei-Gorzo et al. 1998) and the elevated zero maze (Shepherd et al. 1994), its profile has been more variable in tests measuring flight-related behaviours, such as the UEEPM, which aim to model panic as opposed to more generalised anxiety. Thus Mora et al. (1997) found mCPP to reduce the measure of unconditioned escape behaviour in the elevated T-maze. Moreover, Jenck and colleagues (1990) reported anxiolytic properties of mCPP in the dPAG stimulation paradigm, with doses from 0.46 mg/kg significantly increasing the frequency threshold for escape. The finding that the 5-HT$_{2c}$ receptor agonists RO 60-0175, Org 12962 and RO 60-0332 have also proven anxiolytic in this model (Jenck 1998b) has led to the suggestion that such compounds may possess therapeutic potential for panic anxiety (Jenck et al. 1998a; Jenck et al 1998b), a proposition seemingly in conflict with the present results.

Yet, it is important to note that both the elevated T-maze and the dPAG stimulation tests rely on the movement of subjects for their primary measures of escape. Thus, the
increases in the latency to travel the 50cm of the open arm and enter the relative safety of the closed arm of the T-maze following mCPP may, at least partly, reflect a reduction in locomotor behaviour. Likewise, given the main dependent measure of the dPAG stimulation model, operant self interruption behaviour, requires rats to move between compartments separated by a 2cm high barrier, the profiles of mCPP, RO 60-0175, RO 60-0332 and Org 12962 in this test may also be contaminated by hypolocomotor effects. Reductions in locomotor behaviour have been observed in pre-clinical models of anxiety following mCPP, RO 60-0175 and RO 60-0332 at doses comparable to those used in the T-maze and dPAG stimulation tests (e.g. UEEPM, Jones et al. 2002a, social interaction, Geller Seifter, Vögel conflict, Kennett et al. 2000, EPM, Gibson et al. 1994; Martin et al. 1998). Thus, the possible confounding effect of overall behavioural suppression on these results remains unclear.

Furthermore, the predictive validity of both these models has recently been questioned (for review see Blanchard et al. 2001a). Whilst acute doses of the SSRIs fluoxetine and fluvoxamine potentiate panic symptoms in PD patients (Saran and Halaris 1989; Denboer and Westenberg 1990) they do not increase anxiety in the dPAG stimulation model after acute administration (Jenck et al. 1990; Jenck et al. 1998b). Moreover, drugs which have no effect on panic symptoms in patients, such as the CCKB antagonist L-365,260 and the antipsychotic haloperidol, have proven antiaversive in this test (Jenck et al. 1989; Jenck et al. 1996). Along with mCPP, the panicogenic agent caffeine also fails to increase one way escape in the elevated T-maze (Graeff et al. 1998). It is also worthy of consideration that whilst the panicogenic effects of mCPP are well documented, to the best of our
knowledge, the only published data showing any clinical therapeutic effect of mCPP used a sample of six elderly depressed patients and did not assess any measures relating to anxiety or panic (Mellow et al. 1990). These observations, taken with the present results, call into question the suggestion that compounds acting as agonists at the 5-HT$_{2c}$ receptor may possess potential for the treatment of panic and panic-related disorder.

To conclude, it appears that the unconditioned escape responses observed in the UEEPM, a proposed pre-clinical behavioural model of extreme anxiety, are mediated, at least in part, by the 5-HT$_{2c}$ receptor. Given the observed panicogenic effects of mCPP in humans, the role of 5-HT$_{2c}$ receptor function in extreme anxiety disorders is certainly worthy of further investigation.
CHAPTER VI

Experiment 5 – Further Evidence for the Predictive Validity of the Unstable Elevated Exposed Plus Maze: Differential Effects of Fluoxetine and Chlordiazepoxide

Overview

The unstable elevated exposed plus maze (UEEPM) is a novel model of extreme anxiety which elicits unconditioned flight/escape behaviour in rats. The current studies aimed to further investigate the predictive validity of the paradigm by examining the effects of both clinically effective (chronic fluoxetine) and ineffective (acute fluoxetine and chlordiazepoxide) treatments for panic disorder on UEEPM behaviour. In the first experiment male Brown Norway rats received a single injection of fluoxetine (1.0 – 10.0 mg/kg po) or chlordiazepoxide (CDP, 1.0 – 10.0 mg/kg ip) 30 min prior to UEEPM exposure. In the second experiment, to assess the effects of chronic dosing or handling on baseline UEEPM behaviour, subjects received either 21 days vehicle injection (po) or handling, before being exposed to the test. Finally, rats received 21 days fluoxetine (1.0 – 10.0 mg/kg) in their food, before being tested in the UEEPM. Acute CDP and fluoxetine had no effect on UEEPM behaviour. Chronic handling and vehicle administration (po) significantly reduced escape in the UEEPM, hence preventing the effects of chronic fluoxetine administration from being investigated by po dosing. Chronic fluoxetine in subjects' food (10.0 mg/kg) significantly attenuated animals' propensity to escape from the UEEPM. The results further support the pharmacological similarity between
symptoms of panic in humans and escape in the UEEPM and suggest the UEEPM may represent a paradigm to facilitate investigation into the neurochemical basis of extreme anxiety disorders.
Introduction

When faced with a threatening situation, rodents typically display a complex behavioural repertoire ranging from risk assessment through to freezing and flight or escape (Blanchard 1997; Blanchard et al. 1998b) which is dependant on the specific nature and salience of the threat and the available behavioural opportunities (Rodgers 1997). It has been suggested that specific defensive behaviours may be analogous to some of the behavioural symptoms exhibited in discrete anxiety disorders in humans. Thus, an increase in risk assessment behaviour is thought to be more analogous to generalised anxiety disorder (GAD), whereas increases in flight and escape are thought to be more related to panic disorder (Blanchard et al. 2001a).

Unfortunately, attempts to develop flight-based models of panic using rats have proven problematic. Whilst wild subjects typically flee from threatening approaching stimuli, freezing is the predominant behavioural response in laboratory-bred rats (Blanchard et al. 1986b). This is probably due to the domestication effects of selective breeding for reduced defensive threat and attack to human approach and handling (Blanchard et al. 1994). Hence, flight-oriented responses to chemical and electrical modulation of the midbrain defence circuitry have been proposed as simulations of panic in laboratory rats (e.g. stimulation of the dorsal periaqueductal grey [dPAG] Jenck et al. 1995; Mongeau and Marsden 1997; Molchanov and Guimarães 1999; Vargas and Schenberg 2001, Jacob et al. 2002 and tonic inhibition of GABAergic activity in the dorsomedial hypothalamus [DMH] Shekar and Di Micco 1987; Shekhar 1994). Yet, such models lack the
behavioural diversity and ethological validity of tests measuring reactions to situations which pose a threat to survival and are seen under feral conditions (Rodgers and Cole 1994). The predictive validity of these tests has also been called into question. For instance, drugs that both increase (acute doses of the selective serotonin reuptake inhibitors fluoxetine and fluvoxamine), and have no effect on (the antipsychotic compound haloperidol and the cholecystokinin (CCK)B antagonist L-365,260), symptoms of panic in patients have failed to provide similar profiles in the dPAG stimulation model (for review see Blanchard et al. 2001a).

The unstable elevated exposed plus maze (UEEPM, King 1999a, b, c; Jones and King 2001; Jones et al. 2002a, b), a recently developed behavioural model of extreme anxiety, does, however, elicit unconditioned escape behaviour from an aversive test situation in laboratory-bred rats. The model capitalises on many species’ aversion to height, exposure, and instability. Principal components analysis suggests that escape in the UEEPM may model a different type of anxiety from that observed in the elevated plus maze (EPM, King 1999a), a test which is typically sensitive to compounds effective for GAD as opposed to panic disorder (PD, Rodgers et al. 1995). Furthermore, escape in the UEEPM is increased by compounds known to induce panic in humans (e.g. m-chlorophenylpiperazine [mCPP], caffeine, FG 7142, Jones et al. 2002a) and by repetitive electrical stimulation of the rodent midbrain defence circuitry (King 1999b). It has been suggested, therefore, that the UEEPM could form a pre-clinical paradigm of the more extreme anxiety disorders such as PD.
The profiles of compounds which selectively reduce symptoms of PD, such as selective serotonin reuptake inhibitors (SSRI’s), and GAD, such as benzodiazepine receptor agonists (BDZ’s), have not yet been examined in the UEEPM. Hence, the aim of this series of experiments was to further assess the predictive validity of the model.

SSRI’s such as fluoxetine, fluvoxamine, and paroxetine are effective in the treatment of PD when administered chronically (for reviews see, den Boer and Slaap 1998; Kent et al. 1998; Bakker et al. 2000; Figgit and McClellan 2000; Pollack and Marzol 2000; Kasper and Resinger 2001). However, they typically require at least two weeks treatment for clinical improvement and when given acutely can even increase symptoms of panic (Saran and Halaris 1989; den Boer and Westenberg 1990; Catalano et al. 2000). To date, there is a lack of data showing the effects of long-term administration of SSRI’s in proposed pre-clinical models of panic, although chronic fluoxetine has been shown to reduce unconditioned flight behaviour in the Mouse Defence Test Battery (Griebel et al. 1995a) and increase the threshold for dPAG stimulation-evoked unconditioned defensive behaviour in rats (Vargas and Schenberg 2001).

BDZ’s, such as diazepam and chlordiazepoxide (CDP), are well established and widely used treatments for GAD (Nutt 1991; Rickels et al. 1993). However, with the exception of chronic administration of the high potency triazolobenzodiazepines such as alprazolam, they display less efficacy in the treatment of severe anxiety (Taylor 1998; Argyropoulos and Nutt 1999) and are thus not generally recommended for PD (Nutt 1991; Ballenger et al. 1998; den Boer and Slaap 1998; Pollack and Marzol 2000).
Similarly, BDZ's exhibit robust anxiolytic profiles in animal models relying on measures other than flight or escape for indices of anxiety (e.g. EPM, Handley and Mithani 1984; Pellow et al. 1985; Lister 1987, social interaction, File and Hyde 1979; File and Pellow 1984; Gardner and Guy 1984 and light/dark box, Crawley 1981; Costall et al. 1989, Onaivi and Martin 1989). Indeed, it has been suggested such paradigms may be more aptly referred to as models of BDZ psychopharmacology (Green and Hodges 1991).

In the present experiments, the effects of the anxiolytics fluoxetine and CDP on UEEPM behaviour were assessed. Brown Norway rats were used as subjects as they display high baseline levels of escape in the UEEPM (Chapter III of this thesis). In the first experiment subjects received an acute dose of CDP or fluoxetine before being exposed to the UEEPM. As the handling of rats has been shown to significantly affect measures of anxiety (Andrews and File 1991), the possible confounding influence of a chronic oral dosing regime on UEEPM behaviour was then examined. As this regime dramatically reduced escape, the final study examined the profile of 21 days fluoxetine administered to subjects in their food, thus ensuring minimal handling.

Method

Subjects

Subjects were male Brown Norway rats (Harlan UK) aged 10 weeks and weighing between 176 g and 260 g at the start of the experiment. Animals were singly housed
under a normal 12 hour light/dark cycle in a temperature controlled environment (21 ± 1°C) with relative humidity 51 ± 5 %. Food and water were available ad libitum. All in vivo studies were conducted in accordance with the United Kingdom Animal (Scientific Procedures) Act (1986) and conformed to GlaxoSmithKline ethical standards.

Apparatus and Behavioural Measures

The apparatus and behavioural measures used for UEEPM testing were as previously described on pages 59-62 of this thesis.

Procedure

Effect of Acute Fluoxetine and Acute Chlordiazepoxide on UEEPM Behaviour

For the acute fluoxetine experiment subjects were randomly assigned to receive either saline, 1.0, 3.0 or 10.0 mg/kg fluoxetine (po) and for the CDP experiment either saline, 1.0, 3.0 or 10.0 mg/kg CDP (ip). There were 15 subjects in each group. Subjects received their allocated dose (2 ml/kg) 30 mins prior to UEEPM exposure. Subjects were then individually transported to the testing room and exposed to the UEEPM.
Effect of Chronic Handling/Dosing on UEEPM Behaviour

Pre-Treatment Testing

At the start of the experiment all subjects were individually taken to the testing room and exposed to the UEEPM. Subjects were then randomly assigned to one of three groups; vehicle, handled and unhandled for a period of 21 days (n = 15 in each group).

Chronic Handling/Vehicle Dosing

Each day vehicle animals were individually removed from their home cages and weighed before receiving 0.9% saline (po) at a dose volume of 2 ml/kg. Subjects in the handled group were removed from their home cages, weighed and handled as if to be dosed but were returned to their cages untreated. Unhandled subjects remained in their home cages throughout the experiment.

Post-Treatment Testing

On the final day of the study all subjects were re-exposed to the UEEPM. Vehicle and handled animals were placed on the UEEPM 30 min after they had received their dose of vehicle or been handled. Unhandled animals were removed from their home cages and placed on the apparatus immediately.
Effect of Chronic Fluoxetine on UEEPM Behaviour

Food Training

One week before dosing commenced all subjects began a daily food training program. At the beginning of the dark phase the rats' standard diet was removed from their cages. A small plastic dish containing 0.75g of a preferred food (powdered Farleys raspberry flavour baby food) mixed with 4ml saline was then placed into each cage. At the end of the dark cycle the standard diet was put back into the cages for the duration of the light phase. This procedure was repeated for 7 days. Drinking water was available ad libitum. The preferred food was presented to the subjects at the start of the dark cycle as this is when rats display the most consummatory behaviour (Jones and King 2001).

Pre-Treatment Testing

On day 8, all subjects were individually transported to the testing room and exposed to the UEEPM. They were then randomly assigned to one of four experimental groups receiving either vehicle, 1.0, 3.0 or 10 mg/kg fluoxetine (n=15).

Fluoxetine Dosing

At the beginning of the dark phase on day 8 the standard diet was removed as described above for food training. Subjects then received 0.75g of the preferred food mixed with
either 4 ml saline (vehicle group), 3.6 ml saline and 1.0 mg/kg fluoxetine (1.0 mg/kg group), 3.6 ml saline and 3.0 mg/kg fluoxetine (3.0 mg/kg group) or 3.6 ml saline and 10.0 mg/kg fluoxetine (10.0 mg/kg group). Fluoxetine was added at a dose volume of 2 ml/kg. At the end of the dark phase the standard diet was returned to subjects' cages for the duration of the light phase. Drinking water was available *ad libitum*. This procedure was repeated for 21 days (from days 9 – 29). All subjects were weighed on a weekly basis and dose volumes adjusted accordingly. All subjects consumed all of the presented food mixture on each of the 21 days of dosing, typically within 30 min of presentation.

**Post-Treatment Testing**

During the light phase of the day following the final dose of either vehicle or fluoxetine (day 29), subjects were transported to the testing room and re-exposed to the UEEPM.

**Drugs**

Fluoxetine and chlordiazepoxide (CDP) (Sigma, UK) were dissolved in 0.9% saline and administered either ip (CDP) or po (fluoxetine) at a dose volume of 2 ml/kg. All solutions were prepared on test days and saline alone served as vehicle.
Statistical Analysis

In the acute fluoxetine and CDP experiments, scores for each measure were analysed using a one-way ANOVA for independent samples. Any significant main effects were further analysed using a series of Dunnet's t tests comparing each dose with vehicle. Non-parametric data were analysed using a Kruskal-Wallis ANOVA, with any significant effects further examined using Mann-Whitney U-tests comparing each dose to vehicle. The frequency with which subjects jumped or backed off from the UEEPM was analysed using the Chi-Square Test of Association, comparing each dose with vehicle.

In the chronic handling and chronic fluoxetine experiments, group differences in pre-treatment and post-treatment behaviours were examined in the same way as the acute fluoxetine and acute CDP experiments. Pre- and post-treatment comparisons of escape rates and trial duration within each group were compared using the Chi-Square Test of Association (escape) and repeated measures t-tests or Mann-Whitney U-tests (duration).

Results

All subjects escaping from the apparatus did so via jumping, which is henceforth referred to simply as 'escape'. Due to video recorder malfunction the 1.0 mg/kg and 10.0 mg/kg in the chronic fluoxetine experiment consisted of 14 subjects.
Effect of Acute Fluoxetine and Acute Chlordiazepoxide on UEEPM Behaviour

Neither acute CDP nor acute fluoxetine altered subjects' propensity to escape from the UEEPM. All subjects in the fluoxetine study escaped from the apparatus. Although one subject receiving vehicle in the CDP experiment remained on the apparatus, the escape rate of the vehicle group did not differ from the three dose groups which each displayed 100% (15/15) escape (chi [1] = 1.03, p = 0.31). As all but one subject escaped within 15 secs of the trial only group means for trial duration were calculated. These did not differ across the fluoxetine groups (F [3,56] = 1.36 p = 0.27). A main effect of CDP on trial duration (H = 7.83, p < 0.05) was a function of a decrease in the 10.0 mg/kg group compared to the vehicle group. However, this was probably due to one subject from the vehicle group remaining on the UEEPM throughout the 5 min trial. Escape rates and trial duration are presented in tables 6.1 and 6.2.

Table 6.1. Effect of acute fluoxetine on escape and trial duration in the UEEPM

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>1.0 mg/kg</th>
<th>3.0 mg/kg</th>
<th>10.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>% escape</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Trial duration (secs)</td>
<td>0.84 ± 0.21</td>
<td>0.70 ± 0.08</td>
<td>0.56 ± 0.04</td>
<td>0.56 ± 0.05</td>
</tr>
</tbody>
</table>

Data presented as % of group escaping and group means ± SEM (duration).

Table 6.2. Effect of acute CDP on escape and trial duration in the UEEPM

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>1.0 mg/kg</th>
<th>3.0 mg/kg</th>
<th>10.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>% escape</td>
<td>93</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Trial duration (secs)</td>
<td>21.92 ± 19.90</td>
<td>1.32 ± 0.61</td>
<td>0.62 ± 0.06</td>
<td>0.56 ± 0.04*</td>
</tr>
</tbody>
</table>

Data presented as % of group escaping and group means ± SEM (duration). Significantly different from vehicle: * p < .05 by Mann-Whitney U-test.
Effect of Chronic Handling on UEEPM Behaviour

Pre-Treatment Testing

All subjects from the vehicle and unhandled groups escaped from the UEEPM. Although 87% (13/15) of the handled group escaped, this was not significantly different from the other two groups (chi [1] = 2.14, p = 0.14, figure 6.1a). All subjects that escaped did so within the first 15 secs of the trial, therefore only group means for trial duration were calculated. Likewise, these did not differ between groups (H = 5.37, p = 0.07, figure 6.1b).

Post-Treatment Testing

Both chronic treatment with vehicle and chronic handling significantly reduced escape in the UEEPM (figure 6.1a). Post-treatment escape rates were reduced to 26% (4/15) in the vehicle group (chi [1] = 17.37, p < 0.001) and 47% (7/15) in the handled group (chi [1] = 5.40, p < 0.05). In contrast, the unhandled group's escape rates changed little on second exposure to the apparatus (100% [15/15] pre-treatment testing compared to 93% [14/15] post-treatment testing, chi [1] = 1.03, p = 0.31). Post-treatment trial duration also increased in the vehicle (U = 16.50, p < 0.001) and handled (U = 35.0, p < 0.01) groups compared to pre-treatment testing, but was unchanged in the unhandled group (t [14] = -1.14, p = 0.27, figure 6.1b).
Figures 6.1a and 6.1b. Effects of 21 days handling or vehicle administration (po) on escape and trial duration in the UEEPM. Solid bars = pre-treatment and hatched bars = post-treatment. Data are presented as % of group escaping or group medians and interquartile ranges, n = 15 in each group. unhand = unhandled, veh = vehicle and hand = handled. Significantly different from group indicated: * p < .05, ** p < .01, *** p < .001 by Mann-Whitney U-test (duration) or Chi-Square Test of Association (% escape).

As all but one of the unhandled group escaped within the first 15 seconds of the trial, post-treatment group means for all other behaviours were compared between the vehicle and handled groups using independent t-tests or Mann-Whitney U-tests. There were no differences between the two groups for the escape-related behaviours preparing to jump (t [20] = -1.26, p = 0.22), scanning (t [20] = 1.48, p = 0.16), end reaching (t [20] = 0.79, p = 0.44), attempting to back off (t [20] = 0.27, p = 0.80) and turning (t [20] = 0.59, p = 0.56). Likewise the locomotor / exploratory measures zone crossing (t [20] = 0.25, p = 0.80), ratio end time (U = 45.0, p = 0.77) and ratio centre time (t [20] = -0.30, p = 0.77) were consistent between the two groups. Group means for these measures are presented in table 6.3.
Table 6.3. Effects of 21 days handling and vehicle treatment on UEEPM behaviour

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Chronic handling</th>
<th>Chronic vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepare jump / 10 secs</td>
<td>0.24 ± 0.14</td>
<td>0.11 ± 0.05</td>
</tr>
<tr>
<td>Scan / 10 secs</td>
<td>0.93 ± 0.17</td>
<td>1.22 ± 0.11</td>
</tr>
<tr>
<td>End reach / 10 secs</td>
<td>0.26 ± 0.05</td>
<td>0.32 ± 0.05</td>
</tr>
<tr>
<td>Attempt back off / 10 secs</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Turn / 10 secs</td>
<td>1.13 ± 0.17</td>
<td>1.24 ± 0.09</td>
</tr>
<tr>
<td>Zone cross / 10 secs</td>
<td>1.01 ± 0.19</td>
<td>1.15 ± 0.19</td>
</tr>
<tr>
<td>Ratio end time</td>
<td>0.41 ± 0.08</td>
<td>0.46 ± 0.03</td>
</tr>
<tr>
<td>Ratio centre time</td>
<td>0.31 ± 0.09</td>
<td>0.29 ± 0.04</td>
</tr>
</tbody>
</table>

Data are presented as group means ± SEM.

Effect of Chronic Fluoxetine on UEEPM Behaviour

Pre-Treatment Testing

All subjects escaped from the UEEPM within the first 15 secs of the trial (figure 6.2a). Trial duration did not differ between groups (H = 2.87, p = 0.41, figure 6.2b).

Post-Treatment Testing

Following 21 days treatment all groups displayed reduced escape rates (veh chi [1] = 6.0, p < 0.05, 1.0 mg/kg chi [1] = 4.67, p < 0.05, 3.0 mg/kg chi [1] = 10.91, p < 0.01, 10.0 mg/kg chi [1] = 15.56, p< 0.001) and increased trial duration (veh U = 16.0, p < 0.001, 1 mg/kg U = 32.0, p < 0.01, 3.0 mg/kg U = 17.0, p < 0.001, 10.0 mg/kg U = 5.0, p < 0.001) compared to pre-treatment testing (figures 6.2a and 6.2b). Post-treatment comparisons revealed the 10 mg/kg escaped significantly less than the vehicle group (chi [1] = 4.21, p
< 0.05, figure 6.2a). A main treatment effect for trial duration approached significance (F [3,54] = 2.09, p = 0.11, figure 6.2b).

**Figures 6.2a and 6.2b.** Effects of 21 days fluoxetine (1.0 mg/kg – 10.0 mg/kg) on escape and trial duration in the UEEPM. Solid bars = pre-treatment and hatched bars = post-treatment. Data are presented as group means (± SEM). veh and 3.0 mg/kg groups (n=15), 1.0 mg/kg and 10.0 mg/kg groups (n=14), veh = vehicle. Significantly different from group indicated: * p < 0.05, ** p < 0.01, *** p < 0.001 by Mann-Whitney U-test (duration) or Chi-Square Test of Association (% escape).

For the post-treatment escape-related behaviours, main effects were observed for attempting to back off (H = 12.21, p < 0.01) and scanning (H = 21.37, p < 0.001). Follow up tests revealed reductions in scanning for all dose groups and fewer attempts to back off in the 3.0 and 10 mg/kg groups compared to vehicle animals (figures 6.3a and 6.3b). Main effects for preparing to jump (H = 5.96, p = 0.11) and turning (H = 6.47, p = 0.09) approached significance with the data suggesting a reduction in all dose groups compared to vehicle (figures 6.3c and 6.4a). Group means did not differ for end reaching (F [3,30] = 0.24, p = 0.86, figure 6.4b).
Figures 6.3a to 6.3c. Effects of 21 days fluoxetine (1.0 mg/kg - 10.0 mg/kg) on escape-related behaviours in the UEEPM. Data are presented as group medians and interoctile ranges. veh and 3.0 mg/kg groups (n=15), 1.0 mg/kg and 10.0 mg/kg groups (n=14), veh = vehicle. Significantly different from vehicle: * p < .05, ** p < .01, *** p < .001 by Mann Whitney U-test.
Figures 6.4a to 6.4b. Effects of 21 days fluoxetine (1.0 mg/kg – 10.0 mg/kg) on escape-related behaviours in the UEEPM. Data are presented as group means (± SEM) or group medians and interquartile ranges. veh and 3.0 mg/kg groups (n=15), 1.0 mg/kg and 10.0 mg/kg groups (n=14), veh = vehicle.

Chronic fluoxetine did not alter any of the locomotor / exploratory behaviours (table 6.4). No main effects were observed for zone crossing (F [3,30] = 0.18, p = 0.91) or ratio centre time (F [3,30] = 0.82, p = 0.49). Although a treatment effect was observed for ratio end time (F [3,30] = 3.91, p < 0.05), follow up tests revealed no dose group was significantly different from vehicle.

Table 6.4. Effects of chronic fluoxetine on UEEPM exploratory/locomotor behaviours

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Vehicle</th>
<th>1.0 mg/kg</th>
<th>3.0 mg/kg</th>
<th>10.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone cross / 10 secs</td>
<td>2.30 ± 0.28</td>
<td>2.24 ± 0.22</td>
<td>2.54 ± 0.38</td>
<td>2.32 ± 0.26</td>
</tr>
<tr>
<td>Ratio end time</td>
<td>0.44 ± 0.06</td>
<td>0.50 ± 0.04</td>
<td>0.29 ± 0.05</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td>Ratio centre time</td>
<td>0.24 ± 0.04</td>
<td>0.18 ± 0.05</td>
<td>0.23 ± 0.07</td>
<td>0.32 ± 0.05</td>
</tr>
</tbody>
</table>

Data are presented as group means ± SEM.
Discussion

Chronic fluoxetine reduced escape and escape-related behaviour in the UEEPM whilst acute fluoxetine and acute CDP were without effect. Repeated daily handling and oral vehicle administration also significantly reduced escape in the UEEPM. Each of these findings, along with their implications for the pre-clinical study of extreme anxiety states, will be discussed in turn.

Fluoxetine, administered in subjects’ diet for 21 days, displayed an anxiolytic-like profile in the UEEPM. Upon post-treatment exposure to the test both the middle and top doses of fluoxetine reduced escape compared to vehicle, reaching significance at 10.0 mg/kg. The frequency with which animals attempted to back off from the apparatus was also reduced across the dose range, achieving significance at 3.0 mg/kg and 10.0 mg/kg. Scanning was significantly reduced at all doses and a trend towards a dose-dependent reduction in preparing to jump approached significance. Furthermore, these effects were seen in the absence of any change in locomotor/exploratory behaviour.

These decreases in escape behaviour in the UEEPM correspond to the reductions in the symptoms of PD patients seen after chronic fluoxetine treatment (den Boer and Slaap 1998; Kent et al. 1998; Bakker et al. 2000; Figgit and McClellan 2000; Hurst and Lamb 2000; Pollack and Marzol 2000; Kasper and Resinger 2001). Moreover, the profile is opposite to that observed in Hooded Lister rats tested in the UEEPM following acute
doses of the panicogenic compounds mCPP, caffeine and FG 7142 (Jones et al. 2002a), thus providing further evidence for the bi-directional predictive validity of the UEEPM.

Surprisingly, given their well documented efficacy in PD, the effects of chronic SSRI’s in proposed pre-clinical models of panic have rarely been assessed (for review see Blanchard et al. 2001a). However, the present observations are commensurate with those reported previously in two unconditioned paradigms. Vargas and Schenberg (2001) examined the effects of fluoxetine on the dPAG stimulation thresholds required to elicit unconditioned defensive behaviours in the rat. Following 21 days treatment, fluoxetine significantly increased the threshold for ‘galloping’ behaviour. Schenberg et al. (2001) suggest this may be the most ‘panic-like’ behaviour elicited by dPAG stimulation as it is also reduced by the panicolytic agent clomipramine and unchanged by clinically ineffective compounds such as diazepam, midazolam and maprotiline.

Most notably, the profile of chronic fluoxetine in the UEEPM is similar to that reported in the Mouse Defense Test Battery (MDTB). The MDTB allows the measurement of both flight (analogous to PD) and risk assessment (analogous to GAD) behaviours in mice faced with an approaching predator, and displays high levels of predictive validity (Blanchard et al. 2001a). Griebel et al. (1995a) reported significant reductions in the predator-prey distance required to elicit flight, but not in risk assessment behaviours, after 21 days fluoxetine (ip) using a similar dose range to the present experiment.
Conversely, the current findings conflict with the generally ineffective profile of chronic SSRI's in paradigms attempting to model anxiety as opposed to panic (for recent review see Borsini et al. 2002). Hence, fluoxetine has no effect on rats' behaviour in the social interaction test after both 14 days (File et al. 1999) and 21 days (To and Bagdy 1999; To et al. 1999) treatment. Doses ranging from 5 to 20 mg/kg (ip) for up to 3 weeks have failed to effect anxiety-related behaviours in the EPM (Durand et al. 1999; Griebel et al. 1999). Importantly, repeated fluoxetine via a similar route of administration to the one used in the present study, and at the dose shown to significantly reduce escape in the UEEPM, has proven ineffective in the EPM. Silva and Brandão (2000) dissolved 10 mg/kg fluoxetine in rats' drinking water each day for 2 weeks. Post-treatment, neither spatio-temporal indices of anxiety or risk assessment behaviours were altered in the test. Whilst it should be noted that chronic paroxetine has displayed an anxiolytic-like profile in the social interaction test (Lightowler et al. 1994; Duxon et al. 2000) and chronic fluoxetine (Silva et al. 1999) and citalopram (Skrebuhhova et al. 1999) have reduced anxiety behaviour in the EPM, these findings, taken with the profile of chronic fluoxetine in the UEEPM, MDTB and unconditioned dPAG stimulation paradigm, further support the pharmacological similarity between flight behaviour in rodents and panic in humans.

In contrast to repeated administration, an acute dose of fluoxetine had no effect on escape in the UEEPM. The profiles of acute SSRI's in animal models are variable. For instance, fluoxetine has proven both anxiogenic (Silva and Brandão 2000) and ineffective (Griebel et al. 1997b) in the EPM, anxiogenic in the social interaction test (File et al. 1999; Bagdy et al. 2001) and ineffective in the elevated zero maze (Pähkla et al. 2000), four plate
(Hascoët et al. 2000) and black/white box (Sánchez and Meier 1997) tests. Acute fluvoxamine has proven ineffective in the EPM (Griebel et al. 1997b) and black/white box (Sánchez and Meier 1997) and anxiolytic in the four plate test (Hascoët et al. 2000) whilst acute paroxetine is anxiolytic, anxiogenic and devoid of action in the four plate, black/white box and social interaction tests respectively (Hascoët et al. 2000; Sánchez and Meier 1997; Lightowler et al. 1994). Conflicting profiles have also been reported in tests measuring flight/escape behaviour. Thus, acute fluoxetine has attenuated dPAG-evoked defensive behaviours in both conditioned (Jenck et al. 1998b) and unconditioned (Schenberg et al. 2002) situations, yet significantly increased flight in the MDTB (Griebel et al. 1995a).

The latter finding by Griebel and colleagues is in line with the increases in panic symptoms reported upon initiation of SSRI treatment (Saran and Halaris 1989; den Boer and Westenberg 1990; Catalano et al. 2000). Although the reduction in escape observed in the present experiment following chronic, but not acute, fluoxetine mirrors the delayed therapeutic action of SSRI’s in PD, the high escape rates of the vehicle group rendered it impossible to detect any such increases in escape. Thus, the profile of acute fluoxetine in a strain of rat showing lower levels of escape in the UEEPM (e.g. Hooded Lister, Jones et al. 2002a, b; Chapter III of this thesis) would certainly be worthy of investigation.

Acute CDP was devoid of effect in the UEEPM with no dose reducing escape rates in comparison to vehicle treated animals. This supports previous evidence that flight, as opposed to other elements of rodents’ defense strategies such as risk assessment, is not
sensitive to drugs effective against generalised anxiety as opposed to panic (Blanchard et al. 1997). Although the anxiolytic properties of BDZ’s in animal models of anxiety are well established (e.g. File and Pellow 1984; Pellow et al. 1985; Costall et al. 1989) the present findings are again similar to observations in the MDTB. At non-sedative doses similar to those used in the current experiment, CDP reduced risk assessment and defensive threat and attack but had no effect on flight (Blanchard et al. 1997). Moreover, in a earlier defense test battery, wild rats faced with an approaching predator displayed reduced levels of defensive threat and attack, but no change in flight or escape following acute CDP or diazepam (Blanchard et al. 1989).

In the experiment described here, chronic oral administration of vehicle significantly reduced escape and increased trial duration in the UEEPM. Chronic handling produced a similar profile, with no differences between the two groups in any of the recorded behaviours. Given the unhandled group’s response to the UEEPM remained unchanged upon re-exposure, this effect appears to be due to the repeated handling of subjects as opposed to the dosing method per se or habituation to the test procedure. Furthermore, in the current chronic fluoxetine experiment all groups displayed decreased levels of escape during post-treatment testing. Throughout the course of the study animals were removed from their cages and weighed by the experimenter at weekly intervals to adjust individual doses. This minimal handling may be responsible for these, less dramatic, reductions in escape rates. These findings support previous observations that prior handling can decrease anxiety-like behaviour in rats (Andrews and File 1991).
Parallels can be drawn between the present findings and studies of defensive behaviour in wild rats. When threatened, wild rats display much greater levels of flight/escape behaviour compared to laboratory-bred subjects (Blanchard et al. 1986b). However, the selective breeding of feral subjects on the basis of ease of handling results in significant reductions in this response (Blanchard et al. 1994). Laboratory-bred Brown Norway rats also display very high levels of escape in the UEEPM (typically 100% in naïve animals) in comparison to other commonly used, commercially available strains (Chapter III of this thesis). During the current experiment subjects became progressively easier to handle. Initial responses to attempted pick up (pre-treatment testing, high escape) consisted of explosive motor behaviour away from the experimenter’s hand followed by frequent attempts to bite it when contact was imminent. After 21 days handling (post-treatment testing, reduced escape), subjects could be freely picked up at the first attempt. Thus, the unhandled Brown Norway rat may posses a defensive behavioural repertoire more akin to its wild counterparts. Given the general lack of flight behaviour in laboratory-bred rats (Blanchard et al. 1986b), further examination of the range of defensive behaviours exhibited by this strain would be of considerable relevance to pre-clinical research into anxiety and panic.

To conclude, the present set of experiments aimed to further examine the pharmacological predictive validity of the UEEPM, a new pre-clinical model of extreme anxiety. Escape and escape-related behaviour were attenuated by chronic, but not acute, administration of the panicolytic agent fluoxetine. Escape was not reduced by an acute dose of the anxiolytic agent CDP. The current results further confirm that drugs effective
for GAD and PD have differential effects on specific defensive behaviours in rodents, and suggest the UEEPM may represent an unconditioned paradigm which could facilitate investigation into the neurochemical basis of extreme anxiety states.
CHAPTER VII
Some Directions for Future Research

Summary of Findings

The unstable elevated exposed plus maze (UEEPM) is a novel pre-clinical behavioural model of anxiety that elicits unconditioned escape behaviours in rats thought to resemble the symptoms of extreme panic-related anxiety disorders (King 1999a, b, c). As well as this face validity, repetitive stimulation of the rat midbrain defence circuitry results in a chronic potentiation of escape in the UEEPM, potentially analogous to the sensitisation of the neural fear system thought fundamental to the aetiology of pathological anxiety and suggesting elements of construct validity (King 1999b). The broad aim of the experiments reported in this thesis was to assess the predictive validity of the UEEPM and investigate the neurochemical substrates of escape behaviour in this test. Before such studies were undertaken, the effects of a number of potentially confounding methodological variables were quantified. The results of each study have been discussed in the corresponding experimental chapters, however, in brief, they can be summarised as follows;

1) Escape and escape-related behaviours in the UEEPM, and anxiety-related behaviours in the elevated plus maze (EPM), open field and holeboard, were unaffected by changes in circadian phase of testing and level of test illumination (Chapter II). Thus, the construct of ‘anxiety’ as measured by a range of paradigms
appears robust enough to withstand these manipulations. In contrast, the strain of rat used had a dramatic effect on baseline levels of escape, both from the aversive conditions of the UEEPM and from a suddenly looming visual stimulus in the runway test, and anxiety-related behaviour in the EPM (Chapter III). The Brown Norway strain showed consistently high levels of escape- and anxiety-related behaviours, whilst the Hooded Lister strain was the least reactive to all tests. These findings suggest that the flight response, and possibly other aspects of defensive behaviour, seen in wild rats may be more readily elicited in Brown Norways than other commonly used laboratory-bred strains, rendering them more suitable for pre-clinical research into panic-related disorders. The selection of subjects displaying high and low levels of escape may also optimise test situations for the detection of panicolytic and panicogenic drug effects in flight-based models.

2) The UEEPM displayed bi-directional predictive validity as a model of extreme anxiety. Thus, compounds which induce panic attacks and increase panic-related symptoms in patients suffering from panic disorder (PD) and post traumatic stress disorder (PTSD) increased escape and escape-related behaviour in the UEEPM (Chapter IV). Likewise, repeated administration of fluoxetine, a treatment regime efficacious in the treatment of PD and PTSD, significantly reduced escape. Conversely, administration of chlordiazepoxide (CDP), which is used in the treatment of generalised anxiety disorder (GAD) and is anxiolytic in many pre-clinical models of GAD, had no effect on UEEPM behaviour (Chapter VI).
3) Escape in the UEEPM was sensitive to pharmacological manipulation of the serotonin (5-HT)\textsubscript{2c} receptor. The increases in escape caused by the 5-HT\textsubscript{2c/2b} agonist \textit{m}-chlorophenylpiperazine (mCPP) were attenuated by prior administration of the selective 5-HT\textsubscript{2c} receptor antagonist SB-242084 (Chapter V). Given the well documented panicogenic effects of mCPP in humans, these findings also suggest that compounds with antagonistic actions at the 5-HT\textsubscript{2c} receptor may possess therapeutic potential for the treatment of panic-related disorders.

Given the lack of a widely used pre-clinical model of extreme pathological anxiety states in rats, these studies provide valuable preliminary evidence that the UEEPM may form a paradigm to facilitate investigation into the neurochemical substrates of these disorders and aid the development of novel pharmacological treatments. The use of subjects previously undergoing sensitisation of the midbrain defence circuitry in pharmacological experiments, a systematic investigation into the neuroanatomical substrates of escape in the UEEPM and attempts to develop an ethologically-based model of PTSD provide the most salient avenues to extend the current research and possibly gain further insight into these debilitating conditions. Proposals for such experiments are detailed below.

**Pharmacological Research Using Sensitised Animals**

Examination of the UEEPM profiles of panicolytic and panicogenic agents in rats with a sensitised midbrain defense circuitry would go further to developing a pre-clinical paradigm fulfilling the criteria of predictive, face and construct validity for extreme
pathological anxiety disorders. The sensitivity of the UEEPM to panic-, and not anxiety-modulating, agents suggests that escape behaviour in the model more closely resembles the behavioural symptoms of panic (i.e. the ‘urge to flee’) than GAD, which in turn may be more related to other defensive behaviours such as risk assessment (Rodgers et al. 1995; Blanchard et al. 2001a). However, as the subjects used in the current studies were experimentally naïve, it could be argued this protocol lacks construct validity and the UEEPM is only modelling different, albeit more severe, aspects of ‘normal’ anxiety than tests such as the EPM.

Pathological panic-related anxiety is thought to be the result of hyperexcitability of the neural fear circuitry (Rosen and Schulkin 1998) which leads to spontaneous and uncontrollable activation of the flight response (Graeff 1990; Deakin and Graeff 1991; Graeff et al. 1993). As repetitive electrical stimulation of the rat midbrain defence system results in a similar long-term hyper-reactivity in the UEEPM (King 1999b), the use of sensitised subjects would award elements of construct validity to the model. Thus, the logical progression of the current work would be to re-examine the UEEPM behavioural profiles of compounds that clinically increase (e.g. mCPP, caffeine, FG 7142), decrease (chronic fluoxetine) and have no effect on (CDP) panic in animals exhibiting the chronically hyperaroused fight/flight state that typifies PD and PTSD. If, as would be hypothesised from the current findings, these compounds had corresponding effects on escape, and not anxiety-related behaviours measured in models such as the EPM, even closer parallels between the pre-clinical model and the clinical disorders being simulated could be drawn.
The Neuroanatomical Substrates of Escape in the UEEPM

Clinical anxiety states comprise a range of different syndromes. In terms of pre-clinical research, the current studies confirm previous observations (e.g. Blanchard et al. 1993a; Griebel et al. 1995a) that, pharmacologically, the flight component of rodents' defensive behavioural repertoire is more analogous to panic-related disorders as opposed to GAD. The use of immunocyctochemical techniques could be employed to determine if this neurochemical heterogeneity is paralleled by similar neuroanatomical distinctions, thereby providing further insight into the biological bases of these distinct clinical conditions. The expression of fos (the protein product of the immediate early gene c-fos) in the rat brain after presentation of various stimuli is widely used as a marker of cellular activity (Dragunow and Faull 1989). Thus, fos-like immunoreactivity (FLI) has been observed in discrete brain areas following drug administration (e.g. Wirtshafter and Asin 1995; Kurokawa et al. 1997; Singlewald and Sharp 2000), brain stimulation (e.g. Sandner et al. 1993; Silveira et al. 1995; King et al. 1996) and exposure to environmental stimuli (e.g. Silveira et al. 1993; Canteras and Goto 1999; Grahn et al. 1999). Notably, the exposure of rats to aversive situations results in the expression of FLI in brain areas within the neural fear circuitry. Canteras and Goto (1999) observed increased FLI in the dorsolateral and ventrolateral periaqueductal grey (PAG) in the rat brain following a 10 min exposure to a domestic cat. Likewise, dense FLI is apparent in both these areas, along with the dorsal and ventral aspects of the medial hypothalamus and the medial amygdaloid nucleus, after 20 mins exposure to a collar worn by a cat for a period of 3 weeks (Dielenberg et al. 2001). Increased FLI in the dorsomedial hypothalamus,
basolateral and medial amygdala, superior colliculus (SC) and dorsal PAG is also observed following exposure to the EPM for between 5 and 15 mins (Silveira et al. 1993; Duncan et al. 1996).

The examination of FLI in the rat brain following exposure to the UEEPM would allow investigation into the brain structures controlling the escape responses observed in this model. On the basis of previous brain stimulation and lesion literature (see pages 20-26 of this thesis) it could be predicted that, following UEEPM exposure, FLI would be observed in areas such as the dorsal PAG and SC, which elicit rapid escape-related behaviour (e.g. Bandler and Depaulis 1991; Fanselow 1991; King et al. 1996), in comparison to regions of the fear circuitry such as the ventrolateral PAG, which elicit freezing and immobility (e.g. Zhang et al. 1990; Bandler and Depaulis 1991). Moreover, the comparison of neural activation between rats exposed to the UEEPM and EPM or open field would reveal any differences in the structures mediating escape and other forms of defensive behaviours, further investigating the notion that these paradigms model different facets of anxiety. Indeed, similar work by Silveira and colleagues (2001) suggests such a distinction between the neural substrates of specific anxiety-related behaviours in the elevated T maze. Although FLI was apparent in the amygdala after both 'inhibitory avoidance' (moving from the closed to the open arm) and 'one way escape' (moving from the open to the closed arm), FLI in the dorsal PAG was only observed following performance of the latter behaviour, suggesting a preferential role of the structure in active, as opposed to passive, avoidance.
In an extension of this work, the examination of FLI after UEEPM exposure in rats with a sensitised midbrain defence circuitry may provide insights into the specific brain structures mediating the chronically hyperaroused persistent fight/flight state that typifies pathological clinical anxiety. Repetitive electrical stimulation of the SC, which produces chronic increases in escape in the UEEPM, results in elevated levels of FLI in discrete regions of its ipsilateral descending pathway including dorsolateral areas of the PAG, the cuneiform area and the ventrolateral midbrain/pontine reticular formation (King et al. 1996). Anterograde tracing studies have revealed the SC also possesses ascending projections to the amygdala, via the posterior thalamus (Linke et al. 1999). Kindling of the amygdala has been shown to significantly increase anxiety-like behaviour in rats (Adamec 1990; Helfer et al. 1996). It seems likely, therefore, that these regions are involved in the long-term post-stimulation alteration in the behavioural response to the aversive properties of the UEEPM.

Hence, it could be speculated that a comparison of FLI in these target areas, as well as other regions of the fear circuitry, between sensitised and experimentally naïve animals exposed to the UEEPM may reveal quantitative or qualitative differences in the level of cellular activation, thereby implicating discrete structures in the lowering of thresholds for escape behaviour. Selective lesions of areas of interest arising from these studies could then be performed prior to stimulation of the midbrain, with any subsequent disruptions in sensitisation providing further evidence of the structures necessary for the chronic potentiation of escape to occur.
Immunocytochemical techniques could also be applied to pharmacological studies to examine the neuroanatomical targets of compounds modulating escape behaviour in the UEEPM. Thus, if panicolytic and panicogenic agents correspondingly decrease and increase escape in the UEEPM, comparison of FLI between treated and vehicle subjects after UEEPM exposure may indicate the brain areas where these compounds directly, or indirectly, produce their behavioural effects. Moreover, the application of this technique to sensitised rats displaying long-term increases in reactivity to the UEEPM could allow potential extrapolation to the sites of action of drugs which affect symptoms in patients suffering from pathological panic-related conditions, thus facilitating future development of pharmacological therapies.

Towards an Ethological Pre-Clinical Model of PTSD

The lowering of thresholds for escape in the UEEPM in rats following repetitive electrical stimulation of the SC resembles the enduring increases in vigilance, startle reaction and autonomic and physiological responses to traumatic cues seen in PTSD patients (King 1999b). However, given that PTSD arises from catastrophic experiences such as war-time combat, civil disasters such as train crashes and ferry sinkings, and violent sexual assault (Lindsay 1994), it could be argued that the use of direct brain stimulation in the absence of external stimuli lacks ethological validity. To address this issue, a possible analog of such traumatic event in pre-clinical research may be the exposure of rodents to a potentially life threatening event, for example an inescapable encounter with a species-relevant predator. The effects of such an encounter on anxiety-
related behaviours in rats and mice are well documented. For instance, the exposure of rats to a domestic cat or a collar worn by a domestic cat, and mice to tape recorded calls of barn and tawny owls, results in a range of defensive behaviours including immobility, retreat and episodes of rapid running and jumping (Blanchard and Blanchard 1989b; Hendrie and Neill 1991; Eilam et al. 1999, Dielenberg et al. 2001).

Importantly, the increases in anxiety-related behaviour in rats following a single exposure to a cat appear to be chronic in nature. Adamec and Shallow (1993) placed rats into a room containing a cat for a period of 5 min. During the encounter, rats performed a range of defensive behaviours including defensive attack, freezing and escape. When subsequently tested in the EPM, cat exposed subjects displayed significantly more aversion to the open arms than control subjects for a period of 3 weeks, resembling the long-term increases in anxiety seen clinically in PTSD. These increases in anxiety-related behaviour have so far only been measured in the EPM. The integration of a test that elicits behaviours more specifically resembling the hypervigilant, persistent fight/flight state seen in PTSD, such as the UEEPM, into this protocol would be of considerable interest. If cat exposure results in chronic increases in escape in the UEEPM akin to those observed following SC stimulation, and compounds displaying efficacy for the clinical management of PTSD, such as the SSRI sertraline (Brady et al. 2000), attenuate this behavioural sensitisation, this paradigm may form a pre-clinical model of PTSD possessing face, construct, predictive and ethological validity. As well as providing an assay for developing drug treatments for PTSD symptoms, examination of the effects of
compounds administered after cat exposure on subsequent UEEPM behaviour may also facilitate the search for post-trauma prophylactics.

Conclusion

The current thesis provides evidence that the escape behaviours exhibited in the UEEPM are sensitive to compounds which clinically affect the more extreme panic-related anxiety disorders. The testing of similar compounds on sensitised animals exhibiting a chronically aroused hypervigilant state would increase construct validity and enable closer comparisons to the profiles of drugs in pathological anxiety disorders. Immunocytochemical analysis of the brain areas involved in escape in the UEEPM and passive avoidance in tests such as the EPM would reveal any neuroanatomical heterogeneity in different aspects of defensive responses thought to be associated with distinct clinical disorders. Similar comparisons between sensitised and naïve animals exposed to the UEEPM, along with subsequent lesion studies, may provide insights into the actual brain regions mediating the chronic increases in escape, thus identifying possible neural substrates for the shift from ‘normal’ to ‘pathological’ anxiety. Finally, the use of an ethologically relevant external stressor, such as predator exposure, to induce long-term increases in escape in the UEEPM may lead to the development of an ethological pre-clinical model of PTSD.
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APPENDIX

Prior to behavioural testing in experiment 1 (detailed in Chapter II of this thesis) a small pilot study was conducted to investigate any diurnal / nocturnal variations in subjects’ activity in a familiar environment. The method and results of this experiment are detailed below.

Method

Three male Sprague Dawley rats used in experiment 1 and housed under the reversed light cycle condition were recorded in their home cages in the keeping room for 24 hr. To achieve subjective dark conditions, dark phase home cage monitoring was performed under red light using filters (Lee Filters, UK) which blocked light transmission up to 600 nm approx. Activity was analysed by manual recording via video tape of subjects’ behaviour at 10 min intervals. Behaviours were classified as; moving (animal locomoting along the cage floor in any direction), immobility (animal stationary and performing no active behaviour), rearing (the movement of the subject onto its hind legs, either against the side of the cage or unsupported), grooming (cleaning body with tongue, teeth and / or forepaws), feeding (either obtaining food from the hopper or consuming food already obtained) or drinking (animal’s mouth in contact with the spout of the water bottle).
Results

Mean frequencies per hour of each behaviour (see Figure 1) revealed that, with the exception of minimal displays of feeding, subjects performed no active behaviours at any of the 10 min sample points during the light period. In contrast, all behaviours were apparent in the dark and half-light phases. Further analysis of behaviour during the dark and half-light periods revealed an increase in behaviour during the first hour of darkness, which continued until the first hour of the light phase. No marked temporal trends in frequencies of individual active behaviours were observed, suggesting only an overall increase in activity during the dark phase (see Figures 2 and 3). The results confirm previously reported observations that rats display a 24 hr activity rhythm with a general increase in home cage activity during the dark phase (Gorka et al. 1996).

![Figure 1. Home cage behaviours observed during dark (black bars), half-light (grey bars) and light (white bars) periods. Data presented as means per hour (±) SEM, n = 3. Imm = immobility, Mov = moving.](image-url)
Figure 2. Moving, rearing and grooming observed during dark (D) and half-light (H) periods. Values for half-light periods presented as means per hour. Dark values presented as means over consecutive 2 hour periods, n = 3.

Figure 3. Consummatory behaviours observed during dark (D) and half-light (H) periods. Values for half-light periods presented as means per hour. Dark values presented as means over consecutive 2 hour periods, n = 3.