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Themed Issue: Translational Neuropharmacology – Using Appropriate Animal Models to Guide Clinical Drug Development

REVIEW

The age of anxiety: role of animal models of anxiolytic action in drug discovery

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Anxiety disorders are common, serious and a growing health problem worldwide. However, the causative factors, aetiology and underlying mechanisms of anxiety disorders, as for most psychiatric disorders, remain relatively poorly understood. Animal models are an important aid in giving insight into the aetiology, neurobiology and, ultimately, the therapy of human anxiety disorders. The approach, however, is challenged with a number of complexities. In particular, the heterogeneous nature of anxiety disorders in humans coupled with the associated multifaceted and descriptive diagnostic criteria, creates challenges in both animal modelling and in clinical research. In this paper, we describe some of the more widely used approaches for assessing the anxiolytic activity of known and potential therapeutic agents. These include ethological, conflict-based, hyponeophagia, vocalization-based, physiological and cognitive-based paradigms. Developments in the characterization of translational models are also summarized, as are the challenges facing researchers in their drug discovery efforts in developing new anxiolytic drugs, not least the ever-shifting clinical conceptualization of anxiety disorders. In conclusion, to date, although animal models of anxiety have relatively good validity, anxiolytic drugs with novel mechanisms have been slow to emerge. It is clear that a better alignment of the interactions between basic and clinical scientists is needed if this is to change.

LINKED ARTICLES
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Abbreviations
5-HT, 5 hydroxytryptamine (serotonin); 8-OH-DPAT, 8-hydroxy-*N*,*N*-dipropyl-2-aminotetralin; CCK, cholecystokinin; EPM, elevated plus maze; GAD, generalized anxiety disorder; GAT-1, GABA transporter 1; LAB/HAB, low anxiety bred/high anxiety bred; MCH1, melanin-concentrating hormone receptor 1; mCPP, *m*-chlorophenylpiperazine; MDTB, mouse defence test battery; NK1, neurokinin 1; NMDA, *n*-methyl-D-aspartate; PTSD, post-traumatic stress disorder; PTZ, pentylenetetrazol; SSRI, selective serotonin re-uptake inhibitor; TSST, trier social stress test; USV, ultrasonic vocalization

Introduction

'Now is the age of anxiety'.
WH Auden

Despite the passage of more than 60 years since the publication of Auden's Pulitzer Prize-winning text, it can be argued that both at a global and local level that it is the first part of the 21st century that represents the age of anxiety (Auden, 1947). Anxiety disorders are currently the most prevalent psychiatric diseases in Europe and in the USA, and as such represent a grave and ever-increasing strain on healthcare resources (Kessler *et al.*, 2005b; Alonso and Lépine, 2007; Kessler, 2007; Nutt *et al.*, 2007). Separate large-scale epidemiological studies in both Europe (European Study of the Epidemiology of Mental Disorders) (Alonso *et al.*, 2004) and the USA (National Comorbidity Survey – Replication)

(Kessler and Merikangas, 2004) have demonstrated that anxiety disorders have the highest lifetime prevalence estimates (13.6–28.8%) and the earliest age of onset (11 years) of psychiatric disorders (Kessler *et al.*, 2005a,b; Kessler, 2007). Patients suffering from anxiety disorders also frequently present with other comorbid diseases, including not only psychiatric disorders such as depression (Merikangas, 2003; Kessler *et al.*, 2005b), but also medical conditions including functional gastrointestinal disease, asthma, cardiovascular disease, cancer and chronic pain, hypertension and migraine (Härter *et al.*, 2003; Roy-Byrne *et al.*, 2008). As such, anxiety disorders represent a huge burden in terms of both their social impact and their economic cost (Kessler, 2007; Nutt *et al.*, 2007). Our understanding of the pathological processes, aetiology and causative factors underlying anxiety disorders is still unfortunately in its infancy and must be developed if we are to diagnose and treat anxiety disorders more effectively (Wong and Licinio, 2004; Cryan and Holmes, 2005).

In parallel to this, there is a growing realization that the cost of phase II and phase III clinical trials in pharmaceutical drug development is enormous and growing annually (DiMasi *et al.*, 2003), with the cost of central nervous system drug development being higher than that of any other major therapeutic area (Frantz, 2004). Furthermore, clinical trials in psychiatry are burdened, as in many medical disease trials, with very high rates of placebo response (Lakoff, 2002). As a result, before embarking on costly trials, pharmaceutical companies and research-funding agencies increasingly seek assurance that any specific biological target is indeed relevant to the disease (Gomez-Mancilla *et al.*, 2005). Accordingly, there is a growing emphasis on first obtaining proof that a new chemical entity designed to alter the function of a specific target will do so in a predictable and safe manner. Central to this approach, as with all diseases, is the availability of valid preclinical animal models for evaluating the potential utility of novel pharmacotherapeutics. However, as a field, psychiatry has proven to be among the least penetrable clinical disciplines for productively marrying knowledge of human pathology with animal behaviour to develop satisfactory *in vivo* animal models for evaluating novel treatment approaches. In this review, we highlight the contribution of animal models to the current and future development of anxiolytic drugs.

Anxiety disorders

The anxiety response is an important mechanism by which we adapt and respond to real dangers. Dysregulation of this healthy response resulting in 'marked, persistent, and excessive or unreasonable fear' (American Psychiatric Association, 2000), culminating in a significant interference in normal life can be described as an anxiety disorder. From a clinical perspective, anxiety disorders are described by *Diagnostic and Statistical Manual IV* in terms of subtypes distinguished by the nature of the anxiety-provoking stimulus. Most common among these anxiety disorder subtypes are generalized anxiety disorder (GAD), panic disorder (diagnosed with or without agoraphobia), specific phobia, social phobia, obsessive–compulsive disorder and post-traumatic stress disorder (PTSD). It should be noted that the *Diagnostic and Statistical Manual V*, due for publication in May 2013, proposes to expand and modify classification, as well as reclassify obsessive–compulsive disorder in a different diagnostic category (Holden, 2010; Miller and Holden, 2010).

While these subdisorders are to a degree epidemiologically comorbid, they display differential responsiveness to the spectrum of anxiolytic drugs currently in clinical use. This suggests that divergent etiological factors may underlie the different disorders. Rating scales, such as the Hamilton rating scale for anxiety and the clinical global impression scale, are used by clinicians both as tools to quantify symptom severity and as measures of treatment efficacy. These disorders furthermore display distinct neurobiological and neuroendocrine characteristics, indicative of differing underlying pathology (Sramek *et al.*, 2002).

Current drug treatment of anxiety

For millennia, humans have sought out chemical agents to modify the effects of stress and feelings of discomfort, tension, anxiety and dysphoria; the oldest of these being ethanol. In the 19th century, alkaloids, bromide salts and choral hydrate were used for their sedative hypnotic medicine. A major breakthrough came with the introduction of barbiturates into the clinical practice in the early part of the 20th century (López-Muñoz *et al.*, 2005). They induce their effects by facilitating the Cl^- channel of the $GABA_A$ receptor to open, even in the absence of GABA tone. Animal models, especially canine-based paradigms, were particularly useful in identifying the sedative and anticonvulsant properties of such drugs, although self-testing was also very popular in the early days of modern psychopharmacology. While barbiturates were popular as major tranquillizers, their side effects, including sedation and behavioural changes, tolerance, and dependence issues coupled with the fact that their therapeutic dose limit is dangerously close to its toxic level has led to the pharmaceutical industry to seek out safer alternatives (López-Muñoz *et al.*, 2005).

It was in this context that the development of benzodiazepines emerged and revolutionized the treatment of anxiety disorders. The first clinically available benzodiazepine was chlordiazepoxide, which was synthesized by Sternbach in the 1950s at the Hoffman La Roche Pharmaceutical Company (Sternbach, 1979). At a molecular level, benzodiazepines elicit their effects by allosterically activating the $GABA_A$ receptor channel at a site distinct from GABA itself, and thus only induce effects in synapses where GABA is present. Key behavioural studies by Randall and colleagues (Hanson, 2005) indicated that chlordiazepoxide might have a distinct pharmacological profile compared with that of barbiturates and other psychoactive drugs such as the antipsychotic chlorpromazine and anti-hypertensive reserpine. These initial tests were carried out in mice and cats, and included the mouse-inclined screen test indicative of muscle relaxation and sedation, a foot shock test showing 'taming effects', the anaesthetized cat model of muscle relaxation, seizure-based pentylenetetrazol (PTZ) and electroshock tests (Randall, 1960). Later, more sophisticated tests included Skinner box based Sidman avoidance task (Sidman, 1953;

Boren *et al.*, 1959) in rats and monkeys, which provided a sensitive and reliable measure of depressant action on behaviour. Thus, the tests used to illuminate the anxiolytic activity of the first generation of chemically designed anxiolytics were somewhat crude and not selective for anxiety *per se*. Yet, they highlight the crucial role of animal testing in anxiolytics development. It is somewhat ironic that as the tests employed became more sophisticated (See Table 1) the development of anxiolytic drugs has not greatly increased (Figure 1).

Despite the advantages of benzodiazepines over previous drugs, their long-term use is hampered by dependence liability, tolerance, and cognitive and other behavioural side effects. This once again led to a major research effort to try and develop novel non-GABAergic based therapies.

The realization that the serotonergic system plays a role in anxiety has been known for over 50 years since Aprison and Ferster showed that the 5 hydroxytryptamine (serotonin) (5-HT) precursor 5-hydroxytryptophan increased responding in a pigeon conflict model (Aprison and Ferster, 1961). This view mainly arose from some observed activity of 5-HT antagonists in operant conflict paradigms in rats (Robichaud and Sledge, 1969), as well as from an association between reduction in turnover of 5-HT and the anxiolytic effects of benzodiazepines (Goldberg *et al.*, 1967). This research culminated in the development of 5-HT-based therapies for anxiety disorders throughout the 1970s and early 1980s (Taylor and Moon, 1991), chief among them was the azapirone chemical class of which buspirone was the most successful. Buspirone acts as a partial 5-HT_{1A} receptor agonist, and its use confirmed that it was possible to develop novel anxiolytic drugs that lacked the side effects of GABA-based drugs. Also, it opened up the possibility the modulation of the serotonergic system may have clinical benefit in anxiety disorders. Today, buspirone has a somewhat limited use, although it is generally well tolerated with few side effects, its efficacy, is less and onset of action slower than previous drugs such as the benzodiazepines.

The clinical realization that anxiety and depression are co-morbid has led to the clinical observation that selective serotonin re-uptake inhibitors (SSRIs) are effective in treating anxiety disorders following on from observations regarding the efficacy of tricyclic antidepressants in anxiety (Rickels *et al.*, 1974; 1993). Indeed, today SSRIs are first-line therapy for many anxiety disorders (Hoffman and Mathew, 2008). The development of SSRIs for depression and subsequently anxiety was firmly driven by mechanistic studies focusing on the modulation of monoamine neurotransmission *in vitro* and *in vivo* with little input of behavioural models initially (Wong *et al.*, 2005). This is a clear and sobering example where animal models had little to do with the clinical introduction of these treatments for anxiety. Indeed, the reliance on traditional animal models of anxiety shows little positive effects of SSRIs, and indeed anxiogenic effects are often observed (Sánchez and Meier, 1997; Borsini *et al.*, 2002). It should, however, be borne in mind that a transient period of increased anxiety is often reported in patients initiated onto SSRI therapy (Vaswani *et al.*, 2003; Baldwin *et al.*, 2010). This has led to much criticism of the models used. Likewise, there has been a growing discussion focused on whether anxiety and depression should be isolated from a drug development perspective (Shorter and Tyrer, 2003). Moreover, given the relative success of SSRIs, it is becoming clear that many pharmaceutical companies are compelled to develop a 'one pill fits all' approach to anxiety and mood disorders. This propelled research in the area of neuropeptides such as corticotrophin-releasing factor receptor antagonists, neurokinin 1 (NK1) receptor antagonists and melanocortin antagonists, which to date have yet to fulfil its initial promise (Takahashi, 2001; Shimazaki *et al.*, 2006; Ebner *et al.*, 2009). Recent drug discovery efforts have additionally focused on ligands acting at G-protein-coupled receptors for the non-monoaminergic neurotransmitters GABA and glutamate (Chojnacka-Wójcik *et al.*, 2001; Cryan and Kaupmann, 2005). The current status of several promising putative drug classes for anxiety is given in Table 2.

It is clear that while there are certain overlapping factors contributing to the natural history of anxiety and depression, the symptomatic manifestation and treatment of each can be very different; benzodiazepines, for example, have limited efficacy in depression and yet represent a very effective intervention in anxiety disorders, whereas SSRI antidepressants are useful in both disorders. Thus, understanding the neural circuits of both these disorders is crucial to devising novel interventions. Animal models will be critical for such approaches, although it must be remembered that animal models can only be as valid as the clinical knowledge that their translational validity is based on and that a better clinical understanding of the diverging nature of the two disorders is still of the upmost importance.

Endophenotypes

A growing recognition of the complex and heterogeneous nature of anxiety has resulted in an effort to re-evaluate the diagnosis and treatment of anxiety disorders, and develop a novel approach where individual behavioural, physiological and neurochemical end points are specifically considered as opposed to a syndrome-based approach (Geyer and Markou, 2002; Gottesman and Gould, 2003; Hasler *et al.*, 2004). Considered from a genetic perspective, the clinical deconstruction of anxiety can be described in terms of endophenotypes. These are cognitive, psychological, anatomical or biochemical traits which are hereditary and represent reliable markers of both the disease state and disease risk (Hasler *et al.*, 2004). The endophenotypes present in anxiety disorders may allow for a more effective analysis of the neurobiological and genetic factors that contribute to their development in humans, as well as representing facets of disease more amenable to the development of valid animal models (Gottesman and Gould, 2003; Hasler *et al.*, 2004). The behavioural endophenotypes of anxiety disorders such as autonomic hyper arousal, impaired extinction of traumatic memories, sleep disturbances and avoidance of difficulty to escape areas can all be readily modelled in existing behavioural paradigms (Cryan and Holmes, 2005).

Like other medical disciplines, concerted effort is focused on the generation of novel models of anxiety (i.e. an effort to induce in animals a hyper-anxious state), analogous to the state seen in anxiety disorder patients, which can be detected by increased sensitivity to the anxiety-provoking nature of

Table 1

Tests of anxiety in animals

Test	Brief description	Principles	Animals	Clinically available active drugs	References
Ethological tests					
Elevated plus maze	Animals are placed on an elevated plus-shaped maze consisting of two open arms and two enclosed arms connected by a central connecting square.	The open arms of the maze are considered to be more aversive than the closed arms. Anxiolytic drug treatment results in increased entries to and time spent in the open arms.	Mice, rats, gerbils	Benzodiazepines, ondansetron, barbituates, ethanol, prazosin, clonidine, 5-HT_{1A} receptor agonists	(Handley and Mithani, 1984; Lister, 1987; Wilks and File, 1988; Moser *et al.*, 1990; Filip *et al.*, 1992; Hogg, 1996; Braun *et al.*, 2011)
Elevated zero maze	Animals are placed on an elevated circular maze consisting of four segments: two exposed segments and two walled segments of equal length.	The open segments of the maze are considered to be more aversive than the closed segments. Anxiolytic drug treatment results in increased entries to and time spent in the open segments.	Mice	Benzodiazepines, citalopram (repeated dosing)	(Shepherd *et al.*, 1994; Mombereau *et al.*, 2007; Braun *et al.*, 2011)
Light–dark box	Animals are placed in an apparatus consisting of two compartments: an illuminated 'light' compartment and a dark compartment. The compartments are connected by a small opening at floor level. Animals are allowed to freely explore the apparatus.	The light compartment is believed to be more aversive to the mouse than the dark compartment. Anxiolytic drug administration increases the number of entries to the light compartment, time spent in the light compartment and reduces freezing behaviour.	Mice, rats	Benzodiazepines, buspirone, ondansetron, paroxetine, dothiepine, moclobemide, clozapine	(Crawley and Goodwin, 1980; Jones *et al.*, 1988; Onaivi and Martin, 1989; Bourin *et al.*, 1996; De Angelis and Furlan, 2000; Hascoët *et al.*, 2000a; Bourin and Hascoët, 2003)
Open field	Mice are placed in a brightly lit arena and allowed to freely explore.	The brightly lit exposed arena is a highly anxiogenic environment. Anxiety levels are assessed by a variety of ethological parameters, as well as by the amount of time the animal spends in the more anxiety-provoking central compartment.	Mice, rats	Benzodiazepines, phenobarbitol, acute vigabatrin, zolpidem, buspirone, acute fluoxetine, ondansetron, propanolol	(Crawley, 1981; Lucki *et al.*, 1989; Stefański *et al.*, 1992; Sherif and Oreland, 1995; de Angelis, 1996; Schmitt and Hiemke, 1998; Prut and Belzung, 2003)
Defensive marble burying/shock probe burying test	Animals are placed in a novel cage containing bedding and a number of novel marbles or an electrical shock probe.	Burying of the marbles is interpreted as anxiety-like behaviour. Anxiolytic drug treatment reduces the number of marbles buried.	Mice/rats	SSRIs, benzodiazepines, buspirone, chronic desipramine, chlorpromazine, imipramine, haloperidol, morphine, pentobarbitol, progesterone, nitrous oxide, SSRIs	(Treit *et al.*, 1981; Craft *et al.*, 1988; Njung'e and Handley, 1991; Czech and Quock, 1993; Picazo and Fernández-Guasti, 1995; De Boer and Koolhaas, 2003; Joel, 2006)
Ultrasonic vocalizations	Mouse pups are separated from their mothers and the frequency of ultrasonic distress calls is recorded	A reduction in the number of USVs emitted from separated pups is regarded as an anxiolytic effect.	Mice, rats, guinea pigs	Benzodiazepines, 5-HT_{1A} receptor agonists, SSRIs	(Nastiti *et al.*, 1991a,b; Groenink *et al.*, 2008; Scattoni *et al.*, 2009)

Table 1

Continued

Test	Brief description	Principles	Animals	Clinically available active drugs	References
Staircase test	Animals are placed in an enclosed staircase, with the number of steps climbed and the number of rearing behaviours measured.	The climbing of steps in this test is regarded as a measure of exploratory behaviour, with the number of rearing behaviours being regarded as an anxiety-like behaviour.	Mice, rats	benzodiazepines, barbituates	(Molinengo and Ricci-Gamalero, 1970; Hughes, 1972; Cunha and Masur, 1978; Simiand *et al.*, 1984)
Social interaction test	Animals are introduced to a novel animal of the same species, and social behaviour is monitored.	Anxiolytic drugs increase the levels of social interaction with the novel mouse.	Mice	Benzodiazepines	(File, 1980)
Suok ropewalking/ elevated alley test	Animals are placed in the centre of an elevated beam or alley and allowed to freely explore the apparatus. Can be modified to the light–dark Suok test by illuminating one half of the beam and leaving the other half in darkness.	The elevated beam induces anxiety by virtue of its novelty and elevation. Increased horizontal exploration in this test is regarded as a decrease in anxious behaviour. In the light–dark test, increased time spent in the illuminated area is regarded as a decrease in anxiety. The Suok test is also able to detect non-specific drug effects (e.g. ataxia) and is specific use in examining anxiety–vestibular system interactions.	Mice, rats	Benzodiazepines, ethanol	(Kalueff and Tuohimaa, 2005; Kalueff *et al.*, 2007; 2008)
T-maze	The elevated T-maze consists of an elevated platform consisting of two opposing exposed arms and one enclosed arm perpendicular to the open arms. Animals are placed at the distal end of the enclosed arm and are allowed to explore the apparatus. After several trials, the animal is placed on the distal end of an open arm and allowed to explore the apparatus.	Learned fear (inhibitory avoidance) can be assessed by measuring the time taken for the animal to leave the enclosed arm subsequent to initial exposure. Anxiolytic agents decrease the amount of time taken by the animal to leave the open arm. Unconditioned fear is measured using the latency of the animal to escape into the closed arm from the open arm.	Mice, rats	Benzodiazepines, buspirone (inhibitory avoidance only)	(Graeff *et al.*, 1998; Carvalho-Netto, 2004)
Seed-finding test	Hamsters are fasted overnight and are then removed from their home cages to a novel environment. Sunflower seeds are then placed under the bedding of the home cage.	Latency to find the seed when returned to the cage is taken as a marker of anxious behaviour.	Hamster	Chlordiazepoxide, fluoxetine, buspirone	(King *et al.*, 2002)
Anxiety/defence test battery, MDTB	Rats or mice are confronted with a dead or heavily anaesthetized predator (rat) and the defensive behaviour exhibited by the mouse is measured.	Risk assessment, flight responses and defensive attack behaviours are measured, as well as the development of context-dependant defensive behaviours (i.e. in the absence of threat are ethologically examined.	Mice, rats, non-human primates	Benzodiazepines, 5-HT_{1A} receptor agonists, imipramine, chronic SSRIs, chronic TCAs	(Griebel *et al.*, 1995a,b; 1999; Barros *et al.*, 2008)

Table 1

Continued

Test	Brief description	Principles	Animals	Clinically available active drugs	References
(Modified) hole board test	Rats or mice are placed in an open field containing an opaque holed board. The board contains several holes with removable plastic covers.	The number of entries onto the board and duration spent on the board are measures of anxiolyitc activity. Exploratory behaviours (no. of holes visited) and locomotor activity, as well as a number of ethological parameters can be measured.	Mice, rats	Benzodiazepines, chronic tiagabine, chronic paroxetine	(Ohl *et al.*, 2001a,b; Sillaber *et al.*, 2008; Thoeringer *et al.*, 2010)
Conflict tests					
Geller–Seifter task	Food-deprived animals are trained to activate a lever to receive food (unpunished period). Once trained, the delivery of the food reward coincides with an electrical shock (punished period).	The paradigm generates a conflict between hunger-stimulated approach behaviour and fear of the shock. Anxiolytic drugs increase approach behaviour during the punished period.	Rats	Benzodiazepines, barbituates, clozapine, haloperidol	(Geller and Seifter, 1960; 1962; Geller *et al.*, 1962; Wiley *et al.*, 1993; Millan and Brocco, 2003; Paterson and Hanania, 2010)
Vogel punished drinking	Water-deprived rats are given access to water. Drinking behaviour is 'punished' with electrical shocks.	The paradigm generates a conflict between thirst-stimulated approach behaviour and fear of the shock. Increases in 'punished' water consumption is viewed as an anxiolytic effect.	Rats	Diazepam, zolpidem, phenobarbital, propofol, baclofen, buspirone, ondansetron	(Vogel *et al.*, 1971; Depoortere *et al.*, 1986; Shibata *et al.*, 1989; Filip *et al.*, 1992; Matsuo *et al.*, 1997; Dekeyne *et al.*, 2000; Millan and Brocco, 2003)
Four-plate test	Apparatus consists of a cage with a floor comprised of four metal plates. The animal is allowed to freely explore the novel apparatus, but crossing between floor plates results in a mild electrical shock.	In the four-plate test, the aversive shock can only be avoided by immobility. The number of crossings between quadrants in this paradigm is inversely related to the levels of passive avoidance behaviour.	Mice	Benzodiazepines, SSRIs, SNRIs, tricyclic antidepressants, trazodone	(Aron *et al.*, 1971; Hascoët *et al.*, 2000b)
Hyponeophagia tests					
Novelty-suppressed feeding	Food-deprived animals are introduced to a novel anxiogenic environment where food is presented.	Thus, paradigm generated a conflict between the anxiogenic stimulus and hunger-induced approach behaviour. Anxiolytic drugs reduce the latency of the animal to approach the food.	Mice, rats	TCAs (chronic), benzodiazepines, SSRIs	(Bodnoff *et al.*, 1988; 1989)
Novelty-induced hypophagia	Animals are trained to consume a desirable food (e.g. sweetened milk before being presented with the milk in a novel environment.	Hesitation on the part of the animal to consume the highly palatable food in the novel environment is regarded as a measure of both anxiety and of anhedonia.	Mice, rats	Benzodiazepines, buspirone (chronic), SSRIs (chronic), TCAs (chronic), phenobarbitol, valproic acid, propanolol, ondansetron	(Rex *et al.*, 1998; Merali *et al.*, 2003; Santarelli *et al.*, 2003; Dulawa and Hen, 2005)

Table 1

Continued

Test	Brief description	Principles	Animals	Clinically available active drugs	References
Cognitive-based tests					
Pavlovian fear conditioning	Animals are trained to associate a particular contextual or cue-related stimulus (CS) with an unpleasant stimuli such as an electric foot shock, acoustic stimulus or an aversive odour (US). The animal then learns to display an observable startle or freezing response to the CS independent of the presence of the US.	Increased levels of freezing behaviour and enhanced startle responses when the CS is presented in the absence of the US are used as measures of fearful behaviour.	Mice, rats	Benzodiazepines, Barbituates, Opiates, Buspirone, Ethanol, acute SSRIs	(Davis, 1990; Fendt and Fanselow, 1999; Borsini *et al.*, 2002)
Conditioned emotional response	Animals are trained to associate a specific operant, feeding or drinking behaviour (CS) with unpleasant stimuli (US).	Association of the US behaviour results with the CS results in a decrease in the US behaviour. Anxiolytic drugs reduce this effect.	Mice, rats	Benzodiazepines barbituates, opiates	(Davis, 1990; Goddyn *et al.*, 2008)
Conditioned taste aversion	Consumption of sweetened milk is paired with the injection of the malaise inducing compound lithium chloride (LiCl). Animals are then offered a free choice between consumption of water and the same sweetened milk.	Association of the sweetened milk with the LiCl-induced malaise results in a decrease in consumption of the sweetened milk. Anxiolytic drugs reduce this effect.	Rats	Benzodiazepines	(Ervin and Cooper, 1988; Yasoshima and Yamamoto, 2005)
Physiological tests					
Stress-induced hyperthermia	Mice are singly housed overnight prior to rectal temperature measurement. Temperature is measured twice at an interval of 10–15 min.	Rectal temperature measurement is used as a stressor in this procedure. The increase in temperature seen between the first and second measurements is taken regarded as a physiological anxiety response. Drugs regarded as having an anxiolytic effect in this test reduce the magnitude of this temperature increase.	Mice, rats.	Benzodiazepines, alcohol (higher doses are hypothermic), zolpidem, buspirone flesinoxan, chronic SSRI treatment	(Van Bogaert *et al.*, 2006; Bouwknecht *et al.*, 2007; Conley and Hutson, 2007; Vinkers *et al.*, 2008)
Autonomic telemetry	Electrocardiogram transmitters are surgically attached to the mouse, allowing remote monitoring of heart rate, body temperature and locomotor activity	The autonomic response to stressful stimuli, such as heart rate, blood pressure, body temperature and locomotor activity, can be measured and recorded in freely behaving animals.	Mice, rats	Diazepam	(van Bogaert *et al.*, 2006)

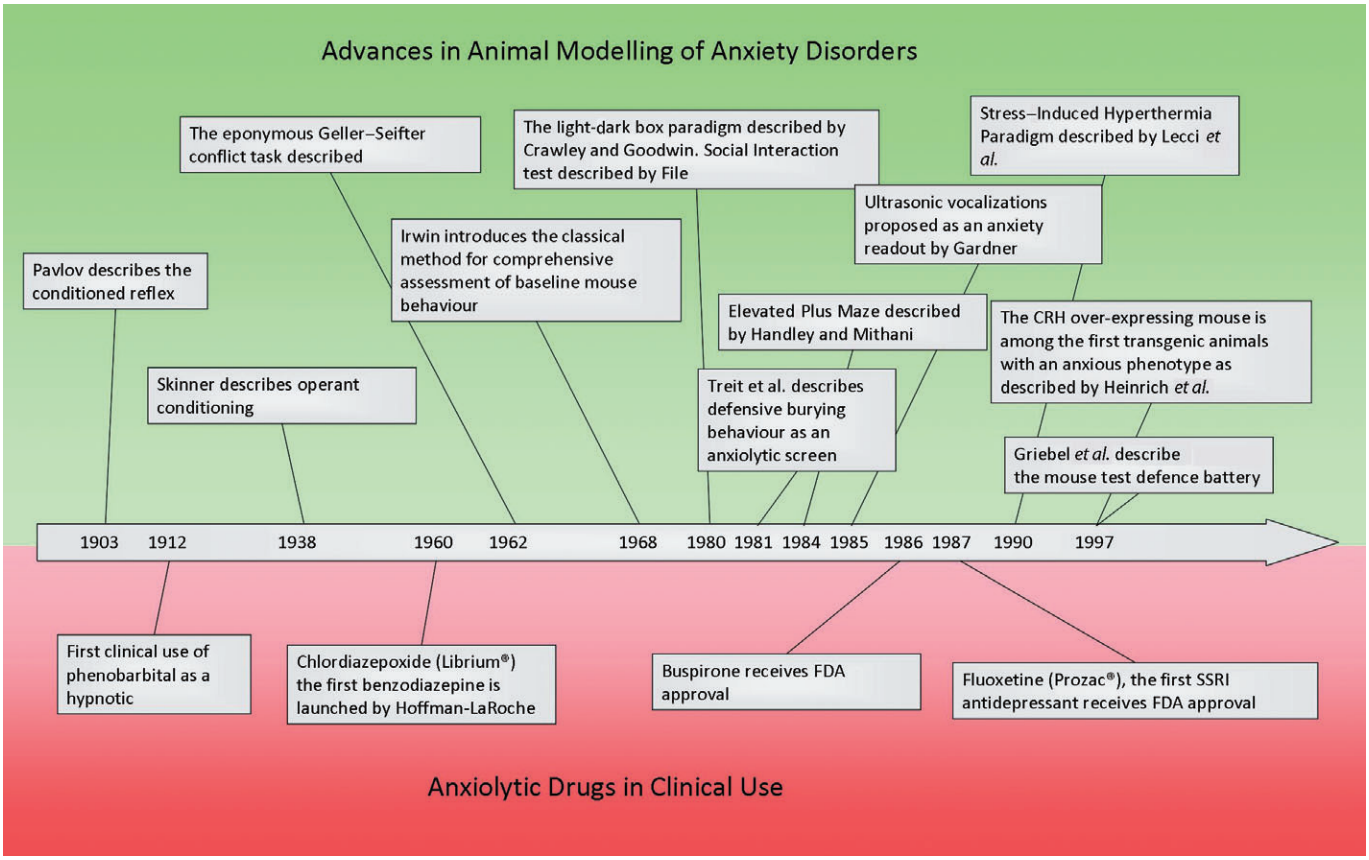

Figure 1

Advances made in the modelling of anxiety disorders in humans compared to the introduction of novel anxiolytic drug classes across the past century. Clinical drug development clearly lags behind the development of novel techniques to model anxiety in animals. A novel class of anxiolytic drug has not entered the market since the approval of fluoxetine, the first SSRI, in 1987, despite numerous advances in the preclinical modelling of anxiety disorders.

behavioural tests (Rodgers, 2010). Development of novel genetic animal models has proven invaluable not only in this regard, but in the dissection of neurobiological basis of anxiety behaviour and in indicating potential therapeutic avenues for treatment of anxiety disorders (Jacobson and Cryan, 2010). Because the demonstration of an anxious phenotype in the corticotrophin-releasing-hormone over-expressing mouse (Heinrichs *et al.*, 1997), knock-out and transgenic mice have played a vital role in both understanding the *in vivo* function of putative drug targets and now represent the definitive target validation strategy. More sophisticated techniques, such as tet-on/off and Cre-lox mediated gene expression systems, as well as siRNA-mediated gene knock down allow temporal and regionally specific control of gene expression in the brain, making transgenic mice an even more useful tool for drug discovery (Gross *et al.*, 2002; Heck *et al.*, 2004; Cryan and Holmes, 2005; Jacobson and Cryan, 2010). Genetic models of anxiety behaviour are listed in Table 3 (Finn *et al.*, 2003). Combining these genetic techniques with endophenotype-based, translationally valid animal models is a central strategy in overcoming the challenges inherent in developing novel treatment strategies for anxiety; however, it is not without its caveats. The genetic models of anxiety listed in Table 3 display anxious phenotypes in some reports, but display phenotypes of reduced or unaltered anxiety in other cases. A prominent example is the GAT1 KO mouse which has been shown to display an anxious phenotype in the open field (Chiu *et al.*, 2005), but also decreased levels of anxiety in several measures of anxiety when generated on a different genetic background (Liu *et al.*, 2007). Background strain can play a highly influential role on the behavioural effects of genetic alterations (Crawley *et al.*, 1997), and is perhaps most notable in genetic models where animals of the 129 strain are used in the generation process. In many cases, anxious behaviour may be more associated with the use of 129 strain mouse during the generation process than the genetic modification itself (Crawley *et al.*, 1997; Võikar *et al.*, 2001; Cook *et al.*, 2002; Eisener-Dorman *et al.*, 2010). This may partly explain the surprisingly extensive list of mutations that result in an anxious phenotype propelling the question as to why so many mutant mice are unhappy (Holmes and Cryan, 2006). Other possibilities include that the test used are conceptually attractive, easy to construct and carry out with minimal requirement for extensive training of either mouse or experimenter (Holmes and Cryan, 2006).

Manipulation of the early life experience of an animal represents an important avenue by which anxiety can be

Table 2

Novel anxiolytic targets

Brain Target	Drug Action	Sample Compound	Behavioural Effects in animal models	Comments	References
GABA					
$GABA_A$ receptors	α2 subunit specific agonists	MRK-409	Anxiolytic effects in rat elevated plus maze, conditioned startle response and punished drinking paradigms, as well as conditioned emotional response paradigm in primates	The safety and tolerability of MRK-409 were examined in humans and it was discovered to possess sedative properties, not observed in preclinical studies. Development was abandoned.	(Atack *et al.*, 2011)
$GABA_B$ receptors	Positive allosteric modulators	GS39783	Anxiolytic effects in the light–dark box, elevated plus maze, elevated zero maze and stress-induced hyperthermia paradigm		(Cryan *et al.*, 2004; Cryan and Kaupmann, 2005)
Glutamate					
NMDA receptor	Partial agonists	D-cycloserine	Facilitates fear extinction in rodents	Effective in reducing conditioned fear responses in acrophobic patients	(Ressler *et al.*, 2004; Amaral and Roesler, 2008)
$mGluR_{2/3}$	Agonists	LY354740, LY314582, APDC	Block expression of fear potentiated startle in rats, anxiolytic effects in the EPM in mice and rats, anxiolytic effects in mouse and rat conflict tests, attenuates lactate-induced panic in rats, anxiolytic in the SIH paradigm reduces startle response following drug of abuse withdrawal in rats.	LY544344, a prodrug of LY354740 with improved oral bioavailabilty, has shown efficacy and tolerability in human GAD patients. Discontinued.	(Monn *et al.*, 1997; Helton *et al.*, 1998; Benvenga *et al.*, 1999; Kłodzińska *et al.*, 1999; Spooren *et al.*, 2002; Dunayevich *et al.*, 2008)
$mGluR_2$	Positive allosteric modulators	4-MPTTS, CBiPES, ADX71149	Block expression of fear-potentiated startle in rats and has an anxiolytic effect in the rat SIH paradigm	ADX71149 is in phase 2 clinical trials	(Johnson *et al.*, 2005, Addex company website)
mGluR4	Agonists	LSP1-2111	Anxiolytic effects in the EPM and reduces the SIH response in mice. Reduction in the SIH response in diminished in 5HT-depleted animals.		(Wierońska *et al.*, 2010)
mGluR5	Antagonists	MPEP, MTEP	MPEP has anxiolytic effects in the Geller–Seifter test, the Vogel punished drinking test, conditioned emotional response paradigm, as well as in the social interaction test, defensive marble burying, the elevated plus maze and the SIH paradigm		(Kuhn *et al.*, 2002; Spooren and Gasparini, 2004; Ballard *et al.*, 2005; Varty *et al.*, 2005)
mGluR7	Agonists	AMN082	Anxiolytic effects in the four-plate test and reduces the SIH response in mice. Facilitates extinction of amygdala-dependent fear conditioning and inhibits acquisition of Pavlovian fear conditioning.		(Fendt *et al.*, 2008; Stachowicz *et al.*, 2008)

Table 2

Continued

Brain Target	Drug Action	Sample Compound	Behavioural Effects in animal models	Comments	References
mGluR8	Agonist/positive allosteric modulator	DCPG (agonist) AZ12216052 (Positive allosteric modulator)	Anxiolytic effects in the elevated zero maze and acoustic startle paradigm (positive modulator only)		(Duvoisin *et al.*, 2010)
Serotonin					
5-HT_7 receptor	Antagonist	SB269970	Anxiolytic in the elevated plus maze, Vogel punished drinking and four-plate test		(Wesolowska *et al.*, 2006)
5-HT_{1A} receptor	Antagonists	WAY100635, p-MPP, ISL88.0338	Anxiolytic in the mouse defensive test battery		(Griebel *et al.*, 1999)
5-HT_{2C} receptor	Antagonists	S32006	Anxiolytic in the defensive marble-burying paradigm, Vogel punished drinking and social interaction tests		(Dekeyne *et al.*, 2008; Wacker and Miller, 2008)
Neuropeptide systems					
CRH_1 receptor	Antagonists	Antalarmin, pexacerfont, GSK561679, CP154-526	Anxiolytic effects across a wide range of behavioural tests and between a variety of species.	Pexacerfont failed in to improve anxiety symptoms in generalized anxiety disorder in a randomized control trial. GSK561679 is currently undergoing clinical trials for anxiety disorders.	(Kehne & De Lombaert, 2002; Takahashi, 2001; Coric *et al.*, 2010)
CRH_2 receptor	Antagonists	Anti-savagine-30	Anxiolytic effects in the elevated plus maze, conditioned freezing and defensive behaviour.		(Takahashi, 2001)
NK_1 receptor	Antagonists	Vestipitant	Anxiolytic effects on fear-induced foot tapping and social interaction in gerbils, USVs in guinea pigs, punished drinking and fear induced USVs in rats and marble-burying behaviour in mice.	Vestipitant is currently in phase II clinical trials in combination with paroxetine for social anxiety disorder.	(Ebner and Singewald, 2006; Brocco *et al.*, 2008; Ebner *et al.*, 2009) (http://www.clinicaltrials,gov)
Translocator protein (18 kD)	Neurosteroid production enhancement	XBD173	Anxiolytic effects in the rat social interaction test and elevated plus maze. Inhibits sodium lactate and CCK-4 induced panic in rats.	Inhibits CCK-4 induced anxiety in healthy human volunteers.	(Rupprecht *et al.*, 2009; 2010)
Other neurotransmitter systems					
MCH_1	Antagonists	SNAP –7941, TPI 1361–17	Increases social interaction in the social interaction test. TPI 1361–17 has an anxiolytic effect in the EPM and light–dark box.		(Doggrell, 2003; Shimazaki *et al.*, 2006; Lee *et al.*, 2010)

Table 3

Genetic and Environmental Models of anxiety (See also Finn *et al.*, 2003)

Model	Description	Anxiety-like behavioural phenotype	Reference
Genetic models			
Selective breeding			
BALB/c mouse	Inbred mouse strain	High Levels of anxious behaviour in the open field, enhanced conditioned fear learning and greater levels of anxious behaviour in the light–dark box.	(Crawley and Davis, 1982; Crawley *et al.*, 1997; Owen *et al.*, 1997; Griebel *et al.*, 2000; Belzung and Griebel, 2001)
BTBR T + tf/J mouse	Inbred mouse strain	Anxious phenotype in the elevated plus maze, as well as increased anxiety like behaviour in measures of social interaction	(Pobbe *et al.*, 2010)
Wistar-Kyoto (WKY) rat	Outbred Rat Strain	Hypoactivity in the open-field test and are more vulnerable to stress-induced gastric ulceration than control Sprague-Dawley rats, hyper vigilant phenotype.	(Paré, 1994; McAuley *et al.*, 2009)
LAB/HAB	Outbred mouse (CD-1) or rat (Wistar) strains selectively bred for high or low anxiety.	Greater level of anxiety behaviour in the elevated plus maze and light–dark box.	(Salomé *et al.*, 2002; Kromer *et al.*, 2005; Landgraf *et al.*, 2007)
High DPAT/Low DPAT	Rat strains bred for low or high levels of responsivity to the hypothermic effects of 5-HT_{1A} receptor agonist 8-OH-DPAT	High DPAT rats display enhanced anxiety behaviour in social interaction tests and conflict tasks compared to low DPAT mice.	(Commissaris *et al.*, 2000)
Roman high-(RHA/Verh) and low-(RLA/Verh) avoidance rats	Rat strains selectively bred for good (RHA) and poor (RLA) performance in two-way, active avoidance paradigms.	RLA/Verh rats display enhanced anxiety behaviour and enhanced neuroendocrine stress response, a tendency towards a passive response to novel environments and higher levels of conditioned fear, as well as increased anxiety in the open field, light–dark box and elevated plus maze than RHA/Verh rats.	(Steimer *et al.*, 1997; Yilmazer-Hanke *et al.*, 2002; Steimer and Driscoll, 2003; López-Aumatell *et al.*, 2009)
Maudsley reactive (MR/Har) and non-reactive (MNRA/Har) rats	Rat strains bred, respectively, for high and low open field-induced defecation	MR/Har rats display a lower level of activity in the open field, a more anxious phenotype in conflict tests which is insensitive to benzodiazepine treatment, reduced exploratory behaviour to novel stimuli, enhanced startle response with reduced within-session habituation to acoustic stimulus, greater levels of stress-induced USVs (maternal separation and air puff) compared to MNRA/Har	(Commissaris *et al.*, 1989; 1992; 1996; Blizard and Adams, 2002)
129 mice	Inbred mouse strain	Impaired Pavlovian fear conditioning. 129/P3 mice fail to habituate to behavioural test of anxiety and display increased vulnerability to chronic mild stress	(Camp *et al.*, 2009; Salomons *et al.*, 2010a,b)
Fawn Hooded (FH/Wjd) rat	Inbred rat strain	The FH/Wjd rat displays lower levels of social behaviour in both anxiogenic and neutral environments than both the Wistar and the Sprague-Dawley rat.	(Kantor *et al.*, 2000)
Single-gene manipulation models			
COMT knock-out	Mice with targeted deletion of the catechol-*O*-methyl transferase gene	Increased levels of anxiety behaviour in the light–dark box seen in females only	(Gogos *et al.*, 1998)

Table 3

Continued

Model	Description	Anxiety-like behavioural phenotype	Reference
Adra2a knock-out	Mice with targeted deletion of the α2A adreno receptor gene	Increased anxiety behaviour in the open field, elevated plus maze and the light–dark box	(Schramm *et al.*, 2001; Lähdesmäki *et al.*, 2002)
5-HT_{1A} receptor KO	Mice with targeted deletion of the $5HT_{1A}$ receptor gene	Increased anxiety behaviour in the open field, elevated plus maze, elevated zero maze, novelty-suppressed feeding and novel object exploration paradigms, as well as increased fear responses to contextual cues in a fear-conditioning paradigm	(Heisler *et al.*, 1998; Ramboz *et al.*, 1998; Gross *et al.*, 2000; Klemenhagen *et al.*, 2006)
Early life 5-HT_{1A} receptor KO (P4-21)	Mice where the 5-HT_{1A} receptor expression is conditionally ablated from postnatal days 4–21	Increased anxiety behaviour in the open field, novelty-suppressed feeding and elevated plus maze in adult life	(Gross *et al.*, 2002)
5-HTT knock-out	Mice with targeted deletion of the serotonin transporter gene	Increased anxiety behaviour in the elevated zero maze, light–dark box test, elevated plus maze, open field and a hyponeophagia paradigm	(Carroll *et al.*, 2007; Line *et al.*, 2011)
GAD65 knock-out	Mice with targeted deletion of the glutamic acid decarboxylase 65 isoform gene	Increased anxiety behaviour in the open field and in the elevated zero maze. Selective alterations in the quality of the mouse response in conditioned fear paradigms	(Kash *et al.*, 1999; Stork *et al.*, 2003)
GAT1 knock-out	Mice with targeted deletion of the GABA transporter (GAT1) gene	Increased anxiety behaviour in the open field	(Chiu *et al.*, 2005)
$GABA_A$ receptor γ_2 knock-out	Mice with heterozygous deletion of the $GABA_A$ γ_2 receptor subunit gene	Increased anxiety behaviour in the open field, elevated plus maze, light–dark box and free choice exploration	(Crestani *et al.*, 1999; Chandra *et al.*, 2005)
$GABA_A$ receptor γ_{2L} knock-out	Mice with targeted deletion of the $GABA_A$ γ_{2L} receptor subunit gene	Anxious phenotype in the elevated plus maze	(Homanics *et al.*, 1999)
$GABA_B1$ receptor subunit knock-out	Mice with targeted deletion of the $GABA_B1$ receptor subunit gene	Panic-like response in the elevated zero maze, as well as increased anxiety behaviour in the light–dark box and staircase test	(Cryan and Kaupmann, 2005)
$GABA_B2$ receptor subunit knock-out	Mice with targeted deletion of the $GABA_B2$ receptor subunit gene	Increased levels of anxiety in the light–dark box	(Mombereau *et al.*, 2005)
CRH over-expression	Transgenic mice which over-express the CRH gene	Increased anxiety in the open field, elevated zero maze and elevated plus maze	(Heinrichs *et al.*, 1997; van Gaalen *et al.*, 2002)
Early life CRH over-expression	Transgenic mice in which CRH is transiently over-expressed from postnatal days 0–21	Increased anxiety behaviour in the open field and light–dark box tests in adult life	(Kolber *et al.*, 2010)
Urocortin knock-out	Mice with targeted deletion of the urocortin gene	Increased anxiety in the open field and the elevated plus maze	(Vetter *et al.*, 2002)
CRH-BP knock-out	Mice with targeted deletion of the corticotrophin-binding protein gene	Increased anxiety phenotype in the open field, elevated plus maze and increases in defensive withdrawal	(Karolyi *et al.*, 1999)
APOE knock-out	Mice with selective deletion of the apolipoprotein E gene	Increased levels of anxietty behaviour in the elevated plus maze	(Raber, 2007)

Table 3

Continued

Model	Description	Anxiety-like behavioural phenotype	Reference
Otsuka Long Evans Tokushima Fatty (OLETF) rat	OLETF rat constitutively lacks the CCK_1 receptor	High levels of anxiety behaviour in the elevated plus maze, light–dark box and open field tests	(Kobayashi *et al.*, 1996; Yamamoto *et al.*, 2000)
$mGluR_5$ receptor knock-out	Mice with a targeted deletion of the $mGlu_5$ receptor gene	Increased anxiety behaviour in the elevated plus maze	(Wu *et al.*, 2007)
Desert hedgehog knock-out	Mice with a targeted deletion of the desert hedgehog gene	Enhanced anxiety behaviour in the Vogel-punished drinking test	(Umehara *et al.*, 2006)
TSC-DN mice	Mice dominant negative for the tuberous sclerosis-associated gene TSC-DN	Increased anxety behaviour in the elevated plus maze, as well as in the open field	(Ehninger and Silva, 2010)
APP transgenic mice	Transgenic mice expressing mutant human β-amyloid precursor protein	Increased anxiety behaviour in the open field and light–dark box as well as greater levels of freezing in a conditioned fear paradigm	(España *et al.*, 2010)
3xTG-AD transgenic mice	Transgenic mice expressing human β-amyloid precursor protein, tau and PS1	Increased anxiety behaviour in the open field and light–dark box, as well as greater levels of freezing in a conditioned fear paradigm	(España *et al.*, 2010)
TgActbetaE mice	Transgenic mice over expressing activin E	Increased anxiety behaviour in the open field test and the elevated plus maze	(Sekiyama *et al.*, 2009)
α-CaMKII transgenic mice	Mice which over-express the Ca2+/calmodulin-dependant protein kinase (α-CaMKII)	Increased anxiety behaviour in open field, elevated zero maze, light–dark transition and social interaction tests	(Hasegawa *et al.*, 2009)
TgNTRK3 mice	Mice over-expressing the full length neurotrophin receptor TrkC	Increased anxiety behaviour in the elevated plus maze and elevated zero maze, as well as a panic reaction in the mouse defensive test battery	(Dierssen *et al.*, 2006)
TGR(ASrAOGEN)680 Rat	A transgenic rat expressing anti-sense RNA to angiotensinogen in the brain	Increased anxiety behaviour in the elevated plus maze, light–dark box and open field	(Voigt *et al.*, 2005)
Hdc knock-out mice	Mice with a targeted deletion of the histidine decarboxylase receptor	Increased anxiety behaviour in the elevated plus maze, light–dark box and open field seen in females	(Acevedo *et al.*, 2006)
SF1 knock-out mice	Mice with a targeted deletion of the steroidogenic factor 1 gene specifically in the CNS	Increased anxiety behaviour in the elevated plus maze, the light–dark box, the open field and the defensive marble burying paradigm	(Zhao *et al.*, 2008)
FMR1 knock-out mice	Mice with targeted deletion of the fragile-X-mental retardation gene 1	Increased anxiety behaviour in the mirror chamber test and in the social interaction test	(Spencer *et al.*, 2005)
Environmental models			
Maternal separation	Mouse or rat pups are separated from their mothers for brief periods during early life	Maternally separated rats display increased anxiety behaviour in the elevated plus maze in adulthood and a heightened neuroendocrine response to stress.	(Plotsky and Meaney, 1993; Wigger and Neumann, 1999)
Maternal separation with early weaning (MSEW)	Mouse pups undergo maternal separation coupled with early weaning	Mice that have undergone MSEW display an anxious phenotype in the open field and elevated plus maze tests	(George *et al.*, 2010)
Social isolation (SI) rearing	Mice pups are singly housed from weaning (3 weeks) until adult hood	SI mice have increased levels of anxious behaviour in the elevated plus maze and novel object recognition test. The SI mice additionally have a depression-like phenotype and display heightened levels of aggression	(Koike *et al.*, 2009)

Table 3

Continued

Model	Description	Anxiety-like behavioural phenotype	Reference
Chemical models			
Pentylenetetrazole (PTZ) induced anxiety	Administration of the $GABA_A$ antagonist PTZ is a highly anxiogenic stimulus. Rats can be trained to discriminate PTZ administration from saline.	PTZ induces an anxiogenic effects in the elevated plus maze, as well as in conflict test. Animals trained to discriminate between PTZ and saline display PTZ appropriated responses to predator stress, alarm pheromones and to social defeat.	(Jung *et al.*, 2002)
Sodium lactate-induced anxiety	Administration of IV sodium lactate produces a panic response in human volunteers and increases in anxious behaviour in rats	Sodium lactate infusion reduces levels of social interaction and increases levels of anxious behaviour in the elevated plus maze	(Johnson *et al.*, 2008; Shekhar *et al.*, 1996; 2010)
m-chlorophenylpiperazine (mCPP)-induced anxiety	Administration of the non-selective serotonin antagonist mCPP is a highly anxiogenic stimulus. Rats can be trained to discriminate mCPP administration from saline.	mCPP administration increases anxious behaviour in the elevated plus maze, elevated t maze, elevated zero maze, Geller-Seifter test, social interaction test and shock induced vocalizations	(Gatch, 2003)
CCK induced anxiety	Administration of cholecystokinin evokes a panic like response in rodents	CCK-8 produces a spontaneous freezing response and evokes anxious behaviour in the elevated T-maze and the elevated plus maze. CCK-4 produces a panic like reaction when injected into the dorsal periaquiductal grey area.	(Mongeau and Marsden, 1997; Netto and Guimarães, 2004; Zanoveli *et al.*, 2004; Rupprecht *et al.*, 2009)

experimentally provoked in a translationally valid manner. Studies of the human population have revealed that adult behaviour is strongly influenced by an interaction of both early life environment and genetic background (Caspi *et al.*, 2002; 2003). In an attempt to study this aspect of development, several experimental protocols have been used to induce anxiety behaviour by modifying early life environment (Plotsky and Meaney, 1993; Wigger and Neumann, 1999; Koike *et al.*, 2009; George *et al.*, 2010), neurochemical function (Ansorge *et al.*, 2004; Depino *et al.*, 2008), as well as altering early life gene expression (Gross *et al.*, 2002; Kolber *et al.*, 2010). These interventions are detailed in Table 3. Similarly to genetic models, the robustness of these environmentally induced models of anxiety varies extensively. In particular, while maternal separation has been shown to generate an anxious phenotype (Plotsky and Meaney, 1993; Wigger and Neumann, 1999) in some reports, this is not the case (Lehmann and Feldon, 2000; Millstein and Holmes, 2007; Savignac *et al.*, 2011). The results achieved here are heavily dependant on procedural factors such as the length of separation and on subject factors such as gender (O'Mahony *et al.*, 2010).

Basic concepts in animal modelling of anxiety disorders

Many of the symptoms of anxiety disorders are dependent on the processing of complex psychological and cognitive concepts that clearly cannot be measured in animals, such as 'fear of losing control or going crazy' or a 'sense of a foreshortened future'. It is thus clear from the clinical presentation of anxiety disorders that they can never be fully emulated as a syndrome in animals (Cryan and Holmes, 2005; Arguello and Gogos, 2006; Crawley, 2007). If, however, we consider the substantial conservation of genetic, neurochemical and neuroanatomical features seen across mammals (Jones, 2002; Tecott, 2003; Arguello and Gogos, 2006), as well as Darwin's observations regarding the conservation of many fundamental, behavioural and pharmacological responses between species (Darwin, 1871; 1872), theoretically, by studying the neural and genetic determinants of animal behavioural response, we can, by inference, develop our understanding of the neural and genetic basis of human behaviour under both normal and pathological states (Geyer and Markou, 2002; Cryan and Holmes, 2005; Crawley, 2007). A necessary extension of this theory is that the validity of any animal model of psychiatric disease is determined by the robustness of the diagnostic techniques used to describe the disease state in the clinic. Translational interspecies comparisons are dependent on combined advances in the fields of both human diagnostics and animal modelling, as well as developments in our understanding of behavioural, genetic and neurobiological function in healthy humans and animals (Geyer and Markou, 2002; Markou *et al.*, 2009). Likewise, novel reverse translational approaches, such as measuring human exploratory behaviour (Perry *et al.*, 2009), may provide novel ways to model anxiety disorder endophenotypes in animals.

To determine the validity of an experimental model of a neuropsychiatric endophenotype, standardized criteria such as those proposed by McKinney and Bunney (1969) for depression, and which are equally applicable to anxiety disorders, can be used. These authors suggest that animal models should bear a reasonable analogy to the human disorder in either manifestation or symptoms, induce a behavioural change that can be objectively monitored, display sensitivity to effective clinical treatments and display inter-researcher reproducibility in order to be considered valid (McKinney and Bunney, 1969). Current thinking on the validity of animal models acknowledges the existence of several types of validity, including face validity (similar symptom manifestation to the clinical condition), construct validity (similar underlying biology), predictive validity (responsiveness to clinically effective therapeutic agents), etiological validity (induced by similar stimuli as the clinical condition), convergent validity (convergent measures with other construct based models) and divergent validity (divergent measures from other construct-based models) while maintaining that the reliability and predictive validity are the most important criteria in determining the overall validity of the system (Geyer and Markou, 2002).

In the context of anxiety, it has been argued by Treit *et al*. (2010) that the validity of behavioural tests of anxiety should be based on three principles arising from the evolutionarily conserved roles the fear response plays in normal survival behaviour. Firstly, a correspondence between the behavioural fear expressions in the animal model biochemical or physiological correlates of these behaviours, and the expression of isomorphic behavioural responses in humans. Secondly, if no isomorphism is present, biological function should be conserved between the anxiety-like behaviour in the animal model and the human fear response. And thirdly, conservation of the neural mechanisms, engaged during the fear response, that underlie anxiety-related behaviour in both animals and humans (Treit *et al*., 2010).

In preclinical psychiatry research, there remains some confusion on the distinction between an animal model versus a test (see Cryan and Slattery, 2007). When describing preclinical anxiety research, it is important to try and draw a distinction between animal models of anxiety and experimental tests of anxiety (Rodgers, 2010). In general, when the term 'animal model of anxiety' is used, it refers to an animal that exhibits a phenotype behaviourally relevant to clinical anxiety disorders. When we use the term 'test of anxiety', we refer to a behavioural paradigm that induces a quantifiable fear-related behaviour related to the normal adaptive fear response (Young and Liberzon, 2002; Rodgers, 2010). We can thus say that a model comprises both an independent variable (i.e. the inducing manipulation) and a dependent variable (i.e. the behavioural/neurochemical readout) (Geyer and Markou, 2000), whereas a test simply comprises a dependent variable. Thorough clinical understanding of the underlying pathophysiology of anxiety disorders is vital to determining appropriate independent variables in preclinical research. Identification of appropriate anxiety endophenotypes has been useful in this regard (Cryan and Slattery, 2007). Tests of anxiety are often described as 'models of anxiety' based on the translationally questionable premise that anxiety disorders represent an exaggerated activation of the normal fear response, when in fact they more accurately represent models of particular behavioural endophenotypes present in anxiety disorders and indeed models of anxiolytic drug activity (Young and Liberzon, 2002; Cryan and Holmes, 2005; Holmes and Cryan, 2006). Rodgers (2010) points to the fact that the distinction between animal test and animal model in anxiety research highlights the crucial difference in the knowledge we can garner from their use in understanding the neural circuitry of anxiety. Studying the induction of fear in an animal test in a normal animal can provide insight into the neurobiology of the adaptive fear response, but may not necessarily be appropriate for investigating the dysregulated fear responses observed in anxiety disorder patients (Rodgers, 2010). It is thus important to remember that symptoms could conceivably arise from pathological processes upstream of the fear response and not from an abnormal fear response *per se*. Knowledge of the dysregulated anxiety response in humans is thus best derived from animals with a translationally relevant dysregulation of their anxiety response, evidenced by greater levels of anxiety in etiologically valid behavioural tests. This may explain why, although our knowledge of the basic fear response has become highly developed over the past number of decades, the pathophysiology of anxiety disorders remains impenetrable (Rodgers, 2010).

Human models of anxiety: translating and adapting

All the abovementioned approaches share in common the precept that the validity of an animal model of anxiety is dependent on solid understanding of the ethological manifestation of anxiety in humans. Vital to this is the pharmacological validation of several fear/anxiety/stress-provoking paradigms that can be used to mimic in humans a state similar to the symptoms experienced by anxiety disorder patients. These include generating classical conditioned fear in humans, generating anxiety via public speaking, measuring attentiveness to threatening cues using the Stroop-word colour task, as well as measuring fear-potentiated startle in humans (Graeff *et al*., 2003).

The provocation of panic attacks using cholecystokinin (CCK) is a well-characterized method for the study of anxiety in humans (Koszycki *et al*., 1991), and has proven to be of use in exploring the neurochemical (Zwanzger *et al*., 2003; Maron *et al*., 2009), genetic (Maron *et al*., 2010) and psychological (Tõru *et al*., 2010) aspects of panic disorder, as well as representing a potentially useful screen for novel anxiolytic drugs (Kellner *et al*., 2005; Kronenberg *et al*., 2005; Eser *et al*., 2007). Anxiety in humans can also be generated experimentally using chemical agents such as caffeine (Nardi *et al*., 2007), *m*-chlorophenylpiperazine (mCPP) (Kahn *et al*., 1990), yohimbine (Charney *et al*., 1984), CO_2 inhalation (Nardi *et al*., 2007), sodium lactate (Liebowitz *et al*., 1984; 1985) and isoproterenol (Pohl *et al*., 1987; Balon *et al*., 1988; Yeragani *et al*., 2007). A detailed summary of these techniques is described in Table 4. The pharmacological validation of these techniques, however, lags far well behind developments in animal modelling of anxiety. It is vital that a greater investment is made into fully validating such paradigms both in

Table 4

Modelling anxiety in humans

Test	Description	Readouts	Behavioural findings	References
Chemical				
Respiratory challenge tests (35% CO_2 induced panic/ hyperventilation induced panic/breath holding-induced panic)	Inhalation of 35% CO_2, hyperventilation and extended breath holding can induce panic attacks in vulnerable patients	Self-reporting of panic type symptoms	Patients suffering from respiratory type panic disorders are more vulnerable to panic attacks in response to these tests.	(Nardi *et al.*, 2006)
Caffeine challenge	Consumption of high doses of caffeine can induce panic attacks in vulnerable patients.	Self-reporting of panic type symptoms	GAD patients, PD patients, performance social anxiety disorder patients are particularly vulnerable to caffeine-induced panic attacks	(Lara, 2010)
Yohimbine Challenge	Administration of the α_2 adrenergic receptor antagonist yohimbine induces panic like symptoms in panic disorder patients and healthy controls	Self reported psychological and somatic panic symptoms, physiological parameters, plasma levels of the noradrenaline metabolite 3-methoxy-4-hydroxyphenylglycol	Yohimbine sensitivity correlates strongly with panic attack frequency in panic disorder patients	(Charney *et al.*, 1984)
Sodium lactate challenge	Alterations in the pH and pCO_2 induced by IV infusion of sodium lactate results in panic-like symptoms	Self-reporting of panic symptoms	Panic disorder patients are more vulnerable to isproterenol-induced panic attacks than healthy controls. The threshold for sodium lactate induced panic attacks is increased by TCA treatment	(Liebowitz *et al.*, 1984; 1985; Yeragani *et al.*, 1988)
Isoproterenol	Infusion of the β-adrenergic agonist isoproternol results in panic-like symptoms in vulnerable patients	Self-reporting of panic response, alterations in cardiac function	Panic disorder patients are more vulnerable to isproterenol-induced panic attacks than healthy controls, as well as greater sensitivity to the arrhythmic effects of the drug	(Pohl *et al.*, 1987; Balon *et al.*, 1988; Yeragani *et al.*, 2007)
mCPP	Administration of the 5-HT receptor agonist *m*-chlorophenylpiperazine results in an anxiety response, largely mediated by activation of the 5-HT_{2C} receptor	Self-reporting of anxiety symptoms, neuroendocrine stress markers	mCPP administration results in an exacerbation of symptoms in obsessive–compulsive disorder at low doses	(Kahn *et al.*, 1990; Erzegovesi *et al.*, 2001)
CCK	Administration of CCK results in an anxiety response	Self-reporting of anxiety symptoms, neuroendocrine stress markers	Patients with panic disorder are more vulnerable to the panic-inducing effects of CCK. Effects reduced by benzodiazepines and tiagabine in healthy volunteers.	(Koszycki *et al.*, 1991; Bradwejn and Koszycki, 2001; Rupprecht *et al.*, 2009; Zwanzger *et al.*, 2009)

Table 4

Continued

Test	Description	Readouts	Behavioural findings	References
Psycho-social				
Trier social stress Test (TSST)	The TSST consists of an anticipatory period and a test period where subjects must perform free speech and mental arithmetic in front of a panel of observers.	Neuroendocrine, plasma neurotransmitter and other physiological parameters can indicate the stress response to the TSST.		(Kirschbaum *et al.*, 1993; von Dawans *et al.*, 2010)
Video Recorded Stroop Colour Word Test (VRSCWT)	Subjects are presented with a cognitive task where they are challenged to name the font colour of a series of words. The performance of this test induces a stress response in humans. Attentional bias to emotionally valent and disorder-specific words can be detected.	Physiological parameters, self-reported anxiety levels, attentional bias to specific word stimuli	GAD patients display enhanced emotional bias towards emotional words, whereas social phobia patients display emotional bias to disorder specific (speech related) words. Non-patients with increased levels of self-reported anxiety also display higher emotional bias towards emotionally valent words.	(Tulen *et al.*, 1989; Williams *et al.*, 1996; Leite *et al.*, 1999; Becker *et al.*, 2001; Sass *et al.*, 2010)
Dot probe task	Subjects are presented a combination of emotional and neutral facial expressions, and are asked to identify a dot sometimes presented in combination.	Time taken to identify the presentation of the dot in combination with emotional faces can indicate an attentional bias.	Anxiety disorder patients display an attentional bias to emotional facial expressions. Diazepam can reduce this attentional bias in healthy volunteers.	(Bradley *et al.*, 1999; Murphy *et al.*, 2008)
Stabilometric analysis of freezing responses	Test subjects are shown a combination of anxiogenic, mutilation and neutral images	Stabilometry is used to detect changes in posture including freezing behaviour and physiological parameters	A freezing-type response is seen in humans confronted by a series of mutilation images. Panic disorder patients display a freezing-like behavioural response in anticipation of seeing the images.	(Azevedo *et al.*, 2005; Lopes *et al.*, 2009)
Human behavioural monitor	The test subject is introduced to a novel room containing no chairs, basic furniture and several small items likely to evoke exploration	Locomotor activity, exploratory behaviour and physiological parameters	Both bipolar mania patients and schizophrenia patients display different behavioural patterns to healthy controls.	(Perry *et al.*, 2009)
Fear-potentiated startle	Test subjects are presented with a neutral stimulus (CS) in combination with a painful stimulus (US). Startle response is then measured in response to a presentation of the CS alone.	Startle response to is measured both at baseline levels and subsequent to expose to the CS.	PTSD and panic disorder patients display enhanced baseline startle responses, as well as in the offspring of anxiety disorder patients.	(Grillon, 2002; 2008)

terms of predictive and face validity. This is one area where the pharmaceutical industries must combine with clinical and basic scientists to really invest substantially in such research (Conn and Roth, 2008). Moreover, with anxiety, we have a clear advantage over depression drug development with the very fact that fear can be relatively easily induced. Moreover, advances in neuroimaging and neurophysiology are unravelling clear circuits that are involved in various anxiety disorders (Schiller and Delgado, 2010; Schiller *et al.*, 2010). The ability of pharmaceutical agents or psychological methods to reverse the patterns of neuronal function associated with anxiety disorders is also at a very limited state, but is a very attractive avenue for future innovation (Murphy, 2010). Moreover, developing this approach to modelling clinical anxiety in humans will greatly facilitate early proof of concept clinical trials.

Animal models and tests used in assessing anxiolytic action

By far, the most commonly used species in preclinical anxiety research are the mouse (*Mus musculus*) and the rat (*Rattus norvegicus*), although as noted early studies in dogs and pigeons have had their use. Traditionally, rats have been the species of choice for behavioural pharmacology due to the practical considerations of their size and amenity to surgical intervention, as well as superior cognitive ability and superior performance in operant and cognitive tasks. Many commonly used behavioural paradigms were initially developed and validated as screens of anxiolytic activity in the rat before adaptation to use with other species (Cryan and Holmes, 2005). The development of novel genetic modification techniques, developed most extensively in the murine models, has led to a surge in the popularity of the mouse in neuropsychiatric research. The mouse additionally has the advantages as regards ease of breeding, low cost, short generation turnover and smaller size from a drug-dosing perspective (Joyner and Sedivy, 2000; Tarantino and Bucan, 2000; Tecott, 2003; Cryan and Holmes, 2005; Crawley, 2007; Jacobson and Cryan, 2007; Phillips *et al.*, 2007). However, this has brought with it its own logistical problems in terms of difficulty in combining blood collection for pharmacokinetic–pharmacodynamic studies or biomarker analysis. Moreover, the enormous interstrain difference in mouse behaviour across many anxiety tests both under baseline conditions and in response to pharmacological manipulation (Jacobson and Cryan, 2010) can make interpretation of data difficult. The question which invariably arises as to which mouse strain is most like human is not an easy question to try and answer. Thus, it is becoming clear that testing of putative anxiolytic drugs requires testing across multiple strains (and species if possible) to ensure the risk of a false negative. It should be noted also that the manner in which a rodent responds to an anxiety-provoking situation may be qualitatively different to that of humans, but it is becoming clear that many of the same neuronal circuits are recruited (Singewald, 2007). Often, efforts at developing of translational models of anxiety are interpreted as forming completely homologous models in both humans and rodents; while this may be possible in certain domains [e.g. startle response, stress-induced hyperthermia (SIH)], it also may be a very narrow approach and disregards the ethological and species-specific aspects of mouse behaviour (Rodgers *et al.*, 1997). In the next section, we will detail some of the more widely used animal tests for assessing anxiolytic action, which are additionally summarized in Table 1.

Approach–avoidance tests in laboratory animals

Several forms of anxiety test have been employed and validated to measure levels of anxiety in rodents, many of which are designed based on the concept that anxiety disorders represent extreme states of a continuum of anxiety-related behaviour (Cryan and Holmes, 2005). Many tests have an ethological foundation based on the conflict that exists in small rodents, such as rats and mice, between the natural exploratory drive in these animals and aversion to exposed brightly lit environments (Rodgers, 1997). These models emerged over the past 40 years or so and relied on an ethological approach to understanding anxiety as opposed to the pharmacological approaches used in the development of drugs such as the benzodiazepines (see above). Behavioural paradigms based on approach–avoidance conflict include the elevated plus maze (Handley and Mithani, 1984; Pellow *et al.*, 1985; Lister, 1987; Rodgers, 1997; Holmes, 2001; Crawley, 2007), elevated zero maze (Lee and Rodgers, 1990; Shepherd *et al.*, 1994), open-field test, light–dark box test (Crawley, 2007), staircase test (Simiand *et al.*, 1984) and mirrored arena (Rodgers, 1997; Rodgers *et al.*, 1997; Belzung and Griebel, 2001; Crawley, 2007) where avoidance of exposed, brightly lit or elevated areas is measured. The modified hole-board test combines the approach–avoidance aspects of the open field with the addition of board containing several holes which allows for the direct measurement of exploratory behaviour (Ohl *et al.*, 2001a,b). Within these approach avoidance procedures, several species-specific behaviours and postures are quantified and used as behavioural readouts. Reductions in these 'ethological parameters' such as head dipping over the edges of elevated apparatuses, rearing and stretch-attend postures regarded as a manifestation of increased anxiety (Shepherd *et al.*, 1994; Rodgers, 1997; Rodgers *et al.*, 1997; Belzung and Griebel, 2001). Ethological analysis is taken to its extreme in the measurement of mouse risk assessment, flight and defensive attack behaviour following threat cue exposure in the mouse defence test battery (MDTB) (Blanchard, 2003). Apprehension and heightened levels of vigilance are frequently a component of anxiety disorders, and measurement of risk assessment behaviour, indexed by relevant ethological parameters, is regarded as a model of this endophenotype (Rodgers, 1997; Blanchard, 2003; Cryan and Holmes, 2005).

Although the constructs underling each of these approach–avoidance tests, it is important to emphasize that the pharmacology and underlying neurobiology are not necessarily identical. To add to the complexity, large species and strain differences occur. Thus, it is very difficult to define which test is the best to model human anxiety responses. This necessitates the use of a battery-style approach for assessing novel pharmacological agents. However, questions always emerge if a compound is showing an anxiolytic effect in more tests, is it going to be more effective in the clinic? The recip-

rocal experiences of researchers with SSRIs [very little activity (Borsini *et al.*, 2002)] and NK1 receptor antagonists [activity in a number of tests (Varty *et al.*, 2002; Vendruscolo *et al.*, 2003; Heldt *et al.*, 2009)] would suggest not.

Conflict-based anxiety tests in laboratory animals

Conflict-based models have been among the most sensitive to GABAergic manipulation and have been played an important role in assessing anxiolytic potential. Since the time of Sigmund Freud, many theories have been introduced to explain the relationship between anxiety and internal conflict (Sato, 2005), especially in relation to psychodynamic theories. Freud (1966) discussed internal conflict in relation to the three structures of the mind. Anxiety according to this view is caused by the psychic tension among the forces representative of the id, ego and superego. Another commonly discussed theory concerning the relationship between internal conflict and anxiety is Alfred Adler's (1954) theory of inferiority. In his work, Adler discussed the process of how our primary internal conflicts are caused by various feelings of inferiority in great detail. Adler suggests that these feelings of inferiority are also assumed to be one of the common causes of anxiety. Sato (2005) describes conflict situations within a framework that when something is consistent with our desires, we feel comfortable. When something is inconsistent with our desires, we feel anxiety. Therefore, internal conflict can be conceptualized using two constructs: (i) what we desire; and (ii) what has, is or could happen. When what we desire matches what has, is or could happen, we feel comfortable. When what we desire does not match what has, is or could happen, we feel anxiety. As is evident from the way the model is worded, this applies regardless of whether we are dealing with events in the past, present or future.

Therefore, conflict situations, in which a subject experiences two opposing impulses, are a common and clinically relevant feature of anxiety, and therefore employed in many models employed for the detection of anxiolytic agents in rodents. In conflict-based tests of anxiety in laboratory animals, subjects receive a punishment (mild electric shock) leading to suppression of a conditioned (learned) response for reinforcement (food or water) (Rodgers, 1997). Punishment-based conflict procedures have been employed for over 50 years in the identification and characterization of anxiolytic agents (Geller and Seifter, 1960). Studies demonstrated that when rats are trained to lever press for a food reward, during the 'conflict' component, responses are inhibited by concomitant, mild electric shocks. This paradigm is known as the Geller–Seifter test. Anxiolytic properties are deduced for drugs that selectively enhance punished responses in the presence of shock as compared to unpunished responses emitted in its absence. Benzodiazepines and barbiturates were initially demonstrated to exert specific anxiolytic properties active in the Geller–Seifter test, and, subsequently, many classes of potential anxiolytic agent have been characterized employing this procedure. However, major disadvantages remain: (i) the necessity for long-term (months) and daily training of subjects; and (ii) their repeated utilization. That is, exposure to drugs may modify the actions of those subsequently evaluated.

In an effort to overcome these problems, Vogel *et al.* (1971) developed a novel conflict procedure in which male rats were water deprived for 48 h and, during a test session of 3 min, drinking was punished by a mild, but aversive shock delivered via the spout of the bottle every 20 licks. Accordingly, a specific, drug-induced increase in the number of shocks taken was considered to reflect anxiolytic properties. Today, Vogel *et al.*'s (1971) test is one of the most widely used tests for assessing anxiolytic activity in rodents (Millan and Brocco, 2003).

A similar conflict procedure is the four-plate test, where the drive to explore a novel environment is conflicted with the drive to avoid floor-delivered foot shocks (Ripoll *et al.*, 2006). Defensive marble and shock-probe burying tests, where animals bury novel or aversive items, differ from other tests of anxious behaviour in that an active behaviour (i.e. burying is used as an index of anxiety as opposed to other tests relying on passive avoidance behaviour). It should, however, be noted that controversy exists as to the precise nature of the behaviour elicited in the defensive marble burying assay in mice. It has been argued that this assay may be more ethologically relevant to obsessive–compulsive disorder than to the rest of the anxiety disorders (Witkin, 2008), or may represent a species-specific repetitive and perseverative behaviour with little correlation to anxiety levels of anxiety-like behaviour (Thomas *et al.*, 2009). As such, they make valuable additions to anxiety test batteries (Broekkamp *et al.*, 1986; Sluyter *et al.*, 1996; 1999; Spooren *et al.*, 2000; Jacobson *et al.*, 2007).

Other anxiety tests in laboratory animals

Anxiolytic activity in many of the mentioned tests can be confounded by aspects of altered locomotor activity induced by genetic or pharmacological manipulations (Cryan and Holmes, 2005; Holmes and Cryan, 2006; Jacobson and Cryan, 2010). Thus, it is important to consider other tests in battery-style approaches that are less dependent on motor outputs. The following are some of the more widely used.

Hyponeophagia. Hyponeophagia, the suppression of eating due to anxiety-related states caused by novelty, can be assessed by measuring the latency to begin eating in a variety of potentially anxiogenic situations. Once again, it is a conflict-based model that has a long history in the assessment of emotionality and anxiety with Hall (1934) observing an inverse relationship between feeding and defecation in animals exposed to a novel environment. In hyponeophagia, tests the level of anxiety-related stimuli is manipulated by using novel food and by conducting the experiment in novel, potentially anxiogenic environments (Bannerman *et al.*, 2002; 2003; Deacon and Rawlins, 2005; Dulawa and Hen, 2005; Finger *et al.*, 2010). These paradigms are ethologically relevant and therefore do not require complex training procedures, are not confounded by painful stimuli, are simple to conduct and are relatively cost-effective. Hyponeophagia-based models are conducted either by presenting chow to food-deprived animals, or by presenting a highly palatable and familiar food to satiated animals, and measuring the latency to feed and/or the amount eaten in a novel environment. The same dependent measures should also be assessed

in the home environment to control for effects of the independent variable on appetite. As in all anxiety assays, inbred mouse strains show baseline differences in levels of hyponeophagia (Trullas and Skolnick, 1993). A number of genetic manipulations resulting in anxious phenotypes including leptin-deficient mice (Finger *et al.*, 2010), 5-HT_{1A} receptor (Gross *et al.*, 2000) and the NK1 receptor (Santarelli *et al.*, 2002) have also increased hyponeophagia.

Several variations of hyponeophagia-based behavioural paradigms exist. These include the novelty-suppressed feeding paradigm where animals are presented with normal food in a novel anxiogenic environment, and the latency to begin eating is taken as an index of the anxiety state of the animal (Bodnoff *et al.*, 1988; 1989; Gross *et al.*, 2002). Repeated exposures to hyponeophagia paradigms in different environments and to different food stuffs can be used to modulate the levels of anxiety generated in these paradigms in order to optimize the sensitivity of the behavioural output in this test (Deacon and Rawlins, 2005; Finger *et al.*, 2010). Another variant is the novelty-induced hypophagia model in which animals are trained to consume a highly palatable food, such as sweetened milk, and then later presented this food in a novel aversive environment (Soubríe *et al.*, 1975; Gross *et al.*, 2002; Santarelli *et al.*, 2003).

Separation-induced ultrasonic vocalizations. Rodent pups produce vocalizations in the ultrasonic range when separated from their mother and littermates. This distress behaviour is intended to elicit maternal attention and retrieval, as well as to modulate maternal care behaviour by stimulating prolactin production (Noirot, 1972; Hashimoto *et al.*, 2001; Farrell and Alberts, 2002). These distress behaviours can be recorded and analysed both quantitatively and qualitatively in order to measure levels of distress-like behaviour in both infant mice and rats (Groenink *et al.*, 2008; Scattoni *et al.*, 2009). Suppression of USV emission is an ethologically valid marker of anxiolytic drug efficacy (Groenink *et al.*, 2008). Anxiolytic effects in this test can be elicited with administration of benzodiazepines, 5-HT_{1A} receptor agonists and SSRIs. Agents acting on the noradrenergic system, such as tricyclic antidepressants, are, however, less consistent in their anxiolytic effects (Borsini *et al.*, 2002). USV reduction is also seen with administration of a range of putative anxiolytic agents, including NK1 receptor antagonists (Groenink *et al.*, 2008).

Measurement of USV production in response to painful or stressful stimuli has also been proposed as a potential measure of anxiety behaviour in adult rodents. 5-HT_{1A} receptor antagonists and SSRI antidepressants are effective at suppressing USV emission in this model, although benzodiazepines have limited effects (Sánchez, 2003).

SIH. A key element of the adaptive anxiety response is activation of the autonomic nervous system and subsequent physiological responses including an increase in body temperature. This process is conserved across mammalian species, including rodents and humans. Measurement of the hypothermic response generated subsequent to stressful stimuli represents a translationally valid and useful approach to modelling anxiety disorders (Bouwknecht *et al.*, 2007; Vinkers *et al.*, 2008). The hypothermic response to stress can be attenuated using benzodiazepines, as well as buspirone and ethanol (Spooren *et al.*, 2002), and chronic, but not acute, SSRI treatment (Conley and Hutson, 2007). The SIH paradigm is additionally sensitive to the effects of numerous putative anxiolytic agents (Spooren *et al.*, 2002), as well as providing a useful technique for exploring the role of individual neurotransmitter systems in the anxiety response (Vinkers *et al.*, 2010).

Fear conditioning-based models of anxiety

Alterations in conditioned fear learning and cognitive defects form an important facet of the clinical manifestation of anxiety disorders (American Psychiatric Association, 2000; Lang *et al.*, 2000). These include inappropriate processing of potentially threatening stimuli in GAD, panic disorder and phobias, as well as the long-term salience of traumatic memories seen in PTSD. In order to model these aspects of anxiety disorders, several conditioned tests of anxiety, such as Pavlovian fear conditioning, have been developed and validated (Cryan and Holmes, 2005; Ledgerwood *et al.*, 2005; Delgado *et al.*, 2006; O'Connor *et al.*, 2010). More recently, the discovery that insular cortex dysfunction may play a role in anxiety disorders (Paulus and Stein, 2006) has led to the insular cortex-dependent, conditioned taste aversion paradigm becoming more widely used (Bermúdez-Rattoni *et al.*, 2004; Guitton and Dudai, 2004; Mickley *et al.*, 2004; Yasoshima and Yamamoto, 2005; Jacobson *et al.*, 2006; Hefner *et al.*, 2008).

Conditioned fear paradigms revolve around the association of innocuous stimuli, such as a tone or palatable taste (conditioned stimulus), with a painful or stressful stimulus, such as a foot shock or chemically induced malaise (unconditioned stimulus). Levels of conditioned fear generated in these paradigms are indexed by a number of behavioural outputs, including conditioned freezing, fear-potentiated startle, active defensive behaviours, vocalizations, physiological responses, as well as alterations in sucrose preference in the conditioned taste aversion paradigm (Fendt and Fanselow, 1999; Cryan and Holmes, 2005). Sleep disturbances form part of the diagnostic criteria for several forms of anxiety disorder (American Psychiatric Association, 2000; Prut and Belzung, 2003), and also represent sensitive output in fear-conditioning paradigms (Sanford *et al.*, 2003a,b).

Extinction of the fear response generated in fear conditioning and conditioned taste aversion paradigms is of particular use in modelling the persistence of traumatic memories associated with PTSD and panic disorder (Ressler *et al.*, 2004; Barad, 2005; Cryan and Holmes, 2005; Ledgerwood *et al.*, 2005; Delgado *et al.*, 2006; Jacobson *et al.*, 2006).The efficacy of the NMDA receptor antagonist D-cycloserine in facilitating fear extinction in both the rat fear potentiated startle paradigm and in human acrophobic patients indicate a predictive validity for this approach (Ressler *et al.*, 2004; Ledgerwood *et al.*, 2005; Davis *et al.*, 2006). For further information, see review from Graham *et al.* (2010) in this issue.

Pharmacological validation

A major criticism of many of the behavioural tests discussed here is that while they display robust responsivity to the benzodiazepine anxiolytics and thus display predictive validity in this respect (Cryan and Holmes, 2005; Crawley, 2007), their predictive validity in respect of other classes of clinically used anxiolytic drugs, in particular SSRI antidepressants, is lacking (Borsini *et al.*, 2002). This is based on the variable effects produced by SSRI across the spectrum of anxiety tests with the notable exceptions of the USV suppression, MDTB and defensive marble burying paradigms (Rodgers, 1997; Borsini *et al.*, 2002; Blanchard, 2003; Markou *et al.*, 2009). It should, however, be remembered that many of the currently used behavioural tests were developed based on prevailing clinical practice that clearly distinguished between the anxiety disorders and depression in terms of both symptom presentation and treatment, with the gold standard of predictive validity as benzodiazepine sensitivity (Treit *et al.*, 2010). Nevertheless, a variety of putative anxiolytic compounds have shown efficacy in a variety of SSRI-sensitive behavioural tests. None, however, have made the transition to clinic as of yet (Table 2). Rodgers (2010) outlines several pitfalls in overemphasizing pharmacologically predictive validity as a marker of a valid test. Predictive validity is by its nature a retrospective assessment, a flaw in itself and that putting too much stead on it comes with the risk that active compounds will never be truly novel in their mode of action. An additional risk is that tests whose sole merit is their predictive validity for either benzodiazepine or chronic SSRI treatment may in reality be simple models of SERT or $GABA_A$ receptor pharmacology (Rodgers, 2010).

Pharmacological agents used to induce anxiety behaviour in animals include CCK (Rupprecht *et al.*, 2009), sodium lactate (Shekhar *et al.*, 1996), the serotonin antagonist mCPP (Gatch, 2003), as well as PTZ (Jung *et al.*, 2002). CCK, mCPP and sodium lactate also provoke panic-like responses in humans (Liebowitz *et al.*, 1984; Kahn *et al.*, 1990; Koszycki *et al.*, 1991), and thus are viewed as potential animal models of panic disorder. However, it should be noted that the lactate model needs the added manipulation in animals of surgically manipulating GABA in the dorsomedial hypothalamus to prime for the panic-inducing effects of lactate (Shekhar *et al.*, 1996).

Caveats in preclinical models of anxiolytics action

Alterations across the entire behavioural repertoire following pharmacological or genetic intervention can often confound the analysis of anxiety behaviour (Bouwknecht and Paylor, 2008). It is critical that such confounding behaviours are taken into account in interpreting the effects of any agent in a test. Outputs, such as freezing or exploratory behaviour, and other locomotor-based behaviours are particularly vulnerable to obfuscation by alterations in locomotor function, making the assessment of the locomotor effects of any novel intervention paramount (Cryan and Holmes, 2005; Holmes and Cryan, 2006; Jacobson and Cryan, 2007). However, some tasks, such as the elevated plus maze, have in-built parameters that take into account any locomotor-altering effects of a drug (Hogg, 1996; Rodgers *et al.*, 1997). Disruption of cognitive function by non-specific drug actions can disrupt learning-dependant and exploration-dependant behavioural outputs, confusing the analysis of data from assays of both innate and conditioned anxiety (Jacobson *et al.*, 2007; Bouwknecht and Paylor, 2008). Alterations to the sensory system of the animal can have marked effects where nociception (foot shock-based paradigms) or olfactory (conditioned taste aversion) function is vital to proper responsiveness to the test. Alterations to basal temperature can confound results obtained from the SIH paradigm (Vinkers *et al.*, 2008), whereas alterations to feeding behaviour and satiety function can influence behaviour in hyponeophagia paradigms (Dulawa and Hen, 2005).

Similar advice is needed for the assessment of genetically modified animals in tests of anxiety, and is vital in avoiding erroneous interpretations of behavioural data. A thorough determination of any confounding abnormalities present in genetically modified animals prior to behavioural testing is vital (Crawley, 2007). Other factors that can influence behaviour in anxiety tests are early life experience, previous test exposure and compensatory changes in the case of transgenic animals (Cryan and Holmes, 2005; Holmes and Cryan, 2006; Jacobson and Cryan, 2007). Use of a test battery encompassing multiple anxiety tests exploring different aspects of anxiety behaviour has thus been suggested as an approach to detect genuine behavioural effects (Cryan and Holmes, 2005; Arguello and Gogos, 2006; Bouwknecht and Paylor, 2008). It must be remembered, however, when designing such a battery, as prior test experiences can influence behaviour and markedly alter responsivity to pharmacological agents in a number of behavioural paradigms (Holmes and Rodgers, 1998; Holmes *et al.*, 2001). Battery approaches where tests used progress from least stressful to most stressful, while considering which tests are more vulnerable to the effects of prior testing, and which allow for sufficient recovery time between tests, can overcome this potential caveat (Bouwknecht and Paylor, 2008).

Conclusions and future directions

Despite the present dizzying array of behavioural paradigms for use in anxiolytic drug discovery, recent advances in animal modelling are yet to translate into novel pharmacological therapies in the clinic. A multitude of reasons may underlie this apparent stagnation. These include concerns as to the ethological and predictive validity of the techniques at our disposal. The apparent insensitivity of many of the tests to commonly used clinical anxiolytics, such as the SSRIs, is a major concern (Borsini *et al.*, 2002), and questions still linger as to the fact that the maintenance of anxiety disorders is ultimately based on cognitive processes only present in humans, as evidenced by the efficacy of cognitive treatments in humans (Steckler *et al.*, 2008). In order to advance the efforts in drug discovery, we must both re-assess our concepts of validity (Treit *et al.*, 2010). It is also important to remember that our animal models are only as valid as the knowledge of the neurobiology and pathophysiology of clinical anxiety disorders that underpin their design.

With this in mind, it is clear that animal models will play a role in the emergence of the next anxiolytic drug class, but only as a component of a broad multidisciplinary approach where advances in animal models are informed by insight into pathophysiological basis, as well as the neurobiological and behavioural correlates of the clinical disease. A recent example is that shown by Rupprecht *et al*. (2009), where *in vitro* electrophysiology, animal behavioural experiments and human studies are combined in the investigation of novel therapeutic avenue for panic disorder, translocator protein (18 kD) ligands.

Indeed, at a molecular and electrophysiological level, great inroads are being made into delineating the circuits underlying amygdala-dependent fear memory (Phelps and LeDoux, 2005; Sigurdsson *et al*., 2007; Herry *et al*., 2008; Ehrlich *et al*., 2009; Davis *et al*., 2010; Haubensak *et al*., 2010; Pape and Pare, 2010; Parsons and Davis, 2011; Sierra-Mercado *et al*., 2011), which are already being paralleled with imaging studies in humans (Phelps and LeDoux, 2005; Davis *et al*., 2010). It is only appropriate to mention here that despite the failings of animal research in generating a new anxiolytic drug class to date, it has proven a highly successful platform for advancing our knowledge of the neurobiology of anxiety and fear (Rodgers, 2010). The next step will be to advance such basic neuroscience approaches into clinical drug development. Interestingly, and for reasons not apparently clear, similar approaches in non-cognitive-based models have failed to advance in the same manner. If there is a continued reliance on behavioural outputs in such models for drug discovery efforts, it is crucial that knowledge is gleaned on how certain anxiolytic work (or not) in them. The combination of behaviour with imaging techniques, such as c-Fos immunohistochemistry, is becoming more sophisticated and will also play a role in the future delineation of anxiety circuits in the brain (Reijmers *et al*., 2007; Singewald, 2007).

In conclusion, animal models have played a role in the development of some anxiolytic drugs, such as the benzodiazepines and buspirone; however, their relative contribution to future drug development will only be accentuated as part of a complete research program combining genetic signalling pathways, electrophysiology, brain neurochemistry, neuroimaging and behaviour. For this approach to bear fruit, there is also an onus on preclinical researchers to ensure that novel clinical insights into the aetiology of anxiety disorders appropriately inform both the design and use of animal models and tests (Rodgers, 2010). It is imperative to state that in addition to refining the predictive efficacy of the animal tests used in anxiety research, preclinical scientists are impressed on ethical grounds to actively innovate in replacement of current models and tests with lower species or non-animal techniques, refinement of procedures in order to minimize animal suffering and reducing the number of animals required to generate data – the 3*R*s (Goldberg *et al*., 1996). Moreover, while we clearly point out some of the drawbacks of animal tests of anxiolytics action in this review, it would be remiss not to mention the impediment to preclinical research posed by the lack of conceptual clarity and stability at the clinical level. Reverse translation, from clinic to animal model in psychiatry, is not yet as fully developed as it should, and this will be crucially important in determining the true translational nature of animal models and aid in the development of novel treatment strategies that will counter the vast health problems in this age of anxiety.

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Conflict of interest

The authors declare no conflict of interest.

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