50 years of hurdles and hope in anxiolytic drug discovery

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Abstract

Anxiety disorders are the most prevalent group of psychiatric diseases, and have high personal and societal costs. The search for novel pharmacological treatments for these conditions is driven by the growing medical need to improve on the effectiveness and the side effect profile of existing drugs. A huge volume of data has been generated by anxiolytic drug discovery studies, which has led to the progression of numerous new molecules into clinical trials. However, the clinical outcome of these efforts has been disappointing, as promising results with novel agents in rodent studies have very rarely translated into effectiveness in humans. Here, we analyse the major trends from preclinical studies over the past 50 years conducted in the search for new drugs beyond those that target the prototypical anxiety-associated GABA (γ-aminobutyric acid)–benzodiazepine system, which have focused most intensively on the serotonin, neuropeptide, glutamate and endocannabinoid systems. We highlight various key issues that may have hampered progress in the field, and offer recommendations for how anxiolytic drug discovery can be more effective in the future.

Anxiety disorders are chronic, disabling conditions that impose enormous costs both on individuals and on society[1–5]. These disorders are the most frequently diagnosed neuropsychiatric diseases in Western countries. According to a recent 3-year multi-method study covering 30 European countries and a population of 514 million people, anxiety disorders had the highest 12-month prevalence estimates (a total of 14%) compared to all other psychiatric conditions[2].

There are currently seven recognized anxiety syndromes: panic disorder, agoraphobia, social anxiety disorder (SAD), generalized anxiety disorder (GAD), specific phobias, obsessive compulsive disorder (OCD) and post-traumatic stress disorder (PTSD) (TABLE 1). However, it should be borne in mind that the categorization of anxiety disorders is constantly evolving and very recently changed with the pending revision of the Diagnostic and Statistical Manual of Mental Disorders. There has also been renewed debate about the validity of imposing strict categorical boundaries between neuropsychiatric disorders; some

authors have argued that these boundaries fall along a dimensional spectrum[6,7]. The ever-changing diagnostic landscape clearly complicates attempts to model and develop drugs for specific disorders. This may be compounded by failings in the design of the clinical trials for novel anxiolytics. Although it is beyond the scope of our expertise to adjudge the fidelity of clinical trials, other authors have critically analysed whether trials for anxiolytics have been optimally designed to detect a reasonable efficacy of novel treatments for mood and anxiety disorders[8].

The other widely discussed issue that confounds neuropsychiatric drug discovery is the lack of an adequate account of the pathogenic mechanisms underlying neuropsychiatric conditions such as anxiety disorders. Although there has been a growing appreciation of how emotional disorders result from a combination of genetic and environmental risk factors[9], identifying reliable biochemical biomarkers or genetic variants that can be used to diagnose anxiety disorders and help predict treatment outcomes remains a major challenge[10].

Beyond these issues, the key challenge of this field ultimately remains the identification of new medications that are devoid of the limitations in efficacy and tolerability that characterize existing anxiolytics. Drugs that act on the prototypical anxiety-associated GABA (γ-aminobutyric acid)–benzodiazepine system have been a benchmark for anxiolytics since their discovery in the mid-1950s and, as discussed briefly below, efforts have been made to develop new compounds that target this system (for a review, see REF. 11). The strong need for new, alternative treatments for anxiety has also fuelled the generation of a vast amount of preclinical data on agents targeting other neurotransmitter systems and led to the advancement of many drugs from the laboratory to the clinic. FIGURE 1 shows the major trends over the past 50 years, involving more than 10,000 experiments on nearly 1,500 novel drugs (for a full list, including the drug, preclinical model, results and references, see Supplementary information S1 (box)). This analysis illustrates the steady increase in preclinical anxiety research from the 1980s onwards, leading to a peak in activity around the end of the 1990s and a robust ongoing effort up to now.

As gauged from the number of preclinical experiments conducted over the past 50 years, four other neurotransmitter systems beyond the GABA–benzodiazepine system stand out as being a principal focus of anxiolytic drug discovery research. Owing to the remarkable success of the selective serotonin reuptake inhibitors (SSRIs) as anti-anxiety treatments, the 5-hydroxytryptamine (5-HT; also known as serotonin) system has received much attention and accounts for more than half of all preclinical studies. Neuropeptides, in particular corticotropin-releasing factor (CRF), cholecystokinin (CCK) and the tachykinins, have also been intensively studied and comprise a further one-third of the studies. In addition, in recent years there has been an increase in preclinical research on the anxiety-related properties of the glutamate and endocannabinoid systems.

Despite this intense preclinical research effort to find new anxiolytics, the field has largely been perceived as a failure. In this Review, we assess the current state of anxiolytic drug discovery at this critical juncture. To provide some context to the preclinical literature, we

first introduce the tests and models of anxiety-like behaviours that have been most commonly used to identify and evaluate novel anxiolytic agents. We then turn to the main aim of this Review, which is to analyse a database comprising virtually all published preclinical studies over the past 50 years using animal models to identify novel anxiolytic drugs beyond those that target the GABA–benzodiazepine system. We focus on the most comprehensively studied neurotransmitter systems: the serotonin, neuropeptide, glutamate and endocannabinoid systems. After reviewing this literature, we highlight some of the key issues that may have hampered progress and offer recommendations for how anxiolytic drug discovery could be improved in the future.

Preclinical measures of anxiety

Numerous preclinical tests for anxiety have been developed, and the specifics of these tests have been described in many comprehensive reviews[12–14]. Here, we only briefly introduce the most frequently used tests (FIG. 2; TABLE 2) to illustrate the strengths and weaknesses of current approaches.

One general consideration from the outset is validity. The validity of a test for anxiety in an animal rests on three criteria: face validity (does it measure something analogous to one or more human anxiety symptoms?), predictive validity (is it reliably sensitive to clinically efficacious anxiolytics?) and construct validity (does it involve some of the same pathophysiological mechanisms found in human anxiety disorders?)[15]. None of the currently available tests or models of anxiety (see below) can be said to unequivocally meet these criteria.

Approach-avoidance conflict tests — a group of tests that have been a mainstay of preclinical anxiety research for many years[16] — assay anxiety-like behaviour in rodents by generating a conflict between a drive to approach novel areas and, simultaneously, to avoid potential threat therein. These simple tests, which include the well-known novel open-field test, the elevated plus-maze test and the light/dark exploration test, were invented in the 1980s to exploit the natural tendency of rats[17] and mice[18,19] to prefer enclosed areas over exposed and/or elevated places. Among the different anxiety disorders, the tests are thought to most closely model GAD and specific phobias, largely based on their perceived face validity and sensitivity to benzodiazepine anxiolytics. These tests have been used in nearly 4,000 drug discovery experiments and continue to be very popular. Indeed, well over half of the rodent-based experiments on anxiety-related drugs have used one or more of these tests; among them, by far the most commonly used ones have been the elevated plus-maze test and the light/dark exploration test.

The term 'conflict-based test' is also often used to describe measures of behaviour suppression by mild electric shock. This group includes the Vogel conflict test[20] and the Geller-Seifter[21] test, which measure anxiolytic-like activity via the maintenance of a behavioural response (for example, licking or bar pressing) despite the receipt of a shock. These putative GAD-related tests were part of many drug discovery programmes in the 1980s and 1990s but have since fallen out of favour, perhaps because they require animals to

be trained over multiple days and are more labour-intensive and time-consuming than the approach-avoidance tests.

Some anxiety tests have been designed to tap into the fundamental defensive responses shown by animals in the face of immediate danger. Such defensive or 'fear' behaviours can be conceptually distinguished from the anxiety states produced by less imminent and more ambiguous threats[16], and may be most relevant to anxiety disorders such as panic disorder and PTSD. For example, the 'Mouse Defense Test Battery' (MDTB) was designed to provide multiple measures related to fear and anxiety, based on observations of how wild rodents respond to danger[22]. In this task, mice are placed in an oval runway and tested for their responses (fight, flight, freeze, vocalize or scan) to an approaching anaesthetized rat (a natural predator). Specific behavioural measures in the MDTB are sensitive to specific classes of anxiolytic medication. For example, benzodiazepines that are effective in GAD reduce mouse risk assessment, whereas serotonergic agents that are efficacious in panic disorder and PTSD attenuate fight and flight behaviours[23]. In spite of these promising results, however, the MDTB has not been widely adopted, again probably owing to the training and technical demands involved. As a practical compromise, researchers have incorporated measures derived from the analysis of defensive behaviours, such as risk assessment, into anxiety-related tests such as the elevated plus-maze test; in some cases, this has resulted in improved sensitivity to certain anxiolytic drug classes[24].

Another set of fear-based tests that are relevant to PTSD and specific phobias involve variations on classical Pavlovian fear conditioning. Here, an animal learns to associate a context or a specific environmental stimulus (for example, a light or a sound) with electric shock to produce a conditioned fear response that can be quantified in various ways (for example, freezing, escape, avoidance or startle). Studies of Pavlovian fear conditioning have contributed greatly to our understanding of the basic neural circuitry and molecular mechanisms of memory, but they have not been traditionally considered to be among the 'classical' tests in anxiolytic drug discovery. This may be changing, however, with the recent focus on devising ways to pharmacologically attenuate fearful memories through the process of reconsolidation or extinction (see below)[25] and, more generally, through a growing appreciation of abnormal learning and cognition in anxiety.

Preclinical anxiety models and endophenotypes

Tests or assays for anxiety, in which the animal is placed in an experimental situation to evoke an acute anxiety-like response, can be distinguished from models of anxiety, in which an animal has been manipulated in some way to produce a more lasting or permanent increase in anxiety. The goal of anxiety models is to produce a form of abnormally elevated anxiety that more closely resembles, by definition, the pathological nature of human anxiety disorders. This can be achieved, for instance, by acutely or chronically subjecting animals to stressors before testing[26,27]. Another approach involves identifying genetic populations (inbred and selectively bred strains)[28,29] or engineering mutant mice with innate anxiety-like phenotypes (TABLE 3). This approach has proven to be valuable for screening novel anxiolytics[30–32] and testing the pharmacoselectivity of putative anxiolytics[33]; emerging

genetic technologies such as optogenetics[34] will be integral to future basic anxiety research[35,36].

The term endophenotype (an immediate phenotype or biomarker) describes a premorbid or symptomatic behavioural, neural or biological feature of an anxiety disorder that, in principle, is more easily quantified than the disorder as a whole. An example of a neural intermediate phenotype in panic disorder and certain other anxiety disorders is the exaggerated blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) amygdala response to threatening stimuli[37]. In rodents, specific behavioural measures can also be viewed as endophenotypes of anxiety symptoms; for example, risk assessment and flight in the MDTB task may relate to threat avoidance and hypervigilance in GAD and panic disorder, respectively[22].

There is growing interest in identifying anxiety endo-phenotypes that are comparable across rodents and humans with a view to foster translation (TABLE 4). A good example that has grown in popularity is the extinction of fear memories. Extinction is an extension of the aforementioned conditioned fear paradigms and is typically assessed by measuring the decrease in a fear-related behaviour (for example, freezing or a startle response) following repeated presentation of an environmental cue or context that is associated with an aversive event (for example, electric shock). Given that extinction has a close therapeutic analogue in the form of exposure therapy, preclinical studies have applied extinction to test for drugs that function as adjuncts to strengthen extinction and reduce intrusive fear memories in PTSD and specific phobias[38,39]. There has been encouraging progress in the development of anxiolytics (for example, D-cycloserine) based on preclinical findings that have used extinction as a paradigm[40].

Below, we assess the preclinical evidence that has accrued — using these and other preclinical approaches — on the neurotransmitter systems that have been the main targets of anxiolytic drug discovery.

GABA–benzodiazepine system

Benzodiazepines such as chlordiazepoxide and diazepam have been reference anxiolytics for over 50 years. These drugs exert their effects by allosterically activating specific $GABA_A$ receptor subtypes to promote inhibitory neurotransmission in the brain. Benzodiazepines are efficacious in the acute treatment of GAD, SAD and panic disorder but have limited to no efficacy in other anxiety conditions[41,42]. In addition, the long-term use of benzodiazepines is hampered by the occurrence of troublesome side effects, including sedation, memory disturbances, tolerance and dependence liability[43] (TABLE 1).

The inherent therapeutic limitations of benzodiazepine anxiolytics led to the search for compounds that were chemically unrelated to the benzodiazepines, with more specific therapeutic actions and without their concomitant unwanted effects. As a result, novel compounds were developed to preferentially bind to specific $GABA_A$ receptor subtypes, to combine preferential affinity and differential intrinsic activity at these receptors or to display low efficacies at each $GABA_A$ receptor subtype[44]. A comprehensive programme of preclinical research provided very encouraging results and led to clinical studies of partial

agonists of $GABA_A$ receptors or agonists of $GABA_A$ receptor α2 or α3 subunits for GAD[44]. However, none of these drugs has reached the market.

The development of some compounds, such as the benzodiazepine receptor partial agonist bretazenil (a benzodiazepine derivative)[45,46] and the $GABA_A$ receptor α2 and α3 subunit agonist SL651498 (REF. 47), was discontinued owing to unexpected sedative and/or amnestic effects. Ocinaplon[48], which combines preferential affinity and differential intrinsic activity at $GABA_A$ receptors, failed clinically owing to toxicity, as did the $GABA_A$ receptor α2 and α3 subunit agonist TPA023 (REF. 49), despite exhibiting anxioselective activity in GAD. The mitochondrial benzodiazepine receptor agonist XBD-173 (REF. 50) also failed in a Phase II trial for GAD, although this may have been attributable to the choice of outcome measure (CCK-induced panic) and because the trial was not controlled for the presence of a genetic polymorphism moderating the binding of the drug[51]. Nonetheless, these disappointments have been a major reason why pharmaceutical companies seem to have abandoned the development of drugs targeting the GABA–benzodiazepine system for anxiety; to our knowledge there are no drugs targeting this system currently under development.

5-HT

The serotonin (5-HT) system has long been implicated in the mediation of anxiety[52]. For example, genetic variation in the human 5-HT transporter and in the $5-HT_{1A}$ receptor influences anxiety traits[53,54], and knockout mice lacking the genes encoding the 5-HT transporter and the $5-HT_{1A}$ receptor show increased anxiety-related behaviour[30,55,56]. 5-HT is also a primary target of existing anxiolytic medications. Indeed, the $5-HT_{1A}$ receptor partial agonist buspirone was the first pharmacotherapeutic alternative to benzodiazepines for the treatment of GAD. It was first described by Goldberg *et al.*[57] and later shown to have anxiolytic efficacy in controlled clinical studies[58] before being launched in 1985 by Kwizda Pharma. Buspirone and other partial agonists of the $5-HT_{1A}$ receptor may exert anxiolytic activity via the activation of $5-HT_{1A}$ heteroreceptors in forebrain areas[59–61]. However, drugs targeting the $5-HT_{1A}$ receptor have failed to demonstrate efficacy in other anxiety disorders, such as panic disorder or OCD[62], and their utility is further limited by extensive first-pass hepatic metabolism[63].

The serendipitous observation that antidepressants such as tricyclic antidepressants or monoamine oxidase inhibitors have anxiolytic properties[64] stimulated research on the anxiolytic properties of newer-generation, better-tolerated antidepressants such as SSRIs[65,66]. SSRIs are thought to exert their therapeutic effects by increasing extracellular 5-HT levels[67]. This class has proven to have efficacy across a range of anxiety disorders, and fluoxetine was the first SSRI to be approved for GAD in 1999 (REFS 41,42). Today, SSRIs are a first-line treatment for many anxiety disorders and are some of the most commonly prescribed medications in the field of psychiatry. However, many patients do not respond to SSRIs, and adverse effects such as sexual dysfunction and a delayed onset of action — sometimes associated with a transient period of increased anxiety — have reduced the acceptability of SSRIs in clinical practice[68].

A vast amount of preclinical pharmacological data has been accumulated on the effects of 5-HT-interacting drugs in anxiety-related procedures (Supplementary information S1 (box)). FIGURE 1 shows that the number of experiments focusing on 5-HT was the highest during the 1990s and, despite a decrease since the early 2000s, 5-HT remains the primary focus of drug testing in pre-clinical anxiety research. Not surprisingly, given their clinical success, studies on the anxiety-modulating actions of 5-HT-targeting drugs predominantly examined 5-HT$_{1A}$ receptor agonists and SSRIs, typified by buspirone and fluoxetine, respectively. The former (buspirone) has been, by far, the most studied anxiolytic outside the benzodiazepine class. Anxiolytic-like properties of buspirone and other 5-HT$_{1A}$ receptor agonists have been reported in about two-thirds of experiments. However, there are also reports that 5-HT$_{1A}$ receptor agonists induce pro-anxiety effects, and several studies did not reveal any modification of anxiety-like behaviours by these drugs (FIG. 3).

The effects of SSRIs are also inconsistent. Anxiolytic-like actions were observed in approximately 40% of the experiments conducted, whereas 20% reported anxiogenic-like effects and the remainder failed to detect any behavioural changes. 5-HT$_2$ and 5-HT$_3$ receptors have also been proposed as potential targets for anxiolytics but, again, compounds with high affinity and selectivity at these receptors produced equivocal results in preclinical experiments (FIG. 3).

What might account for these inconsistencies? Variability in experimental conditions across laboratories has often been cited as a potential influence on rodent anxiety-like behaviour[69]. In the case of 5-HT$_{1A}$ receptor agonists, increasing lighting levels in the elevated plus-maze test can switch an anxiogenic-like effect to anxiolytic-like activity[70], and manipulating shock associations in a conditioned suppression task can transform an inactive drug profile into an anxiolytic-like profile[71]. Findings such as these raise the possibility that certain key procedural factors, notably those affecting stress, determine the magnitude and direction of the anxiety-related effects of drugs acting on the 5-HT system. Although it remains to be thoroughly investigated, this attractive hypothesis is in line with the known, complex, stress-modulating role of the 5-HT system[72] and could have important implications for the design and choice of the animal model used in studies of 5-HT-targeting anxiolytics.

Overall, despite the intense focus on 5-HT receptor ligands, only the 5-HT$_{1A}$ receptor agonist tandospirone has made it to the market, and only in Japan and China. Agomelatine is an agonist of melatonin MT$_1$ and MT$_2$ receptors and an antagonist of the 5-HT$_{2C}$ receptor that has been developed and launched in Europe as an antidepressant; it has also demonstrated efficacy in a Phase II trial in GAD[73], but the exact contribution of 5-HT$_{2C}$ receptor antagonism to these anxiolytic effects is unclear. Some other drugs that either selectively or non-selectively target 5-HT receptor subtypes or modulate 5-HT reuptake are in active clinical development for anxiety disorders (TABLE 5). However, the anxiolytic effects of these drugs in preclinical settings have not been reported in the published literature.

Neuropeptides

The field of neuropeptide research has seen considerable progress in the past two decades, with the identification of new centrally expressed peptides and the elucidation of their functions using genetic manipulations and newly developed specific receptor ligands[74–76]. Almost 20 different peptide systems have been suggested to have a role in the modulation of anxiety (FIG. 4). This line of research was driven by the finding that these neurotransmitters and neuromodulators, as well as their receptors, are found in areas of the brain that are implicated in the control of anxiety. Further support emerged from studies showing that the central infusion or genetic manipulation of neuropeptides modified anxiety-related behaviours[77,78]. A detailed review of the vast preclinical literature in this area is beyond the scope of this article (for a full summary of experiments, see Supplementary information S1 (box)). Below, we consider three of the most intensively studied neuropeptides — CCK, CRF and tachykinins — and we also mention some other promising neuropeptides such as neuropeptide Y (NPY).

CCK was the first peptide to be discovered in the central nervous system (CNS)[79], where it is abundantly distributed and binds to two receptor subtypes, the CCK_1 receptor and the CCK_2 receptor, with the latter having a much broader distribution pattern[80]. Initial research focused mainly on CCK and the development of selective CCK_2 receptor antagonists as potential anxiolytics; this generated much interest in the late 1980s through to the 1990s. These compounds produced anxiolytic-like effects in less than two-thirds of the experiments; the remainder of experiments failed to detect any behavioural changes, and some even showed pro-anxiety effects (FIG. 3). The results of CCK_2 receptor deletion in animal models of anxiety are similarly discrepant, with both anxiolytic- or anxiogenic-like effects being reported, and about half of studies have shown no clear change in anxiety-like behaviour (FIG. 3). This questioned the idea that CCK represents a valid target for anti-anxiety medications. Clinical trials undertaken with CCK_2 receptor antagonists in anxiety disorders, including GAD and panic disorder, have also been unsuccessful[77,81] and no CCK-based drugs have yet been approved.

CRF is the major physiological regulator of the stress response[82] and has been one of the most studied neuropeptides in anxiety (FIG. 4). CRF binds to at least two receptors: the CRF_1 receptor and the CRF_2 receptor. Most preclinical studies have focused on the CRF_1 receptor because it is expressed at high density in corticotropic cells in regions of the brain that mediate anxiety[83]. Anxiolytic-like effects of CRF_1 receptor antagonists have been reported in the majority of preclinical experiments (one-third of these experiments failed to detect effects), which is consistent with the anxiolytic-like phenotype of *Crfr1*-null mutant mice (FIG. 3). Echoing the literature data on 5-HT, stress might have a strong influence on the effects of these drugs. CRF_1 receptor antagonists most reliably produce anxiolytic-like effects under conditions of elevated stress (for example, in tests involving predator or shock exposure) or in animals displaying excessive CRF–CRF_1 receptor signalling (for example, CRF-overexpressing mice)[78,81]. As in the case of drugs that target 5-HT, the choice of experimental models is therefore critical for the accurate assessment of the anxiolytic potential of targeting CRF. This issue could also extend to clinical studies. Controlled trials with CRF_1 receptor antagonists in anxiety disorders such as GAD and SAD have yielded

negative results, but these studies were carried out in heterogeneous patient groups[81], raising the question of whether effects would be more readily detected in patient subpopulations with the highest levels of anxiety.

The tachykinins substance P (also known as neurokinin 1), neurokinin A (NKA; also known as tachykinin precursor 1) and neurokinin B (NKB; also known as tachykinin 3) are widely distributed in the CNS[84]. Substance P and NKA, along with their respective receptors tachykinin receptor 1 (TACR1; also known as the NK_1 receptor) and TACR2 (also known as the NK_2 receptor), are especially well expressed in structures of the brain that are implicated in anxiety, including the amygdala and septum[85]. Several non-peptide antagonists at NK_1 and NK_2 receptors produced anxiolytic-like effects in a little more than half of the experiments (FIG. 3; Supplementary information S1 (box)). Variability in the outcomes of these studies seems to be highest when certain behavioural assays are utilized, in particular the elevated plus-maze test and social interaction tests. NK_2 receptor antagonists seem to have more reliable anxiolytic effects in tests involving strong or explicit stressors, such as in the MDTB. However, late-stage clinical trials with NK_2 receptor blockers have shown either negative or inconclusive results in GAD, SAD and PTSD[81]. Thus, selective blockade of tachykinin receptors may be insufficient to achieve therapeutic efficacy[81,86]. Differences in tachykinin receptor physiology between rodents and humans have also been suggested to account for at least some of the failure to translate preclinical data on this target to the clinic[81].

Other neuropeptides that have been studied for their anxiolytic potential include NPY[87], nociceptin[88], galanin[75,89], melanin-concentrating hormone (MCH)[90,91] and neuropeptide S (NPS)[92] (FIG. 4; Supplementary information S1 (box)). These peptides and their receptors are densely expressed in various regions of the brain that mediate anxiety. Preclinical experiments have investigated the administration of nociceptin, galanin, NPS or non-peptide ligands of their receptors either directly into the brain or — in the case of putatively brain-penetrant compounds — systemically; however, these experiments have not produced consistent effects on anxiety-related behaviours. Perhaps more promising are the results from the administration of MCH receptor antagonists, which have demonstrated anxiolytic-like effects in about three-quarters of preclinical experiments conducted to date (FIG. 3). The literature data on NPY is also encouraging[93]. Based on around 100 pharmacological and gene mutant experiments, many of which have been conducted in recent years (FIG. 4), the preclinical evidence supports the potent anxiolytic actions of NPY (BOX 1). However, although there are some promising lead compounds, there are no drugs targeting NPY, or any other neuropeptide, currently undergoing clinical evaluation for anxiety disorders (TABLE 4).

Box 1

Neuropeptide Y: an attractive system for the discovery of new anxiolytics

Neuropeptide Y (NPY) appears to act as an endogenous anxiolytic based on the numerous findings demonstrating that the central application of NPY produces consistent anxiolytic-like actions — effects that correspond well with the low level of anxiety observed in NPY-overexpressing transgenic mice. NPY and at least four of its receptors

(NPY receptor 1 (NPY1R), NPY2R, NPY4R and NPY5R) are found in the brain, with significant levels in regions that are believed to be implicated in anxiety, such as the amygdala and the hippocampus (see the figure). NPY pathways originating in the arcuate nucleus of the hypothalamus (Arc) project to the lateral septum (LS), amygdala and periacqueductal grey matter (PAG). Major NPY-containing neurons in the amygdala also innervate the PAG and locus coeruleus (LC) — regions that have been shown to have a crucial role in emotional processes. However, there are no NPY-based compounds currently in development. The major challenge associated with targeting the NPY system is obtaining non-peptide brain-penetrant ligands, and it is not clear at present which NPYR should be targeted, as peptide ligands of NPY1R, NPY2R and NPY5R have been shown to produce anxiolytic-like effects. In principle, the simultaneous targeting of all three NPYRs would represent the optimal approach.
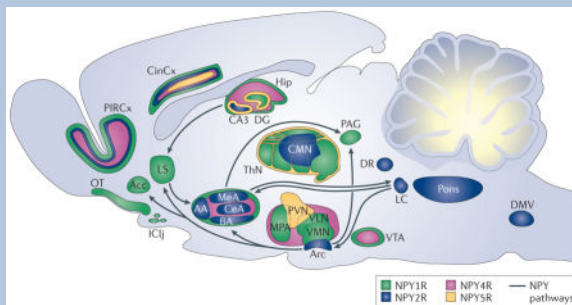
AA, anterior amygdaloid area; Acc, nucleus accumbens; BA, basolateral amygdala; CeA, central amygdala; CA3, hippocampal field CA3; CinCx, cingulate cortex; CMN, centromedial thalamic nucleus; DG, dentate gyrus; DMV, dorsal motor nucleus of the vagus and the trigeminal ganglion; DR, dorsal raphe; Hip, hippocampus; IClj, island of Calleja; MeA, median amygdala; MPA, medial preoptic area; OT, olfactory tubercle; PIRCx, piriform cortex; PVN, paraventricular nucleus of the hypothalamus; ThN, thalamic nucleus; VLN, ventral lateral nucleus; VMN, ventromedial nucleus; VTA, ventral tegmental area.

Glutamate

Multiple lines of evidence strongly implicate glutamate — the major excitatory neurotransmitter system in the brain — in anxiety disorders. There are abnormal levels of glutamate and various glutamate receptor classes in the brains of patients with anxiety disorders, and glutamate levels are altered in rodents by stressors[94]. However, delineating the contribution of the glutamate system to anxiety is a formidable task, given the large number of signalling receptors involved in glutamate neurotransmission. The glutamate system has nonetheless emerged as an increasingly active area of preclinical research within the past decade, with around 100 experiments conducted in 2012 alone (FIG. 1).

Metabotropic glutamate receptors (mGluRs), particularly mGluR1, mGluR2, mGlu3 and mGluR5, have been well studied preclinically and shown to have a role in anxiety behaviour. Orthosteric agonists, negative allosteric modulators or antagonists at mGluR1 (for example, JNJ16259685 and LY456236), at mGluR2 and mGluR3 (for example,

LY354740) or at mGluR5 (for example, 2-methyl-6-(phenylethynyl)pyridine (MPEP)) have shown anti-anxiety effects across various rodent assays[94]. Although there have been some negative results, around 80% of studies have been positive, with MPEP being particularly notable for its robust anxiolytic-like activity (FIG. 3; Supplementary information S1 (box)). MPEP was in preclinical development by Merz Pharmaceuticals but the drug was discontinued (for as yet undisclosed reasons) before entering clinical trials. Drugs acting at other mGluRs, including mGluR7 agonists (AMN082), have not been studied in as much depth and their effects still need to be clarified[94]. Clinically, some mGluR compounds, such as the mGluR2 and mGluR3 orthosteric agonist LY354740 (or its pro-drug LY544344), have produced encouraging preliminary results in GAD[95] (but not in panic disorder)[96], which have been somewhat tempered in some cases by pro-convulsant activity in animals[95]. Clinical trials are currently underway for the mGluR2 positive allosteric modulator ADX-71149 and for the mGluR1 and mGluR5 antagonist RGH-618 in anxiety disorders (TABLE 5).

The NMDA (*N*-methyl-D-aspartate) receptor antagonist ketamine was recently found to exert rapid antidepressant effects in treatment-resistant major depression[97]. This has generated considerable interest in NMDA and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors as targets for depression and is likely to provide insights into the anxiety-related effects of these compounds, for example, based on the effects observed in patients with comorbid depression and anxiety who receive ketamine[97]. In addition, the preclinical literature on the anxiolytic-like effects of NMDA and AMPA receptor antagonists has substantially grown in recent years. For example, the non-selective NMDA receptor channel blocker MK-801 has shown anti-anxiety effects across several assays, and NMDA receptor blockers have shown anxiolytic effects in around three-quarters of studies (FIG. 3; Supplementary information S1 (box)). Because indiscriminate blockade of NMDA receptors is unlikely to be a well-tolerated option for an anxiolytic, compounds that target specific NMDA receptor subunits (for example, the NMDA receptor subunit NR2B antagonist ifenprodil) have been studied but they do not produce comparably robust effects (Supplementary information S1 (box)). Similarly, the anxiety-related preclinical effects of AMPA receptor antagonists such as NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f] quinoxaline-2,3-dione) have overall proven to be inconsistent (Supplementary information S1 (box)).

Out of the other potential glutamate-acting targets for anxiety, D-cycloserine — which potentiates NMDA receptor signalling via the glycine co-agonist site — has, as already noted, shown efficacy as a therapeutic adjunct in various anxiety disorders[98,99]. Another glycine-acting drug, bitopertin (RG1678), which inhibits glycine reuptake by glycine transporter 1, is currently being investigated for efficacy in OCD (TABLE 5), but the class of glycine transporter 1 inhibitors has produced mixed preclinical data. Last, pregabalin, riluzole and topiramate are three drugs that exert glutamatergic effects as part of a complex pharmacological profile; pregabalin is approved (in Europe) for GAD, whereas all three are undergoing proof-of-concept studies for PTSD and SAD, with the caveat that the precise contribution of glutamate to their anxiolytic actions remains unclear.

Endocannabinoids

Endocannabinoids represent another system that has attracted attention in recent years as a potential target for novel anxiolytics (FIG. 1). The endocannabinoids anandamide (also known as *N*-arachidonoylethanolamide) and 2-arachidonoylglycerol, and their principal CNS receptor (the cannabinoid 1 (CB_1) receptor), are densely expressed in the brain, particularly in regions mediating anxiety[100]. Further implicating this system as a relevant translational target, there is growing evidence that abnormalities in the CB_1 receptor and other endocannabinoid systems are implicated in anxiety disorders such as PTSD[101,102]. The effects of CB_1 receptor agonists, inverse agonists and antagonists on anxiety-related behaviours have been intensively studied across a range of preclinical assays and models, with mixed results. There are examples of CB_1 receptor ligands and gene mutations producing either anxiolytic-[103] or anxiogenic-like[104,105] effects in rodents[106] (FIG. 3).

Part of the complexity of the anxiety-related effects associated with manipulating CB_1 receptors is very likely to stem from the ubiquitous expression of CB_1 receptors in different anxiety-mediating regions and circuits of the brain, some of which may have opposing roles in anxiety (for example, cortical regions versus the amygdala, and GABAergic circuits versus glutamatergic circuits)[107]. In addition, the enthusiasm for developing agents that target the CB_1 receptor was tempered by the withdrawal of the CB_1 receptor antagonist rimonabant (also known as SR141716) from the market as an anti-obesity medication owing to depression, suicidal ideation and anxiety symptoms in the patient populations receiving the drug[108].

An alternative approach for pharmacologically modulating endocannabinoids is to target their post-release reuptake and degradation. Endocannabinoids are thought to be primarily released 'on demand' as a function of physiological requirements. Therefore, pharmacologically inhibiting their reuptake or degradation could augment functionally relevant recruitment of endocannabinoids and produce more selective effects on anxiety than CB_1 receptor agonists. Although this is an attractive hypothesis, preclinical studies have not shown robust anxiety-related effects of, for example, compounds that augment anandamide via inhibition of the catabolic enzyme fatty acid amide hydrolase (FIG. 3; Supplementary information S1 (box)).

More promising are the recent findings that both anandamide transporter blockers (such as AM404) and fatty acid amide hydrolase inhibitors (such as AM3506 and JNJ-5003) promoted the extinction of rodent fear[101] and prevented stress-induced anxiety-like behaviour[109]. These preliminary observations suggest that this class of compounds may be preferentially active under conditions of high stress and abnormal endocannabinoid tone[110]. The anxiolytic potential of fatty acid amide hydrolase inhibitors is currently being investigated in early-phase clinical trials, and it remains to be confirmed whether this or other approaches to targeting endocannabinoids[111] will prove to be an effective translational strategy.

Lessons learned and future perspectives

Taking stock of half a century of intensive research, where does the effort to find effective medications for anxiety disorders now stand? Clearly, there are promising targets in the various neurotransmitter systems discussed above, and there is reason to be optimistic that one or more of these will yield a novel, safe and clinically efficacious anxiolytic. Considering the huge amount of data that has amassed, however, the drug discovery efforts in this field can be, and often have been, viewed as a failure.

However, this conclusion is not unique to the anxiety field; in fact, it has been levelled at most of the drug discovery efforts in psychiatry[112]. It is worth reiterating the point that finding medications for psychiatric illnesses is made all the more daunting by fluid diagnostic end points that are based almost entirely on behavioural symptomatology rather than on a deep mechanistic understanding of the underlying biology. Indeed, this and various other issues have been offered as explanations for why the search for new anxiolytics has stalled. Some of the issues were reinforced by our systematic analysis of the literature, and below we expand upon three issues that came to the fore.

Current tests have limited predictive and postdictive validity

An oft-cited explanation for the poor translational track record of preclinical anxiety studies is the lack of validity of the available rodent tests and models. On the one hand, the fact that the field has found the need to continually devise new procedures (well over 100 by recent counts)[13] to assess rodent anxiety-like behaviour reflects innovation, but on the other hand this indicates the dissatisfaction with the tools available. Still, as our analysis illustrates, the vast majority of studies have relied on a limited subset of tests. Many of these tests are excellent for demonstrating the effects of benzodiazepine anxiolytics but much less reliable in their sensitivity to drugs acting on the 5-HT system, including the SSRIs. This is concerning in view of the fact that several SSRIs (including escitalopram, paroxetine, fluvox-amine and sertraline) are approved for various anxiety disorders and are now the most successful drugs in this class. This means that, with the exception of the benzodiazepines, many preclinical anxiety tests lack not only predictive validity (the ability to predict new drugs) but also postdictive validity (sensitivity to existing drugs).

Some authors have contested that the available tests have skewed the anxiety field towards detecting new 'benzodiazepine-like' anxiolytics[13,113]. This argument has been levelled most forcefully at the approach-avoidance conflict tests (such as the elevated plus-maze test), which have been, by far, the most frequently used tests and have therefore shouldered most of the blame. These tests have clear intuitive appeal, are inexpensive to construct and ostensibly quick and easy to run, but they also produce the most inconsistent findings. This may be due to inadequate optimization: the elevated plus-maze test, in particular, is known to be highly sensitive to laboratory conditions[14]. However, our examination of the literature does not reveal any systematic differences in results across models, or any obvious experimental variables (including strain, species, dose or route of administration), that predict the effects of any class of drugs. To give just one example, buspirone has been found to exhibit both anxiolytic- and anxiogenic-like properties after either acute or repeated treatment across a large dose range, and there is no indication of more reliable results being

obtained in any particular species, assay or model. This does not preclude the possibility that, with careful scrutiny of the methods used across studies, ostensibly contradictory findings could be reconciled and attributed to key procedural variables (for an example, see REF. 114); however, at present the field does not have a clear grasp of what these variables may be.

The literature is biased towards acute treatments in 'normal' male rodents

FIGURE 5 illustrates the main characteristics of the animal models used in preclinical anxiety studies. The majority of studies have used rodents, mainly rats and somewhat less frequently mice, with only a small fraction of tests conducted in other species, ranging from zebrafish to monkeys. Anxiety is a highly adaptive response in many situations and, to the extent that they are understood, neural mechanisms appear to be fairly well conserved across species. However, differences between animals and humans cannot be ignored in any type of translational research. In fact, even among different strains of rats and mice, there is profound variation in anxiety-related phenotypes. This underscores the importance of careful model selection and provides the opportunity to make use of strains that are innately anxious[29,55]. Disease-susceptible animal models are commonplace in many non-psychiatry drug discovery programmes (for example, diabetes and cancer) but, despite their conceptual appeal, only a minority of anxiety studies use such models.

Another potentially important statistic is that although anxiety disorders are diagnosed in twice as many women as men[115], there has been a greater than 10:1 bias in favour of using male over female animals in anxiolytic drug discovery[116]. The basic neurobiology of anxiety may be similar between males and females, but there is a significant degree of sexual differentiation in the formation and function of anxiety circuits, as well as a significant influence of steroid hormones on anxiety behaviour[117]. Females also metabolize and respond differently to certain drugs[118]. As such, the generalizability of literature data to both sexes may be limited if these data are predominantly derived from male animals.

Finally, regardless of the species, strain or sex, most studies have relied on acute drug administration in testing for anti-anxiety effects. There may be good practical reasons for this, given that it is more difficult to deliver drugs repeatedly without stressing animals and confounding an experiment. Certain anxiolytics can reduce anxiety symptoms in patients following a single administered dose, but many effective interventions involve long-term treatment to deal with these chronic conditions. The possibility that preclinical results from acute treatments could be misleading is exemplified by the profile of SSRIs, which can transiently exacerbate anxiety symptoms yet produce anxiolytic activity with chronic dosing.

The focus has been on single targets in poorly defined neurobiological systems

A guiding principle of anxiolytic drug discovery over the past 50 years has been that identifying compounds that affect specific molecular targets would lead to more effective treatments with fewer side effects. The reductionist approach has considerable appeal but has not yielded significant successes. Indeed, current anxiolytics — the benzodiazepines and the SSRIs — are relatively non-selective. Benzodiazepines do not discriminate among $GABA_A$ receptor subtypes, whereas SSRIs globally enhance 5-HT transmission. This raises

the question of whether the concept of designing maximally selective ligands to act on individual molecular targets is the best — or at least the only — paradigm for anxiolytic drug discovery.

Polypharmacology has gained traction in other areas of drug discovery, including other CNS disorders[119,120]. It is based on the idea that superior efficacy can be achieved by designing new chemical entities that simultaneously act on multiple pathogenic targets. The design of a desired multi-target drug remains a complex and exceedingly difficult task for medicinal chemists. However, new approaches are emerging for improving the design of ligands against profiles of multiple drug targets[121,122].

Anxiolytic drug discovery, whether it is focused on a single target or on multiple targets, will be greatly facilitated by concerted efforts to elucidate the underlying neurobiology of anxiety. A better understanding of anxiety at this level would provide the foundation for a rational, mechanism-based approach for designing anxiolytics. Fear extinction has already been mentioned as an exemplar of a measure that is behaviourally underpinned by an excellent understanding of the underlying neural systems and circuits. The neural circuitry subserving behaviour in the classic anxiety tests has, by contrast, not been well defined. This may be changing, however, with the application of powerful new techniques, such as optogenetics[35,123,124], and could be further bolstered by the incorporation of advances in the imaging of the living brain of rodents. In parallel, evolving technologies for studying the neuropathophysiology of anxiety in humans, from diffusion tensor imaging and fMRI to genome sequencing, will serve to inform and direct the preclinical research. An optimal strategy will integrate findings from humans and animals in an effort to synergize convergent, cross-translational support for the clinical potential of an anxiolytic target. Other simple and actionable — rather than idealized — suggestions for how preclinical anxiety can be improved are detailed in FIG. 6.

Concluding remarks

Anxiolytic disorders are serious medical problems that are commonplace and becoming more prevalent in many parts of the world. The growing burden of anxiety disorders demands better treatments but, although the field has promising leads, the efforts to identify new anxiolytics seem to have reached an impasse. Here, we have offered a comprehensive analysis of the published preclinical research conducted to date with the aim of providing an objective analysis of the major trends, biases and limitations within the field in order to help direct a more effective translational approach in the future. We are optimistic that a new generation of preclinical studies that are built around circuit-informed, pathogenic rodent models and strong, bi-directional translational links to clinical research can move us out of the age of anxiety and into the age of discovery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Glossary

Validity	A feature that is assessed (for a test or model of anxiety) by determining how closely the model or test resembles human anxiety symptoms (known as face validity); by determining whether the model or test reliably responds to clinically efficacious anxiety medications (known as predictive validity); and by determining the degree to which the model or test recruits the same underlying neurobiology as implicated in human anxiety (known as construct validity)
Approach-avoidance conflict tests	Tests that generate anxiety-related behaviours in rodents by posing a conflict between a natural drive to explore a novel place and an inherent tendency to avoid new — particularly well-exposed — areas that may be dangerous
Pavlovian fear conditioning	A learning process by which neutral environmental stimuli, by virtue of association with a stressful event, evoke anxiety reactions. Fear extinction involves the learned inhibition of these reactions. Abnormalities in fear conditioning and extinction are thought to underlie anxiety disorders, notably specific phobias and post-traumatic stress disorder
Neural circuitry	A network of interconnected regions of the brain that mediate anxiety, including cortical structures (for example, the prefrontal cortex), limbic structures (for example, the amygdala, lateral septum and hippocampus) and the midbrain (for example, the dorsal raphe)
Anxiety models	Models that generate lasting or permanently heightened anxiety; for example by subjecting animals to chronic stress or by identifying or engineering 'high-anxiety' rodent strains. By contrast, simple tests or assays only transiently evoke an anxiety-like behaviour
Intermediate phenotype	A specific behavioural or neural feature of an anxiety disorder that might be more easily modelled in rodents than the whole constellation of symptoms found in an anxiety disorder
Anxiety traits	Persistent anxiety characteristics that manifest across a variety of situations and are considered to be an enduring feature of an individual

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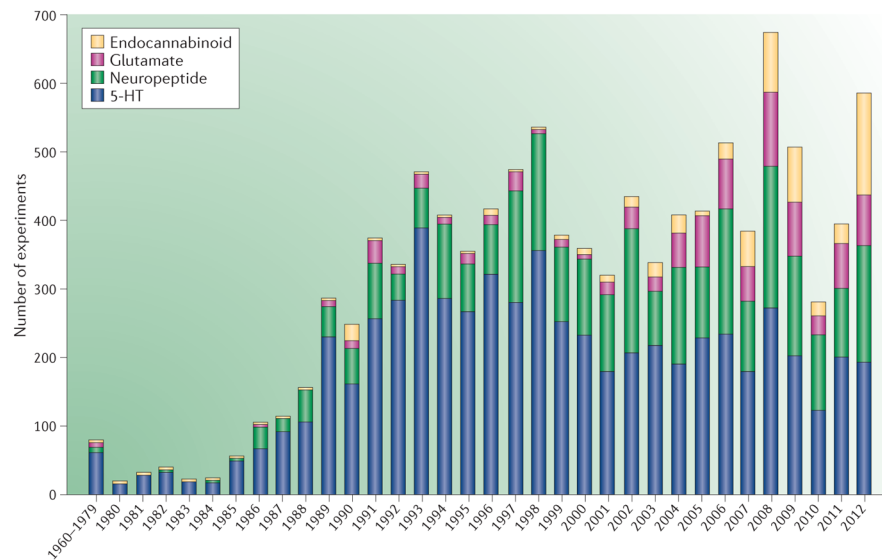
Figure 1. Fifty-year trends in preclinical anxiolytic drug discovery

The values represent the number of experiments investigating the anxiety-related effects of targeting the 5-hydroxytryptamine (5-HT; also known as serotonin), neuropeptide, glutamate and endocannabinoid systems between 1960 and 2012. The graph shows that the volume of research steadily increased from the 1980s onwards, peaking at the end of the 1990s, and has remained relatively constant up to now. More than half of the experiments focused on the 5-HT system, but neuropeptide drugs have also been a major focus of anxiolytic drug discovery, accounting for about one-third of all experiments. Over the past decade, the field has seen a rise in studies focusing on the glutamate and endocannabinoid systems. In this figure, an experiment refers to one drug (single or multiple dosing) that is tested in one assay or model. For more information on each experiment, including the drug, preclinical model, results and references, see Supplementary information S1 (box).
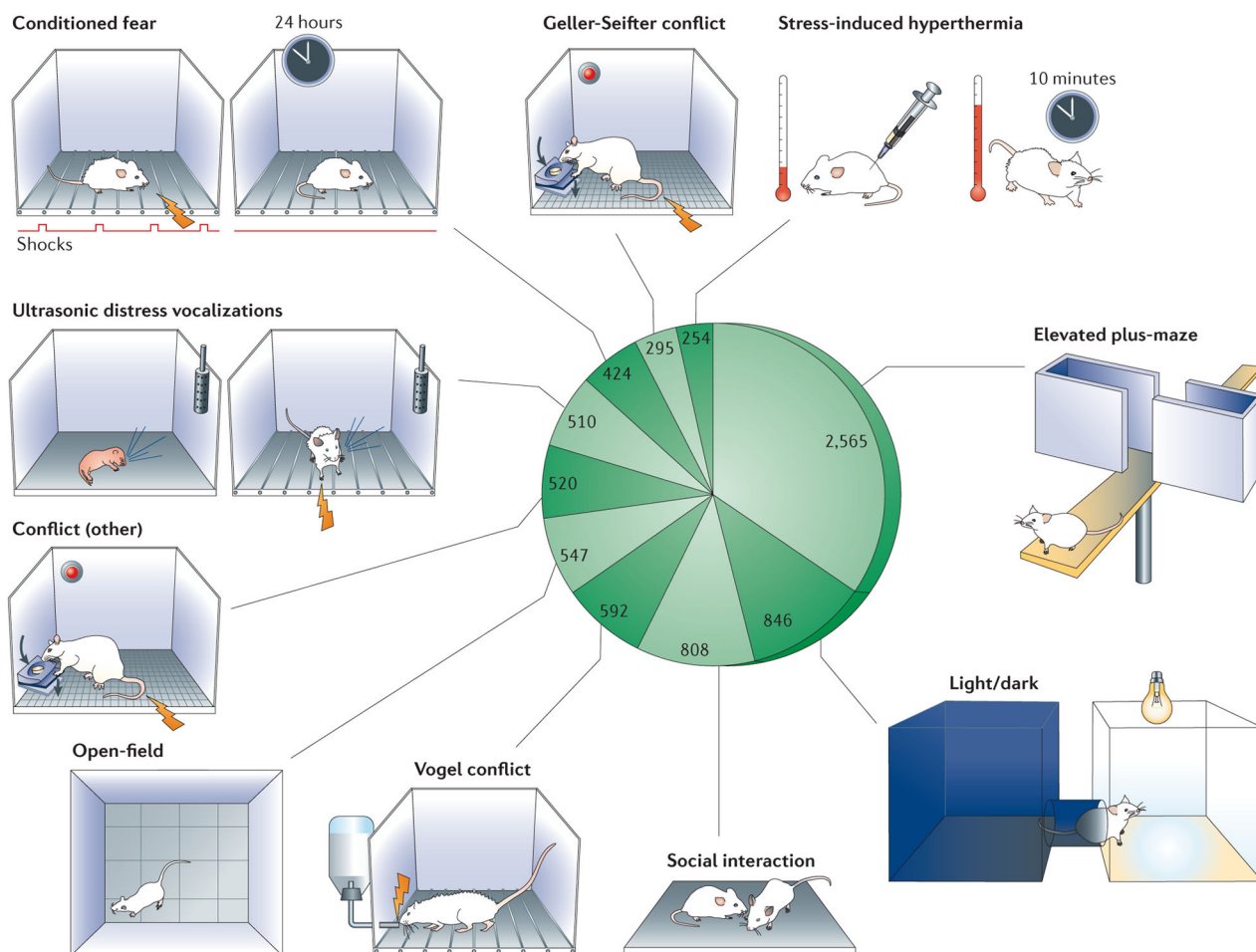
Figure 2. The ten most commonly used tests in anxiolytic drug discovery

The values represent the number of experiments performed with each test between 1960 and 2012. The elevated plus-maze test, the light/dark test and the open-field test have been a mainstay of anxiolytic drug discovery research for many years. They assay anxiety-like behaviour by generating a conflict between a drive to approach novel areas and, simultaneously, to avoid potential threat therein. They have clear intuitive appeal, are inexpensive to construct, and ostensibly quick and easy to run. The term 'conflict-based test' is also often used to describe measures of behaviour suppression by mild electric shock. This group includes the Vogel conflict and Geller-Seifter tests, which measure anxiolytic-like activity as the maintenance of a behavioural response (for example, licking or bar pressing) despite the receipt of a shock. Another set of fear-based tests involves variations on classical Pavlovian fear conditioning. Here, an animal learns to associate a context or specific environmental stimulus (for example, a light or a sound) with electric shock to produce a conditioned fear response that can be quantified in various ways (for example, freezing, escaping, avoidance or startle). Although the elevated plus-maze test, the light/dark test and the open-field test continue to be very popular, conflict-based tests — which were part of many drug discovery programmes in the 1980s and 1990s — are less frequently used today,

perhaps because they require animals to be trained over multiple days and are more labour-intensive and time-consuming than the approach-avoidance tests.
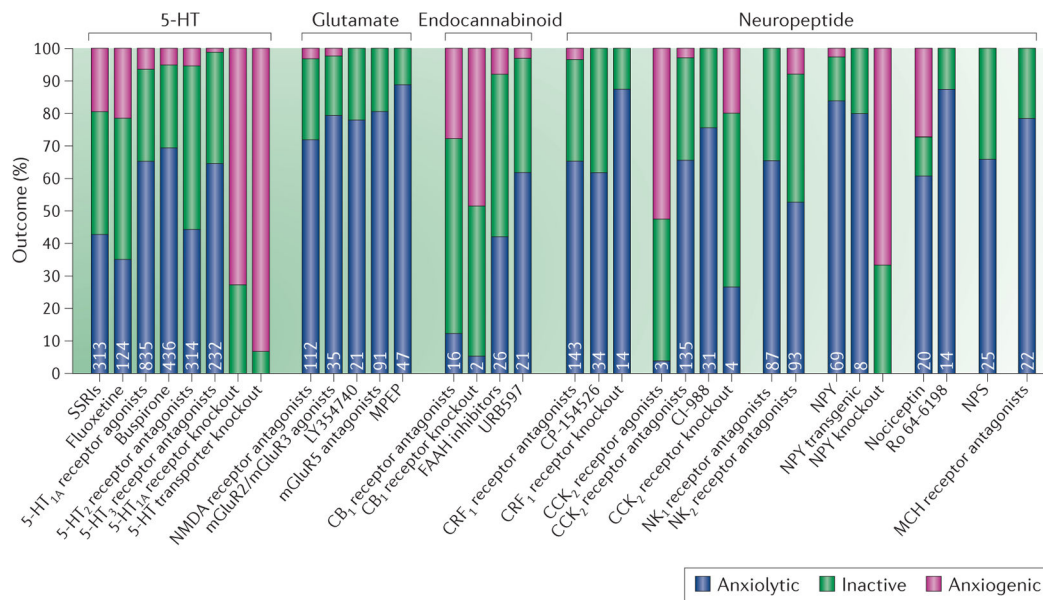
Figure 3. Anxiety-related effects of drugs targeting the 5-HT, neuropeptide, glutamate and endocannabinoid systems

Findings from experiments conducted between 1960 and 2012 are shown as the percentage of experiments that showed anxiolytic-like, anxiogenic-like and inactive effects. The number of experiments reporting anxiolytic-like effects is shown on the graph. This figure shows that although compounds modulating the 5-hydroxytryptamine (5-HT), corticotropin-releasing factor (CRF), cholecystokinin (CCK), endocannabinoid and tachykinin systems have shown variable effects, compounds acting at several glutamatergic receptors (that is, metabotropic glutamate receptor 2 (mGluR2) and mGluR5), compounds targeting neuropeptide Y (NPY) and compounds that block melanin-concentrating hormone (MCH) receptors have all produced relatively consistent anxiolytic-like effects. CB$_1$, cannabinoid 1; FAAH, fatty acid amide hydrolase; MPEP, 2-methyl-6-(phenylethynyl)pyridine; NK$_1$, neurokinin 1; NMDA, N-methyl-D-aspartate; NPS, neuropeptide S; SSRI, selective serotonin reuptake inhibitor.
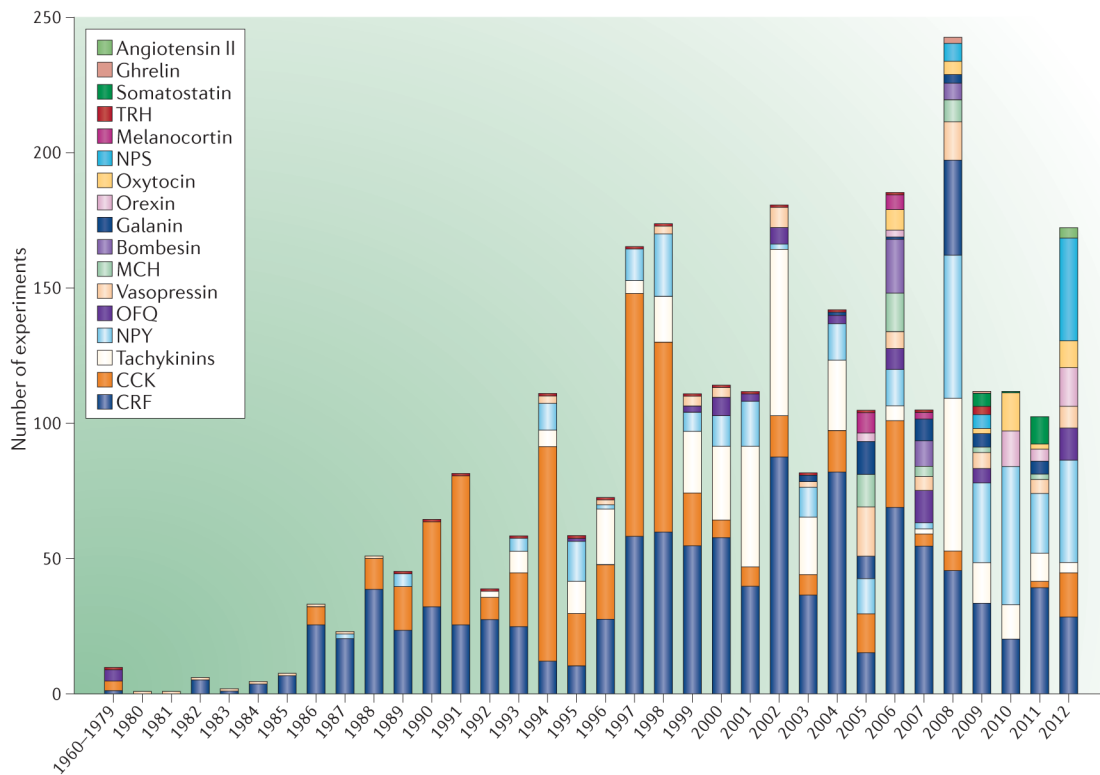
Figure 4. Experiments in animal models that investigated the effects of drugs modulating neuropeptide systems in models of anxiety disorders from 1960 to 2012

Seventeen different peptide systems have been suggested to have a role in the modulation of anxiety behaviours. This graph shows that, among them, corticotropin-releasing factor (CRF), the tachykinins and cholecystokinin (CCK) have been a major focus of anxiolytic drug discovery, accounting for about one-third of all experiments. MCH, melanin-concentrating hormone; NPS, neuropeptide S; NPY, neuropeptide Y; OFQ, orphanin FQ/ nociceptin; TRH, thyrotropin-releasing hormone.
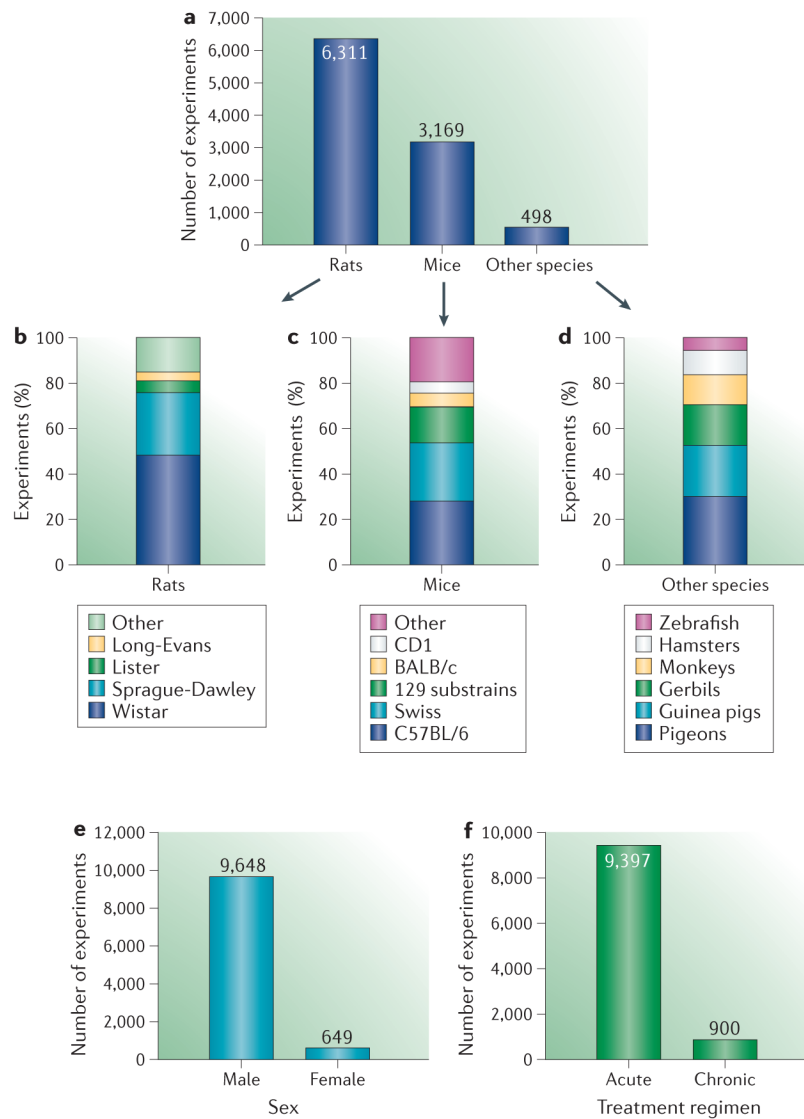
Figure 5. Fifty-year trends in the species, strain, sex and chronicity of drug treatment in anxiolytic drug discovery studies

The values represent the absolute numbers and percentages of experiments performed with different species (part **a**), strains (parts **b, c, d**) and sexes (part **e**), regardless of whether these involved acute or chronic treatment (part **f**), between 1960 and 2012. Rats represented the species of choice for anxiety tests, but mice have been extensively used as well. In addition, the majority of studies have used male subjects (part **e**) rather than females, and tested the effects of drugs following acute treatment (part **f**) rather than chronic treatment.

Target validation

Key aspects to consider when addressing a new anxiolytic drug target:
• Alteration in neurotransmitter signalling is one of the pathogenic factors
• Homologies between animal and human target, as well as the pharmacology
• Genetically engineered animals that overexpress or lack the target or neurotransmitter and display phenotypic features of the targeted anxiety disorder using approach-avoidance conflict tests

Primary screening (large libraries of molecules)

• Approach-avoidance conflict tests have proven to be of limited utility for detecting non-benzodiazepine anxiolytic activity, but they have a high throughput
• As many anxiety tests are highly sensitive to procedural variables and environment factors, the tests should be validated in-house and methods fully reported
• Examples include the elevated plus-maze test and the light/dark test

Secondary screening (preclinical candidates)

• Tests that assess multiple anxiety-related behaviours and/or are based on abnormal learning and cognitive processes in anxiety disorders may offer a tractable and translatable approach
• Genetic populations or engineered animals that are inherently anxiety-prone can be used
• Examples include the Mouse Defense Test Battery (MDTB), conditioned fear paradigms, BALB/c mice and 5-HT transporter knockout mice

Advanced profiling and integration (clinical candidate)

• The use of multiple techniques in addition to pharmacological tools, such as neuroimaging, optogenetics and *in vitro* electrophysiology will provide the convergence of information and deepen the mechanistic understanding of the putative anxiolytic target

Low — High
Throughput

High — Low
Translatability

Figure 6. Recommendations for improving anxiolytic drug discovery
The figure details simple and actionable, rather than idealized, suggestions and points to keep in mind. Although the early stages of the anxiolytic discovery process require high-throughput tests, these have generally limited predictive validity. Later-stage profiling using behavioural models with increased translatability potential could confirm or reject the initial findings, thereby increasing the probability of having selected the drug candidate with the highest anxiolytic potential. 5-HT, 5-hydroxytryptamine.

Table 1

The five main anxiety disorders as described in the DSM-IV-TR

Symptoms	Prevalence	Treatment
Generalized anxiety disorder (GAD)		
The existence of chronic feelings of excessive worry and anxiety are the main symptoms; these are accompanied by somatic symptoms such as elevated blood pressure, increased heart rate, muscle tension, sweating and shaking[125,126]	• GAD is one of the most common anxiety disorders • Approximately 3% of people in the United States will develop GAD during a given year, and 5% will have the disorder at some point in their lives • Approximately 25% of the people who attend anxiety treatment clinics have GAD[125]	Several different types of medications are used to treat GAD, including SSRIs, 5-HT–noradrenaline reuptake inhibitors, benzodiazepines, the 5-HT$_{1A}$ receptor partial agonist buspirone and the calcium channel $\alpha 2\delta$ subunit ligand pregabalin[127]
Post-traumatic stress disorder (PTSD)		
The essential feature is the development of distinct symptom clusters triggered by a terrifying event, which may include re-experiencing or flashbacks, nightmares and severe anxiety, as well as persistent thoughts about the event[125,126]	• The experience of a traumatic event is common in the general population, but the majority of individuals recover without developing PTSD • The NCS-R, conducted between 2001 and 2003, estimated the lifetime prevalence of PTSD among adult Americans to be nearly 7%[128], and the 12-month prevalence was estimated at 3.5%[129] • Current past-year PTSD prevalence in Europe was also estimated at 3.5%[2]	• Although evidence-based, trauma-focused psychotherapy is the preferred treatment for PTSD, pharmacotherapy is also an important treatment option • First-line pharmacotherapy agents include SSRIs and the selective 5-HT–noradrenaline reuptake inhibitor venlafaxine • Second-line agents include the α_2-adrenergic receptor antagonist mirtazapine, tricyclic antidepressants and monoamine oxidase inhibitors[130]
Panic disorder		
The main feature is the panic attack, which is defined as an abruptly developed feeling of intense anxiety or fear that has been present for a discrete period of time; panic disorder involves a set of cognitive and physical symptoms, such as choking feelings, fear of losing control or dying, depersonalization, accelerated heart rate and trembling or shortness of breath; agoraphobic avoidance can often be an additional condition[125,126]	Lifetime prevalence estimates of panic disorder (with or without agoraphobia) range from 1–3.5%, whereas the 12-month prevalence rates are 0.5–3.1%[2,125]	• The main treatment options for panic attacks are psychotherapy and medications • SSRIs and venlafaxine are generally used as first-line pharmacological agents in panic disorder, followed by tricyclic antidepressants such clomipramine and imipramine • Some benzodiazepines (such as alprazolam, clonazepam, diazepam and lorazepam) are also efficacious in the acute management of panic disorder[131]
Social anxiety disorder (SAD)		
The vast majority of individuals will experience mild anxiety in some social situations (for example, public speaking), but in SAD, public situations cause irrational anxiety, fear, self-consciousness and embarrassment, as well as avoidance and anxious anticipation[125,126]	Lifetime prevalence estimates of SAD as determined by the NCS for the American adult population range from 3–13%, whereas the 12-month prevalence rates as reported in a recent pan-European landmark study are 0.6–7.9%[2,132]; this makes SAD the most common anxiety disorder and the third most common of all psychiatric conditions	• The two most common types of treatment are medications and psychological counselling • Although several types of medications are used to treat SAD, SSRIs and venlafaxine are generally used as first-line treatment • Other medications for SAD include the benzodiazepines bromazepam and clonazepam • Some beta blockers are used to control symptoms for a particular situation, such

Symptoms	Prevalence	Treatment
		as giving a speech, but they are not recommended for the general treatment of SAD[133]
Obsessive compulsive disorder (OCD)		
OCD is an anxiety disorder that is characterized by unreasonable thoughts and impulses that lead to stereotyped behaviours with the aim of reducing the distress caused by the obsession[125,126]	Lifetime prevalence estimates of OCD range from 0.5–2%, whereas the 12-month prevalence rates are 0.1–2.3%[2,125]	• OCD treatment can be difficult; treatment with SSRIs is generally used but it is only effective in about half of patients • Management of the remaining patients is challenging, but can include augmentation with antipsychotics, as well as the use of 5-HT–noradrenaline reuptake inhibitors and monoamine oxidase inhibitors • Non-pharmacological interventions such as cognitive behavioural therapy can also be effective[134]

5-HT, 5-hydroxytryptamine (serotonin); DSM-IV-TR, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision; NCS, National Comorbidity Survey; NCS-R, National Comorbidity Survey Replication; SSRI, selective serotonin reuptake inhibitor.

Table 2

The fifteen most commonly used tests in anxiolytic drug discovery by order of importance[*]

Test	Anxiety disorder	Species	Setting up	Throughput	Anxiolytic pharmacology	Refs
Elevated plus-maze or zero-maze	GAD	Rats, mice, gerbils, guinea pigs	Easy	+++	$5\text{-}HT_{1A}$ receptor agonists or antagonists; $5\text{-}HT_2$ receptor antagonists; $5\text{-}HT_3$ receptor antagonists; AMPA receptor antagonists; benzodiazepines; CB_1 receptor agonists; CCK_1 and CCK_2 receptor antagonists; CRF_1 and CRF_2 receptor antagonists; FAAH inhibitors; mGluR2 and mGluR3 agonists; mGluR5 antagonists; NMDA receptor antagonists; NK_1 receptor antagonists; ORL1 agonists; SSRIs[‡]; V_{1A} and V_{1B} receptor antagonists	17,19, 135–137
Light/dark exploration	GAD	Rats, mice, hamsters	Easy	+++	$5\text{-}HT_{1A}$ receptor agonists or antagonists; $5\text{-}HT_2$ receptor antagonists; $5\text{-}HT_3$ receptor antagonists; benzodiazepines; CCK_1 and CCK_2 receptor antagonists; CRF_1 and CRF_2 receptor antagonists; SSRIs[‡]	18,138
Social interaction	GAD, SAD	Rats, mice, gerbils	Easy	++	$5\text{-}HT_{1A}$ receptor agonists; $5\text{-}HT_2$ receptor antagonists; $5\text{-}HT_3$ receptor antagonists; benzodiazepines; CRF_1 and CRF_2 receptor antagonists; NK_1 receptor antagonists; NMDA receptor antagonists; SSRIs[‡]	139,140
Conflict	GAD	Rats, mice, pigeons, squirrel monkeys, hamsters	Difficult[§]	+	$5\text{-}HT_{1A}$ receptor agonists or antagonists; $5\text{-}HT_2$ receptor antagonists; $5\text{-}HT_3$ receptor antagonists; benzodiazepines; CCK_1 and CCK_2 receptor antagonists; CRF_1 and CRF_2 receptor antagonists; mGluR5 antagonists; NMDA receptor antagonists	20,21,141
Open-field	GAD	Rats, mice, zebrafish	Easy	+++	$5\text{-}HT_{1A}$ receptor agonists $5\text{-}HT_2$ receptor antagonists; $5\text{-}HT_3$ receptor antagonists; benzodiazepines; SSRIs[‡]	16
Ultrasonic distress vocalizations	GAD	Rats, mice, guinea pigs	Somewhat difficult[∥]	+++	$5\text{-}HT_{1A}$ receptor agonists; $5\text{-}HT_2$ receptor antagonists;; benzodiazepines; CRF_1 and CRF_2 receptor antagonists; mGluR5 antagonists; NMDA receptor antagonists; SSRIs; V_{1B} receptor antagonists	142
Conditioned fear	PTSD, specific phobia	Rats, mice	Somewhat difficult[∥]	++	$5\text{-}HT_{1A}$ receptor agonists, $5\text{-}HT_2$ receptor antagonists; CB_1 receptor agonists; CRF_1 and CRF_2 receptor antagonists; mGluR5 antagonists; NMDA receptor antagonists; NMDA receptor glycine B agonists; SSRIs	38

Test	Anxiety disorder	Species	Setting up	Throughput	Anxiolytic pharmacology	Refs
Stress-induced hyperthermia	GAD	Rats, mice	Easy	+++	5-HT$_{1A}$ receptor agonists; benzodiazepines; MCH$_1$ receptor antagonists; mGluR2 agonists, antagonists or potentiators; mGluR5 antagonists	143
Four-plate	GAD	Mice, gerbils	Somewhat difficult//	+++	5-HT$_2$ receptor agonists; benzodiazepines; CRF$_1$ receptor antagonists; SSRIs	144
Defensive burying	GAD	Rats, mice	Somewhat difficult//	+++	5-HT$_{1A}$ receptor agonists; 5-HT$_2$ receptor antagonists; benzodiazepines; CRF$_1$ and CRF$_2$ receptor antagonists; MCH$_1$ receptor antagonists; mGluR5 antagonists; SSRIs; V$_{1B}$ receptor antagonists	145,146
Fear-potentiated startle	GAD	Rats, mice, monkeys	Difficult§	++	5-HT$_{1A}$ receptor agonists or antagonists; 5-HT$_3$ receptor antagonists; benzodiazepines; CRF$_1$ and CRF$_2$ receptor antagonists; mGluR2 and mGluR3 agonists; mGluR5 antagonists; NK$_1$ receptor antagonists; NPY1R and NPY2R agonists	147
Holeboard	GAD	Rats, mice	Easy	+++	5-HT$_{1A}$ receptor agonists; benzodiazepines	148
Novelty-suppressed feeding	GAD	Rats, mice	Easy	+++	5-HT$_{1A}$ receptor agonists; benzodiazepines; mGluR5 antagonists; SSRIs‡	149
Elevated T-maze	GAD, panic disorder	Rats, mice	Easy	+++	5-HT$_{1A}$ receptor agonists or antagonists; benzodiazepines; SSRIs‡	150
Mouse Defense Test Battery	GAD, panic disorder, PTSD	Mice	Somewhat difficult//	++	5-HT$_{1A}$ receptor agonists or antagonists; CRF$_1$ and CRF$_2$ receptor antagonists; NK$_2$ receptor antagonists; SSRIs‡	22,23

+low (requires several weeks to achieve a dose response); ++, medium (one dose response per week); +++, high (at least one dose response per day); 5-HT, 5-hydroxytryptamine (serotonin); AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; CB1, cannabinoid 1; CCK, cholecystokinin; CRF, corticotropin-releasing factor; FAAH, fatty acid amide hydrolase; GAD, generalized anxiety disorder; MCH, melanin-concentrating hormone; mGluR, metabotropic glutamate receptor; NMDA, *N*-methyl-D-aspartate; NK1, neurokinin 1; NPY1R, neuropeptide Y receptor 1; ORL1, opiate receptor-like 1 (nociceptin/orphanin FQ receptor); PTSD, post-traumatic stress disorder; SAD, social anxiety disorder; SSRI, selective serotonin reuptake inhibitor; V1A, vasopressin V1A receptor; V1B, vasopressin V1B receptor.

*
This table indicates the relevance of each test for modelling various aspects of anxiety disorders based on face and/or predictive validity, the species that have been used, the difficulty in implementing the procedure, its throughput and the pharmacological classes that have shown anxiolytic-like activity in these tests in at least five studies.

‡
Only following repeated treatment.

§
Requires highly specialized equipment (for example, operant conditioning chambers), software and training.

//
Requires specific equipment (for example, a shocker or a non-commercially available apparatus).

Table 3

Genetic mouse and rat[*] models of anxiety

Model	Description	Tests	Refs
Single-gene engineered models			
3xTg-AD[‡]	Transgenic	Conditioned fear stress test, light/dark test, open-field test	151
5-HT$_{1A}$ receptor	Knockout	Conditioned fear stress test, elevated plus-maze test, elevated zero-maze test, light/ark test, novelty-suppressed feeding, open-field test, stress-induced hyperthermia	152–163
5-HT$_{1A}$ and 5-HT$_{1B}$ receptor	Knockout	Elevated plus-maze test, novelty-suppressed feeding, open-field test	164
5-HT$_{2C}$ receptor	Knockout	Elevated plus-maze test	165
5-HT$_3$ receptor	Knockout	Conditioned fear stress test, defensive withdrawal test	166,167
5-HT transporter	Knockout	Conditioned fear stress test, elevated plus-maze test, emergence test, light/dark test, novelty-suppressed feeding, open-field test, successive alleys, shock-escape paradigm	168–175
CaMKIIα	Transgenic	Elevated zero-maze test, light/dark test, open-field test, social interaction test	176
Adenosine A$_{2A}$ receptor	Knockout	Elevated plus-maze test, light/dark test	177
Adrenergic α$_{2A}$ receptor	Knockout	Elevated plus-maze test, light/dark test, marble burying test, open-field test	178,179
Angiotensin II receptor type 2	Knockout	Elevated plus-maze test, light/dark test	180,181
Apolipoprotein E	Knockout	Elevated plus-maze test	182
APP	Transgenic	Conditioned fear stress test, light/dark test, open-field test	151
CB$_1$ receptor	Knockout	Conditioned fear stress test, elevated plus-maze test, light/dark test, open-field test, social interaction test	107,183–192
FAAH	Knockout	Elevated plus-maze test	105
COMT	Knockout	Light/dark test	193
CCK; OLETF, CCK$_1$ receptor	Knockout[*]	Elevated plus-maze test, light/dark test, open-field test	194,195
CCK; CCK$_2$ receptor	Knockout	Elevated plus-maze test	196–198
CCK; CCK$_2$ receptor	Transgenic	Conditioned fear stress test, open-field test, social interaction test	199
CRF	Transgenic	Conditioned fear stress test, elevated plus-maze test, light/dark test, open-field test	200–204
CRF-binding protein	Knockout	Elevated plus-maze test, open-field test, defensive withdrawal test	205,206
CRF$_1$ receptor	Knockout	Light/dark test	207
CRF$_2$ receptor	Knockout	Elevated plus-maze test, light/dark test, open-field test, Vogel conflict test	208–210
Desert hedgehog	Knockout	Vogel conflict test	211
Dopamine D4 receptor	Knockout	Open-field test	212
Oestrogen receptor-α	Knockout	Light/dark test	213
FMR1	Knockout	Mirror chamber, social interaction test	214
FYN tyrosine kinase	Knockout	Elevated plus-maze test, light/dark test, open-field test	215
GABA$_A$ α$_1$ subunit receptor	Knockout	Conditioned fear stress test	216
GABA$_A$ α$_2$ subunit receptor	Knockout	Conditioned emotional response	217
GABA$_A$ β$_3$ subunit receptor	Knockout	Elevated plus-maze test, marble burying	218,219

Model	Description	Tests	Refs
GABA$_A$ γ_2 subunit receptor	Knockout	Conditioned fear stress test, elevated plus-maze test, free exploration test, light/dark test, novelty-suppressed feeding	220–222
GABA$_A$ γ_2 subunit receptor	Knockdown	Elevated plus-maze test, forced novelty exploration	223
GABA$_{B1}$ receptor	Knockout	Elevated zero-maze test, light/dark test, staircase test	224–226
GABA$_{B2}$ receptor	Knockout	Light/dark test	224
GABA GAD65	Knockout	Conditioned fear stress test, elevated plus-maze test, light/dark test, open-field test	227–231
GAT1	Knockout	Elevated plus-maze test	232
GALR1	Knockout	Elevated plus-maze test	233
Glucocorticoid	Transgenic	Elevated plus-maze test, light/dark test	234
DAO	Knockout	Elevated plus-maze test, novel object test, open-field test	235
NMDA receptor subunit NR2B	Knock-in	Elevated plus-maze test	236
mGluR4	Knockout	Elevated zero-maze test, open-field test	237
mGluR5	Knockout	Elevated plus-maze test	238
mGluR8	Knockout	Acoustic startle, elevated plus-maze test, elevated zero-maze test, open-field test	239–243
HDC	Knockout	Elevated plus-maze test, light/dark test, open-field test	244
Interferon-γ	Knockout	Elevated plus-maze test	245
Interleukin-6	Knockout	Elevated plus-maze test	246
MAS oncogene	Knockout	Elevated plus-maze test	247
Midkine	Knockout	Elevated plus-maze test	248
NCAM	Knockout	Elevated plus-maze test, light/dark test	249
Nicotinic AChR α4 subunit	Knockout	Elevated plus-maze test	250
Nociceptin	Transgenic	Acoustic startle, light/dark test	251
NOS	Knockout	Elevated plus-maze test, open-field test	252
Nociceptin	Knockout	Elevated plus-maze test, light/dark test, open-field test	253
Nociceptin receptor	Knockout	Elevated plus-maze test, elevated T-maze test, light/dark test	254
NPY	Knockout	Acoustic startle, elevated plus-maze test, open-field test	255–257
NPY	Transgenic	Elevated plus-maze test	258
NPY1 receptor	Knockout	Light/dark test	259
Preproenkephalin	Knockout	Elevated plus-maze test	260
Puromycin-sensitive aminopeptidase	Knockout	Elevated plus-maze test	261
SF1	Knockout	Elevated plus-maze test, light/dark test, marble burying test, open-field test	262
Single-minded homolog 2	Transgenic	Elevated plus-maze test	263
TRH receptor 2	Knockout	Novelty-suppressed feeding	264
Activin βE	Transgenic	Elevated plus-maze test, open-field test	265
NTRK3	Transgenic	Elevated plus-maze test, elevated zero-maze test, Mouse Defense Test Battery	266
Tumour necrosis factor	Transgenic	Light/dark test	267
TSC-DN	Transgenic	Elevated plus-maze test, open-field test	268

Model	Description	Tests	Refs
Vasopressin V_{1A} receptor	Transgenic	Light/dark test	269
Selective breeding			
BALB/c	Inbred	Conditioned fear stress test, free exploration test, light/dark test, elevated plus-maze test, open-field test	55, 270–272
BTBR T + tf/J	Inbred	Elevated plus-maze test, social interaction test	273
Fawn-hooded	Inbred[*]	Social interaction test	274
LAB/HAB	Outbred[*]	Elevated plus-maze test, light/dark test	275–277
MR/Har and MNRA/Har	Outbred[*]	Acoustic startle, conflict test, open-field test, ultrasonic distress vocalizations	278–280
RHA/Verh and RLA/Verh	Inbred[*]	Elevated plus-maze test, light/dark test, open-field test	281–283
Wistar-Kyoto	Outbred[*]	Open-field test	284,285

5-HT, 5-hydroxytryptamine (serotonin); AChR, acetylcholine receptor; APP, amyloid precursor protein; CB1, cannabinoid 1; CCK, cholecystokinin; CaMKIIα; calcium/calmodulin-dependent protein kinase IIα; COMT, catechol-*O*-methyltransferase; CRF, corticotropin-releasing factor; DAO, D-amino-acid oxidase; FAAH, fatty acid amide hydrolase; FMR1, fragile X mental retardation 1; GABA, γ-aminobutyric acid; GAD65, 65 kDa glutamate decarboxylase; GAT1, GABA transporter 1; GALR1, galanin receptor 1; HAB, high anxiety behaviour; HDC, histidine decarboxylase; LAB, low anxiety behaviour; mGluR, metabotropic glutamate receptor; MNRA/Har, Maudsley non-reactive; MR/Har, Maudsley reactive; NCAM, neural cell adhesion molecule; NOS, nitric oxide synthase; NMDA, *N*-methyl-D-aspartate; NPY, neuropeptide Y; NTRK3, neurotrophic tyrosine kinase receptor type 3; OLETF, Otsuka Long-Evans Tokushima Fatty; RHA/Verh, Roman high avoidance; RLA/Verh, Roman low avoidance; SF1, steroidogenic factor 1; TRH, thyrotropin releasing hormone; TSC-DN, tuberous sclerosis dominant negative.

[*] The column tests indicate the procedures in which these animals displayed increased anxiety-like behaviours.

[‡] 3xTg-AD: transgenic mice expressing human mutant amyloid-β precursor protein (APP[Ind] and APP[Sw,Ind]) and tau.

Table 4

Translatable measures of anxiety endophenotypes

Measure	Example of relevant anxiety disorder	Refs
Impaired fear extinction	Post-traumatic stress disorder	25
Elevated startle response	Generalized anxiety disorder	286
Fear generalization	Post-traumatic stress disorder	25
Increased BOLD amygdala response to threat	Panic disorder	37

BOLD, blood-oxygen-level-dependent.

Table 5

Compounds in clinical development for anxiety disorders

Drug	Companies	Properties	Disorder	Phase
Vortioxetine (LU-AA-21004)	Lundbeck/Takeda	receptor antagonist, 5-HT$_3$ receptor agonist 5-HT$_{1A}$ and 5-HT enhancer	GAD	Pre-registration
Agomelatine (S 90098)[*]	Servier	Melatonin 1 and melatonin 2 receptor agonist, 5-HT$_{2C}$ receptor antagonist	GAD	III
Pregabalin[‡]	Pfizer	Calcium channel α2δ subunit ligand	SAD	III
Vilazodone (EMD 68843)	Merck KGaA	5-HT$_{1A}$ receptor agonist and SSRI	GAD	III
ADX-71149	Addex/Johnson & Johnson	Positive allosteric modulator of mGluR2	NA	II
Androstadienol (PH-94B)	Pherin	Vomeropherin	GAD, SAD	II
AVN-101	Avineuro Pharmaceuticals	5-HT$_6$ receptor antagonist	NA	II
AVN-397	Avineuro Pharmaceuticals	5-HT$_6$ receptor antagonist	GAD	II
Bitopertin (R-1678)	Roche	Glycine transporter 1 inhibitor	OCD	II
Guanfacine (SPD-503)	Shire	Unknown	GAD, SAD	II
Orvepitant	GlaxoSmithKline	NK$_1$ receptor antagonist	PTSD	II
Pivagabine (CXB-722)	CeNeRx BioPharma	Hypothalamic–pituitary–adrenal axis modulator	NA	II
TGFK-08AA	Fabre-Kramer Pharmaceutical	5-HT$_{1A}$ receptor partial agonist	GAD	II
Verucerfont (GSK561679)	GlaxoSmithKline	CRF$_1$ receptor antagonist	PTSD	II
YKP-3089	Sunkyoung Group Holdings	Undisclosed	NA	II
BNC-210	Bionomics	GABA$_A$ receptor modulator	GAD	I
JNJ-19385899	Johnson & Johnson	OPRL1 agonist	NA	I
RGH-618	Gedeon Richter	mGluR1 and mGluR5 antagonist	NA	I
SPD-554	Shire	α$_2$-adrenergic receptor agonist	NA	I
SRX-246	Azevan Pharmaceuticals	Vasopressin V$_{1A}$ receptor antagonist	PTSD	I
TriRima (CX-157)	CeNeRx BioPharma	MAO inhibitor	NA	I

5-HT, 5-hydroxytryptamine (serotonin); CRF, corticotropin-releasing factor; GABA, γ-aminobutyric acid; GAD, generalized anxiety disorder, NA, information not available; NK$_1$, neurokinin 1; MAO, monoamine oxidase; mGluR, metabotropic glutamate receptor; OPRL1, opiate receptor-like 1 (nociceptin/orphanin FQ receptor); OCD, obsessive compulsive disorder; PTSD, post-traumatic stress disorder; SAD, social anxiety disorder.

[*] Agomelatine has been launched as an antidepressant in Europe.

[‡] Pregabalin has been launched for the treatment of GAD in Europe.