Expert Opinion



# Experimental models for anxiolytic drug discovery in the era of *omes* and *omics*

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***Introduction:*** Animal behavioral models have become an indispensable tool for studying anxiety disorders and testing anxiety-modulating drugs. However, significant methodological and conceptual challenges affect the translational validity and accurate behavioral dissection in such models. They are also often limited to individual behavioral domains and fail to target the disorder's real clinical picture (its spectrum or overlap with other disorders), which hinder screening and development of novel anxiolytic drugs.

***Areas covered:*** In this article, the authors discuss and emphasize the importance of high-throughput multi-domain neurophenotyping based on the latest developments in video-tracking and bioinformatics. Additionally, the authors also explain how bioinformatics can provide new insight into the neural substrates of brain disorders and its benefit for drug discovery.

***Expert opinion:*** The throughput and utility of animal models of anxiety and other brain disorders can be markedly increased by a number of ways: i) analyzing systems of several domains and their interplay in a wider spectrum of model species; ii) using a larger number of end points generated by video-tracking tools; iii) correlating behavioral data with genomic, proteomic and other physiologically relevant markers using online databases and iv) creating molecular network-based models of anxiety to identify new targets for drug design and discovery. Experimental models utilizing bioinformatics tools and online databases will not only improve our understanding of both gene–behavior interactions and complex trait interconnectivity but also highlight new targets for novel anxiolytic drugs.

**Keywords:** animal models, anxiety, behavioral phenotyping, bioinformatics, neurobehavioral domains

*Expert Opin. Drug Discov. [Early Online]*

## 1. Introduction

Anxiety and anxiety-spectrum disorders are becoming increasingly prevalent in modern society, requiring new therapeutic approaches and treatments [1-3]. Affective disorders are also complex, showing high co-morbidity within and outside the anxiety spectrum [4-7]. As drug discovery shifts toward targeting specific pathways and molecular determinants, versatile translational experimental models are important for preclinical drug screening [8]. Although constant refinement of existing experimental paradigms is necessary [9], it is crucial to make further conceptual advances in this field [10,11], especially because of the domination of single-domain animal models of anxiety and the lack of complex models that target several different domains and their interplay (see **Table 1** and [10,12-13] for details).

Recently, we outlined strategic directions for experimental modeling of affective disorders [10]. While there has been remarkable progress in this field, new challenges

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**Article highlights.**

- Increasing the range of model species is critical for affective research.
- Targeting physiological phenotypes of affective disorders warrants greater focus on pathogenic complexities.
- Assessing systems of integrated domains helps characterize complex neurobehavioral phenotypes.
- Innovative approaches including data-dense video-tracking and data-mining technologies are emerging as useful new methods to characterize anxiety-like phenotypes.
- Experimental models utilizing bioinformatics tools and online databases are needed to understand the gene–behavior interactions, complex trait interconnectivity and new targets for novel anxiolytic drugs.

This box summarizes key points contained in the article.



have arisen [14] and are briefly addressed here. We argue that multi-dimensional and multi-domain neurophenotyping of anxiety can be facilitated by the combined application of modern video-tracking and data-mining technologies to maximize the validity and accuracy of animal models. We also discuss how bioinformatics enables further unprecedented insights into the neural substrates of brain disorders, creating interactomes, connectomes and other *omes* to apply to affective disorders. Together with high-throughput *-omics*, they complement multi-domain behavioral analyses to advance our understanding of affective pathogenesis and reveal novel targets for anxiolytic drugs (**Table 1**).

## 2. Strategic directions of basic anxiety research

### 2.1 Moving farther beyond traditional rodent paradigms

Critical for translational cross-species analysis (**Figures 1** and **2**), the increase in the range of model species is an important direction of affective research [8]. Exhibiting a significant physiological homology to humans, zebrafish (*Danio rerio*) are rapidly gaining popularity due to their robust anxiety-like responses to various experimental manipulations [15-19], including novelty or predator exposure [20-22] and drug withdrawal [23-26]. Mounting evidence shows that zebrafish are sensitive to a wide range of psychotropic compounds, confirming their utility to study anxiety [17]. The responses to such compounds generally parallel rodent and clinical observations, further confirming the translational value of zebrafish tests [16,23]. Combined with rapid development, high fecundity and low costs, the potential of both larval and adult zebrafish for high-throughput anxiolytic drug discovery is becoming widely recognized [16,17,19].

Recent reports have also demonstrated the validity and utility of the chick (*Gallus gallus*) as an animal model of anxiety under both acute and repeated administration [27,28]. While many clinical signs of anxiety and depression exist along a temporal continuum, chicks have been a useful model in exemplifying this construct by showing distress vocalizations that sequentially model anxiety- and depressive-like states [29,30]. Importantly, this assay is fast, inexpensive and has been repeatedly validated as a pharmacological screen of substances that modulate anxiety behavior [29-31].

Anxiety-like behavior has also been extensively studied in non-human primates [32], whose marked behavioral complexity resembles that of humans [33-35]. Despite high costs associated with primate research, monkeys are increasingly used to model social anxiety-related behaviors, aggression [33,36-37] and post-traumatic stress [38]. They also show homology of the neural circuits of fear and anxiety between monkeys and human adults with childhood history of extreme behavioral inhibition [39,40]. Given these promising traits, expanding the range of animal model species continues to be a strategic priority in experimental modeling of anxiety disorders.

### 2.2 Focus on pathogenetic complexities

In addition to behavioral paradigms, there is a growing need for targeting physiological phenotypes of affective disorders [41]. 5-HT, GABA and corticotropin-releasing hormone (CRH) have been shown to mediate anxiety and stress-related behaviors [41], and the disruption of genes associated with these systems has been linked to altered anxiety [42-47]. The function of CRH-mediated genes in stress-related psychopathology has been of particular interest recently, revealing maladaptive stress responses following experimental alterations in these genes [48-51]. Similarly, *c-fos* expression is a marker of neuronal activation in rodents [52-54] and zebrafish [55-57] and has been shown to correlate with environmentally or pharmacologically induced anxiety [53,54,58].

Numerous reports have demonstrated alterations in gene expression corresponding to anxiety-like behavior in various species from rodents [59-61] to primates [62-64], and can help identify appropriate targets for therapeutic intervention [65-67]. For example, differential expressions of guanine nucleotide-binding protein β 1, cadherin 7 and calcium-calmodulin-dependent protein kinase II (CaMKII) inhibitor have recently been associated with anxiety-like behavior [68]. In line with this, anxiety-like behavior induced by microinjection of CRH receptor agonists into rat brain is blocked by CaMKII inhibitors, exemplifying the potential for therapeutics based on the overlap between the two candidate pathways [69]. Furthermore, the effects of gene disruption on anxiety and drug responsivity have also been evaluated [70-73]. For example, the deletion of the prodynorphin gene in mice increases anxiety-like behaviors and $GABA_A$ receptor subunit expression, while attenuating the anxiolytic action of bromazepam [70]. Similarly, deletion of mouse cannabinoid CB1 receptors increases anxiety-like behaviors and decreases

**Table 1. Glossary of terms.**

- Bioinformatics is the application of statistics and computational techniques to the field of biology, with the primary goal of increasing our understanding of the mechanisms and interconnectivity of biological processes
- Domain is the specific cluster of behavioral phenotypes based on contextual similarity within a disorder. Typical domains affected by pathogenesis include locomotor, emotionality, cognitive, neurological or sensory components. In biological psychiatry, each disorder (such as anxiety) can be deconstructed into multiple endophenotypes, which can then be clustered into larger groups (domains). The domain interplay concept [12,13,79] postulates that various domains overlap within a specific disorder or even between different disorders, thereby underlying complex spectra of psychiatric disorders. The domain interplay concept also posits that a similar approach – targeting a system of overlapping, clinically relevant domains – may be applied to animal models. The more overlapping domains are observed in both clinical and experimental models, the more valid these experimental models of human disorder would be according to this concept. We apply the term domain in this paper in the same context as it is used in the domain interplay concept [12,13,79]. For example, in rodent models of anxiety, research assessing multiple domains typically analyzes not only affective, but also locomotor and cognitive domains and their interactions. For the model to be valid, these domains would have to be similarly affected in the disorder between clinical and experimental data
- The concept of *omes* is used to describe a system of interacting entities of biological information (see **Table 3** for examples of specific *omes*). Similarly, the term *-omics* pertains to the study of an *ome*-based system. A primary aim of *-omics* analyses is mapping the interactions and relationships among the biological objects comprising an *ome*



proopiomelanocortin gene expression, while reducing the anxiolytic action of bromazepam [71].

Due to the development of microarray techniques, a rapid high-throughput analysis of the activity of multiple genes became possible [61,74-75]. Occasionally described as 'fishing expeditions' by some critics, such high-throughput genome-wide screens have tremendous potential for uncovering new gene networks and pathways of anxiety [76-78]. We argue that global genome-wide interrogation needs further appreciation from the scientific community studying affective disorders and should be encouraged in anxiety research to complement more specific, mechanistically driven single-gene or single-pathway analyses.

### 2.3 Applying new behavioral analyses

As already mentioned, current behavioral phenotyping methods are often limited to examining individual domains within a multifaceted disorder. However, such myopic focus in a model may fail to describe the complex dynamics of the disorder in question, and is inconsistent with clinical diagnoses representing an integral continuum with common genetic and environmental factors [12]. The domain interplay concept (**Table 1**) offers a new strategy to dissect complex neurobehavioral phenotypes, assessing systems of integrated domains rather than individual behaviors [12,79]. For example, with common neural, genetic and environmental determinants, it is not surprising that anxiety and depression are highly comorbid [80]. While these disorders have traditionally been modeled individually, the recent conceptualization of anxiety and depression as a common affective spectrum calls for models to mimic this continuum [29,30]. By virtue of focusing on the interplay of already integrated domains, the domain interplay concept is also suitable for the integration of affective and non-affective phenotypes, given the complex 'continuum' nature of brain pathogenesis which is becoming widely recognized in biological psychiatry [81-84].

With the advent of video-tracking and data-mining technologies, new methods are emerging to characterize and quantify anxiety-like phenotypes [85]. For example, three-dimensional (3D) imaging has been recently applied to zebrafish behavior [86] using data-mining IT tools to extract and integrate manual and automated anxiety-related end points (see [18,87] for details). While the field of zebrafish neurobehavioral research is rapidly expanding, fast and objective quantification of behavior is needed to supplement the often time-consuming and variation-prone manual registration. The 3D approach allowed a dissection of complex behavioral responses across multiple automated end points, identifying previously undetectable behavioral events sensitive to anxiolytic and anxiogenic drugs and mapping them within 3D coordinates [18,86-87], and applying intuitive visualization to globally evaluate and interpret the observed affective states [86-90].

Mounting research has also focused on temporal aspects and global assessment of animal anxiety-related behavioral activity. For example, near-infrared illumination in the PhenoScan system (CleverSys, Inc., Reston, VA, USA) enables tracking of animals over a 24-h period, without detriment to light-cycle behavior [91-93]. A similar approach has been used with TSE (TSE Systems, Bad Homburg, Germany) phenotyping tools [94,95]. Such constant monitoring complements multi-dimensional and multi-domain neurophenotyping to maximize model validity and accuracy, and enables a less invasive assessment of acute or delayed behavioral responses to pharmacological challenge. The latter may be particularly useful for testing strains with high basal anxiety, preventing ceiling/floor effects or performing more ethological assessment of drug action. Notably, these methodologies have only become possible due to the recent availability of technologies and tools, and further exciting developments in this field will emerge soon.

### 2.4 Applying bioinformatics for analyses of anxiety behaviors and *-omics*

In models attempting to reproduce the entire syndrome of complex disorders, the need for multiple simultaneous end points makes it difficult to apply the experimental

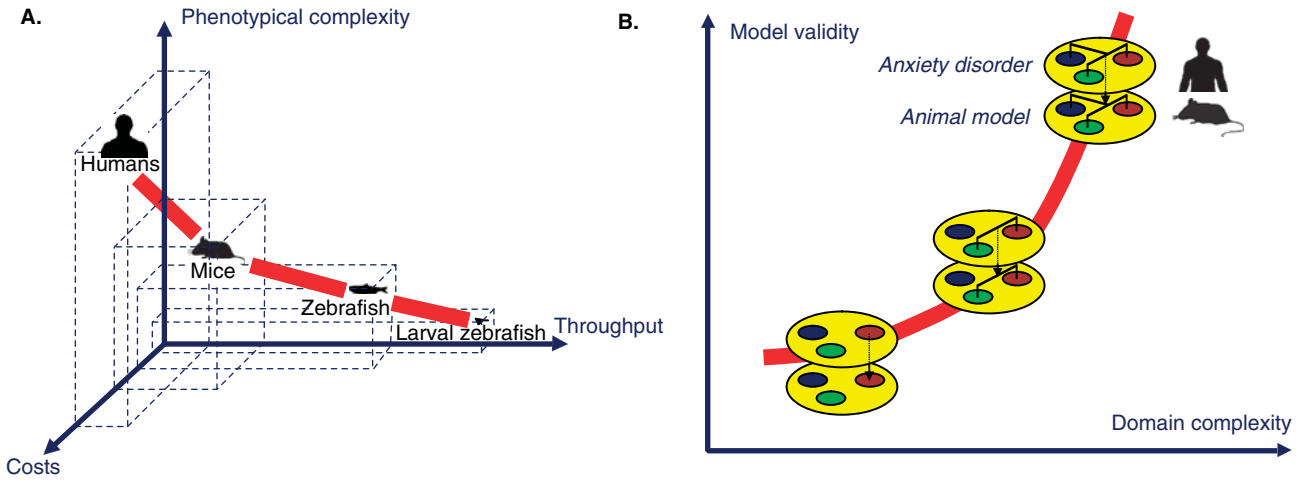


**Figure 1. Translational cross-species modeling approach to anxiety and anxiolytic drug research. Panel A** shows phenotypical complexity, throughput and cost-efficiency of several popular model species (humans, mice, adult and larval zebrafish). Panel **B** illustrates how the validity of various experimental models can increase, based on domain interplay concept (see **Table 1** for details), as modeling shift from focusing on single domains to modeling a system of domains. In this panel, anxiety disorder is shown as a larger circle (two parallel circles represent anxiety-like states in two different species, such as mice and humans).



manipulations to establish underlying mechanisms [96]. The proposed focus on modeling several domains and the use of modern video-tracking tools described above make this task even more challenging due to multiple end points per domain, resulting in a rapidly increasing amount of behavioral data. Therefore, the use of bioinformatics tools becomes crucial to examine this amount of data and identify patterns and phenotypes, as well as to decipher multiple interconnected underlying physiological pathways.

The era of genomics is rapidly impacting experimental anxiety research, as genetic factors play an important role in affective disorders, and complex traits cluster based on their genetics [97-100]. High-throughput genomics has provided an extraordinary view into the genetic architecture of animal and human behavior, the interconnectivity of complex traits [101,102] and 'network' models of animal anxiety phenotypes (powered by bioinformatics analyses and extensive publicly available online databases; **Table 2**), which are crucial to explore affective processes [10,103-105]. For example, web-based tools, such as Lirnet and eQTL Viewer (**Table 2**), offer efficient and intuitive methods to explore transcriptional regulation [106], while the Mouse Phenome Database (MPD), Mouse Genome Informatics (MGI) or PhenoGen (**Table 2**) integrate genetic, genomic and other biological data to facilitate gene characterization, mapping and the identification of inter-strain phenotypes. With the availability of marker information from HapMap or GenBank databases (**Table 2**) and high-density single nucleotide polymorphism (SNP) genotyping platforms, the correlations between candidate genes and their contribution to a behavioral phenotype also becomes possible (**Figure 3**) [107].

The premise of such approaches is that an amalgamation of candidate genes for a particular phenotype may lead to a functional explanation of the etiology of that phenotype [108]. For example, a specific mouse anxiety-related phenotype in one research project (e.g., vertical rears in the open-field test) can now be linked to a gene or chromosomal region through the MPD or to specific gene mutations using MGI (**Table 2**). Furthermore, two different projects (e.g., one behavioral and another microarray-based) performed in two different laboratories on the same mouse strain can be correlated together in the same way for the integrated search of anxiety phenotypes and markers (**Figures 2** and **3**). The identified mouse gene can then be linked to human analogs using the Online Mendelian Inheritance in Man browser or run through a genome-wide association study database to identify a list of candidate SNPs that correlate with human variation in anxiety phenotype susceptibility, thus linking biological data across different species (**Figures 2** and **3**).

More specific intra-species strain analyses are also possible with this approach. For instance, a recent gene expression analysis of C57BL/6J and A/J mouse inbred strains using MPD revealed gene network specificity for different brain regions and limited interaction effects between these strains and brain region [109]. Strain differences in behavioral responses to stress can also be accompanied by differential expression in various anxiety-related genes [104,110-111]. For example, genetically driven variation in corticolimbic function underlies individual differences in anxiety responses [112], as stress upregulates circadian genes in DBA/2J mice but mainly alters plasticity-related genes in C57BL/6J strain. Such *omics*-based analysis shows how the corticolimbic 'stress'

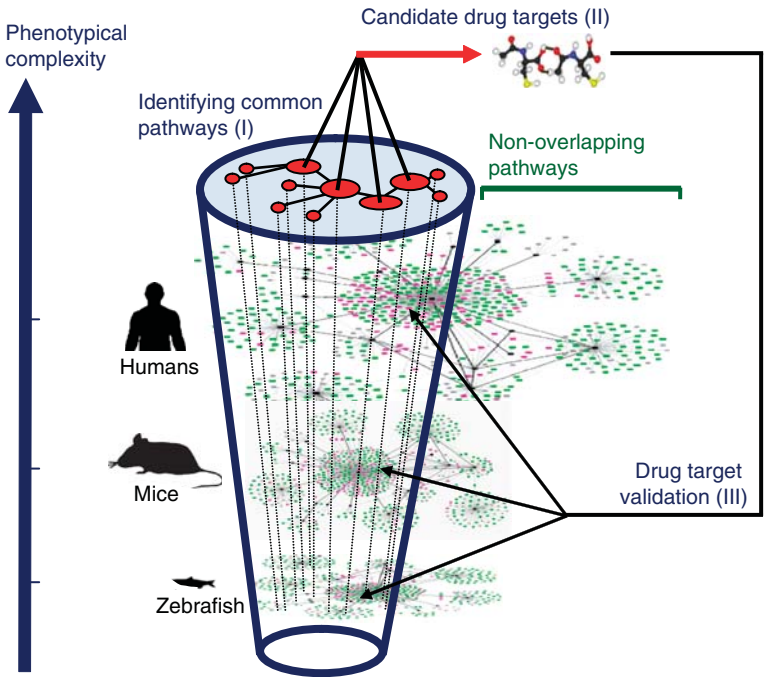


**Figure 2. The strategy of anxiolytic drug discovery based on identifying common, evolutionarily conserved 'core' affected genetic/molecular pathways (I), followed by the development of novel drugs targeting these pathways (II) and their subsequent validation based on the ability to affect anxiety phenotypes and the identified molecular targets (III).**

network may be underscored by various gene sets serving as correlates of the divergent behavioral responses to anxiety in DBA/2J and C57BL/6J mice [111].

Another promising application of genomic approaches is mapping quantitative trait loci (QTLs) for behavioral disorders [113,114]. Numerous reports have already identified QTLs for anxiety behavior in various animal models [115-118], including translationally valuable QTLs with homologous regions on human chromosomes [117,119]. Furthermore, the ability to use mean phenotypic values from inbred strains to map likely genomic locations of QTLs '*in silico*' markedly accelerates genetic analysis of animal disease models [114]. As an alternative to traditional QTL mapping, *in silico* mapping simultaneously exploits phenotypic, genotypic and pedigree data already available in breeding programs [114,120]. This computational method can predict the chromosomal regions that most likely contribute to complex traits of experimental intercross populations for multiple traits analyzed, while exponentially reducing the time required for analysis [114]. Application of this approach to genetics of anxiety-related phenotypes may reveal further clusters of candidate genes, again leading to potential new molecular targets for anxiolytic compounds.

Further development in the integration of heterogeneous data, in particular gene and protein expression pathways, will also be critical for *-omics* data interpretation. Deciphering such networks poses one of the greatest challenges in current systems biology [121], crucial for the successful elucidation of pathways and circuits involved in anxiety. While the typical approach to microarray analysis is to map *a posteriori* the results onto gene networks to dissect pathway-level expression changes, integrating *a priori* knowledge of the gene networks may provide even more powerful analysis [122]. For example, recent work based on the spectral decomposition of gene expression profiles to filter out high-frequency components with respect to known pathways has already produced more biologically relevant results that allow for a direct biological interpretation [122]. Moving beyond technological approaches, public servers such as GraphWeb (**Table 2**) have emerged as another promising avenue, allowing users to integrate heterogeneous and multispecies data in order to construct and interpret individual or multiple merged networks [121,123-124].

### 2.5 Applying various *omes* to anxiety research

In addition to genomic responses, mounting evidence links proteome changes to certain anxiety states, showing altered patterns of protein expression and genotypic differences relevant for anxiety phenotypes [125-127]. For example, alterations in proteins related to serotonin receptors, carbohydrate metabolism, cellular redox system and synaptic docking are involved in anxiety [127]. Thus, research focusing on the receptorome can be useful in identifying molecular targets and characterizing the interactions between interconnected signaling pathways affected by experimental or pharmacological manipulations [128-130]. For example, receptorome screening has been important in determining that the κ-opioid receptor

**Table 2. Selected publicly available online bioinformatics-based databases and data-mining servers.**

| Project name | Maintained by | Description | Can users submit data? | Potential application to anxiolytic drug research |
|---|---|---|---|---|
| Mouse Genome Informatics [156] | The Jackson Laboratory (Bar Harbor, USA) | An extensive database drawing from multiple projects, providing integrated genomic and phenotypic mouse data, including mutations and transgenic models for phenotypes or diseases [157,158] | Yes | Analyzes multiple gene–phenotype correlations for a large number of transgenic and mutant mouse strains |
| Mouse Phenome Database [159] | The Jackson Laboratory, (Bar Harbor, USA) | Contains an extensive repository of genotypic and phenotypic data, which allows for genotype–phenotype association predictions in multiple inbred strains of mice [151,160] | Yes | Assesses gene–phenotype correlations in a large number of inbred mouse strains from raw data from multiple projects |
| PhenoGen Informatics [161] | University of Colorado – Denver (Denver, USA) | A microarray data repository and analysis tool allowing users to research candidate genes and QTL regions [162-164] | Yes | Examines QTL–phenotype correlations related to anxiety behaviors |
| International HapMap Project [165] | National Human Genome Research Institute, NIH (Bethesda, USA) | Continuing to develop a haplotype map of the human genome to describe the common patterns of human DNA sequence variation [166-169] | No | Catalogs common genetic variants (SNPs) in humans, useful for predicting drug responses in animal models, identifying candidate genes or target pathways |
| Reactome [170] | European Bioinformatics Institute (Cambridge, UK) | A database of human biological pathways able to infer orthologous events in other organisms [171-173] | No | Computationally infers biochemical pathways for numerous animal models (based on human homologues) |
| GenBank [174] | National Center for Biotechnology Information (NCBI/NIH, Bethesda, USA) | A comprehensive annotated database of nucleotide sequences and their protein translations for > 300,000 organisms [175] | Yes | Catalogs common genetic variants (SNPs) across multiple species, useful for predicting drug response after animal models reveal candidate genes or target pathways |
| Online Mendelian Inheritance in Man [176] | Johns Hopkins University School of Medicine (Baltimore, USA) | A compendium of human genes and phenotypes, containing information on all known Mendelian disorders and > 12,000 genes [177-179] | Can submit via NCBI | Catalogs disorder-specific genetic variants in humans |
| Online Mendelian Inheritance in Animals [180] | University of Sydney (Sydney, Australia) | A compendium of genes and genetic phenotypes in animal species besides humans and mice [181,182] | Yes | Catalogs disorder-specific genetic variants in numerous animal models |
| GraphWeb [183] | University of Tartu (Tartu, Estonia) | A public web server for graph-based analysis of biological networks, allowing users to integrate heterogeneous and multispecies data [121] | No | Analyzes and visualizes gene–protein–phenotype networks across multiple organisms |
| Lirnet [184] | Stanford University (Stanford, USA) | Estimates how likely a sequence variation is to have a significant effect on gene expression [185] | No | Can show the extent to which the different features influence regulatory potential, including gene function |
| eQTL viewer [186] | North Carolina State University (Raleigh, USA) | Visualizes the relationships between the expression trait genes and the candidate genes in the eQTL regions [187] | No | Can help explore transcriptional regulation patterns and generate hypotheses on the genetic basis of transcriptional regulations |
| PhenomicDB [188] | MetaLife AG (Winden, Germany) | A multi-organism phenotype–genotype database of gene indices and orthologues [189-191] | No | Compares phenotypes of a given gene in several organisms simultaneously |
| Allen Brain Atlas [192] | Allen Institute for Brain Science (Seattle, USA) | A searchable atlas of gene activity patterns throughout the C57BL6/J mouse brain [193,194] | No | Depicts the regional expression of genes; may be used to identify anxiety-related circuits based on expression of specific 'anxiety' genes |

NCBI: National Center for Biotechnology Information; QTL: Quantitative trait locus; SNP: Single nucleotide polymorphism.

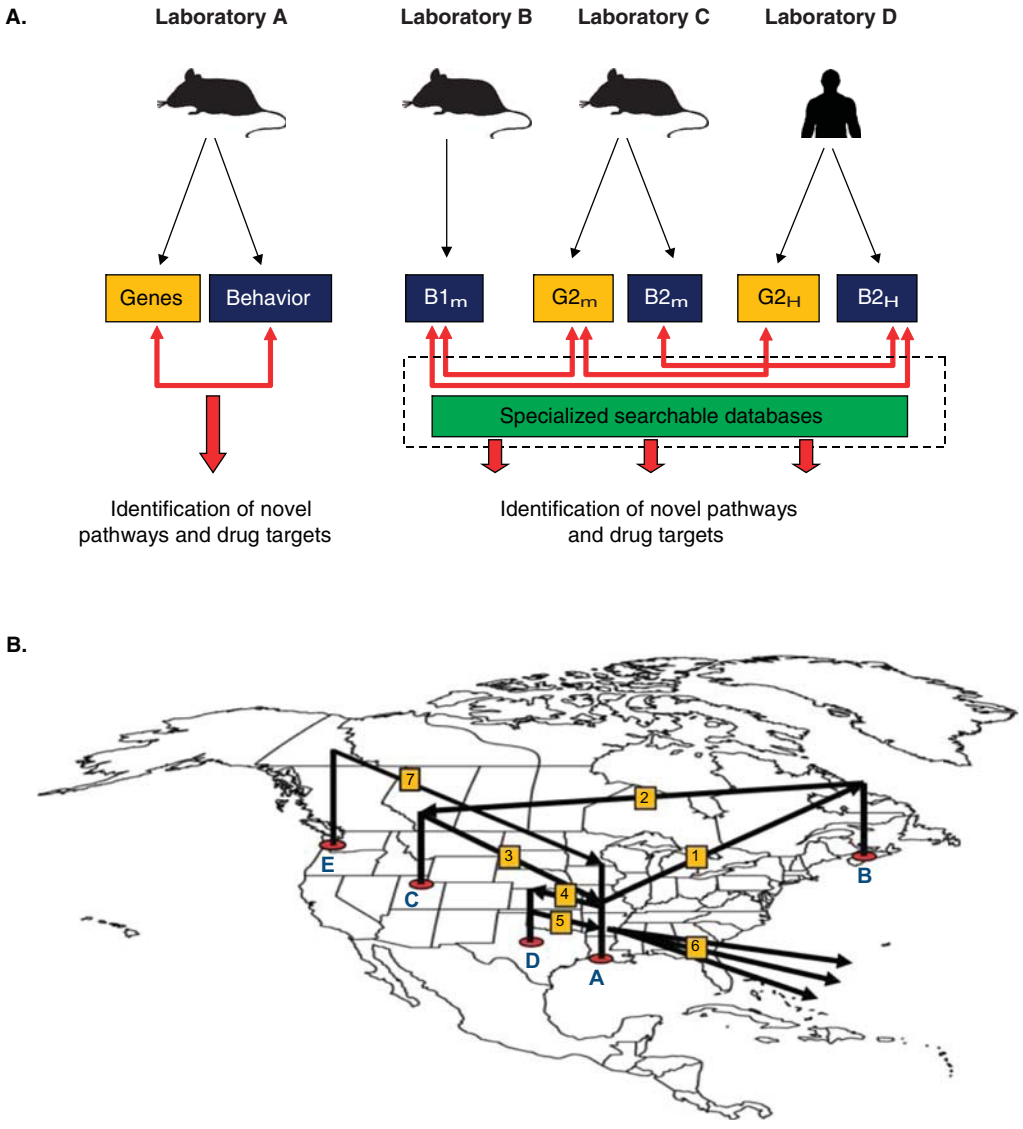


**Figure 3. The role of bioinformatics tools in shaping anxiolytic drug research. Previously, as shown in Panel A** (left), behavioral and *-omic* data (e.g., genomic profiles) were correlated within the same laboratory (laboratory A), leading to a limited number of phenotypes and implicated pathways obtained in a single model species (e.g., mice). As shown in the right part of Panel **A**, currently available searchable databases (see **Table 2** for details) enable complex data integration and interrogation, including correlation of mouse behavioral data ($B1_m$) obtained in one laboratory (Laboratory B) with genomic ($G2_m$) or behavioral ($B2_m$) data obtained in a different laboratory (Laboratory C) working with the same model species. Furthermore, cross-species translational analyses using these databases enable comparison of mouse anxiety-like behavioral phenotypes $B1_m$ (obtained in laboratory A) with human behavior $B2_H$ collected and deposited by a clinical Laboratory D. Similarly, the implicated mouse gene networks $G2_m$ (from Laboratory C) can be paralleled with the homologous human genes $G2_H$ (from Laboratory D) to identify potential drug targets. Panel **B** illustrates how information from various currently available databases (see **Table 2** for details) can be used to advance knowledge about anxiety disorders and their pathways in different species. In this hypothetical case, the Kalueff laboratory (Laboratory A; Tulane University in New Orleans, LA, USA) accessed Mouse Phenome Database (step 1) to obtain data on C57BL6/J mouse open field vertical rears generated in 2005 by the Brown laboratory (Laboratory B; Dalhousie University, Halifax, Canada; step 2) and correlates them with genomic data obtained in 2007 by the Tabakoff laboratory (Laboratory C; University of Colorado in Denver, CO, USA; step 3). This analysis identified 20 – 30 genes showing highly significant and consistent correlation in several behavioral tests of anxiety, suggesting that some of these genes may represent drug targets for novel anxiolytic drugs. Molecular network analysis performed by Lab A identified several specific pathways implicated in mouse anxiety (step 4), which will then be used by a zebrafish group (Laboratory D; the University of Texas Health Sciences Center San Antonio, TX, USA) to parallel mouse genes with zebrafish orthologs and reconstruct zebrafish genes which may be implicated in anxiety behaviors. Subsequent microarray experiments performed in this lab will re-confirm a group of ~ 10 genes from that list, whose expression was altered in zebrafish exposed to anxiety tests (step 5). This information will be deposited by Laboratory D to the Zebrafish Neurophenome Database, maintained by Laboratory A, to become available to a large number of zebrafish researchers worldwide (step 6). Meanwhile, the pattern of expression of the specific mouse genes identified as a 'candidate' during the previous steps 3 - 4 can be examined for their regional distribution using Allen Brain Atlas database (Laboratory E, Seattle, WA, USA), revealing specific affected brain areas which may represent a novel circuit for anxiety-related behaviors (step 7).

**Table 3. Examples of *omes* and *omes*-based approaches (see [195] for details) for integrating biological information potentially relevant to modeling anxiety and anxiolytic drug discovery.**

| Ome | Definition | Potential applications to anxiety research |
|---|---|---|
| Connectome | The neuronal connection matrix of the brain | Can decipher emotionality circuits related to anxiety |
| Interactome | A complete set of macromolecular interactions, such as between protein and other intercellular molecules | Can predict physiological reactions based on a change in another state, including drug responses |
| Metabolome | The complete set of small-molecule metabolites | Can predict changes in metabolic state in response to anxiety and/or drug treatment |
| Pathome | An integrated molecular basis for the pathophysiology of a phenotype subset of a condition | Model pathogenesis, can be applied to the anxiety spectrum |
| Peptidome | A complete set of all peptides in an organism's body | Altered peptide profile due to change in protein expression/regulation, may reveal molecular pathways related to anxiety |
| Physiome | The quantitative description of the physiological dynamics or functions of the intact organism | Model physiological state functions, can be applied (as a systems biology approach) to modeling normal vs abnormal emotionality |
| Proteome | The entirety of proteins expressed by an organism | Can reveal altered protein expression profiles, including protein biomarkers of anxiety disorders |
| Receptorome | The portion of the proteome encoding various receptors | Can identify molecular targets and characterize interactions between signaling pathways implicated in anxiety |
| Regulome | The whole set of regulation components in a cell | Can examine regulatory effects on genetic and protein expression profiles implicated in anxiety |
| Signalome | The identification of all signaling components in all messenger-mediated transduction | Can identify molecular targets and characterize interactions between signaling pathways implicated in anxiety |
| Unknome | A large proportion of unnamed genes, currently without functional information | Can reveal multiple genes with previously unknown functions, which influence anxiety-like behavior |



is the pharmacological target of salvinorin A [131-133]. Receptoromics has also been utilized to discover novel therapeutic treatments, such as the observation that mGluR2/3 agonists can have anxiolytic effects [134].

Recent research has also applied metabolomics, including modeling metabolic networks [135-137] and biomarkers [137,138]. For example, *in utero* labeling of mice using a $^{15}$N-enriched diet has been used for metabolomic analysis to reveal differential levels of metabolites in several mouse strains with different anxiety levels [139]. Moreover, metabolome models may also be used to predict changes in metabolic state in response to drug treatment (i.e., pharmacometabolomics) [140]. The application of a metabolomic analysis is critical in psychopharmacological research, as the understanding of metabolites and their interactions gives insight into the mechanistic pathways affected by experimental challenge. This level of analysis can also elucidate the progression of pathogenic conditions, such as anxiety spectrum disorders, as well as the co-morbidity of such pathology with metabolic syndromes. For example, the mechanism by which neuropeptide Y and the endocrine stress axis (CRH and cortisol) integrate in response to acute or chronic stress has been shown to markedly affect obesity and related metabolic pathways [141].

Growing efforts are also being made to develop a connection matrix to comprehensively map the neural connections of the brain [142-145]. This connectome-based analysis increases our understanding of how affective processes emerge from their morphological substrates, providing new mechanistic insights into how brain function is affected if this structural substrate is disrupted [145]. The connectome has been assessed from the level of single neurons and synapses (microscale) and the level of anatomically distinct brain regions and inter-regional pathways (macroscale) [145]. Recently, clinical functional imaging has revealed a universal architecture of positive and negative functional connections as well as consistent loci of inter-individual variability [146]. While primarily focused on the normal human brain, future work may also expand our knowledge of network topology and dynamics in the developing and diseased brain, as well as the brains of animal models [144]. Taken together, it is becoming important to apply *omes*-based analyses to more comprehensively define anxiety spectrum pathologies, identify the targets affected and characterize their impact on signaling pathways, proteomes, receptoromes and metabolomes, as well as broader connectomes and functionality (see **Table 3** for several *omes*-based approaches).

## 3. Conclusion

Current anxiety behavioral paradigms are often encumbered by an 'artificial' heterogeneity stemming from single-domain and single-gene or single-pathway models, thereby limiting the behavioral dissection of complex phenotypes [10,12,13,79].

However, a comprehensive understanding of the neurobiological mechanisms underlying anxiety-spectrum disorders is essential for developing new effective therapies. Here, we outlined a domain interplay-oriented approach to modeling anxiety disorders, powered with video-tracking, bioinformatics tools and online databases to better understand the interactions and complex trait interconnectivity of affective disorders. We call for further bridging between conceptual innovations in behavioral neurophenotyping and modern -omics approaches, as anxiety researchers today are no longer alone face-to-face with a mouse or a rat tested in a behavioral apparatus. While the behavioral data obtained in this experiment can immediately undergo a sophisticated behavioral analysis to reveal multiple additional endpoints, the *omics*-based data from this experiment can be correlated with these behavioral end points to reveal novel associations, molecular networks and pathways within an interdisciplinary systems biology approach (**Figure 2** and **3**).

## 4. Expert opinion

Human affective states are complex, multifaceted and polygenic disorders that remain poorly understood disorders [147,148]. Animal models have become invaluable to basic research of anxiety disorders, enabling researchers to screen novel pharmacological compounds and study genetic and environmental influences on the implicated neural pathways [9,10,148].

The need to maximize the data density requires improved phenotyping strategies [11] and conceptual innovation focused on integration of animal modeling across several different, clinically relevant domains (**Table 1**) [11]. We have argued previously that the throughput and utility of animal models of brain disorders can be markedly increased by analyzing several domains and their interplay [12,79]. It is critical to identify and describe multiple domains (e.g., locomotor, cognitive, affective) involved in a particular disorder in order to improve diagnostic criteria and preventative techniques (e.g., drug or gene therapy) in clinical settings. Furthermore, it is important to assess novel compounds for their efficacy in treating both single and multiple domains, and how acute and chronic treatment may resolve certain abnormal traits within one domain while not affecting others. However, as we move toward higher-throughput assays, caution must be taken not to trade validity for expeditious results. Indeed, the focus on quick, high-throughput single-domain assays in anxiety research has complicated drug discovery, as a lack of complexity has led to difficulties in translating preclinical findings to clinically active drugs. Therefore, as discussed here, novel approaches using sophisticated video-tracking combined with bioinformatics tools will foster further innovations in the field of anxiolytic drug design and discovery (**Figures 1 – 3** ).

This strategy will also enable a more comprehensive global behavioral characterization of anxiety-related responses, increased throughput and more thorough identification of biological markers. Molecular genetics and bioinformatics-based techniques, in combination with the extensive new body of genome information (**Table 2**), are currently revolutionizing the way in which physiological processes are investigated [105,149-153]. Publicly available online resources allow the researchers around the globe to rapidly evaluate possible correlations between candidate genes and their potential contribution to a particular pathogenic phenotype (**Table 2**) [151,154-155]. It is now time to more actively apply these approaches to animal modeling of anxiety and the search for new anxiolytic drugs (**Figures 2** and **3**).

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## Declaration of interest

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Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.


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