Published in final edited form as:

Curr Opin Neurobiol. 2015 February ; 0: 122–130. doi:10.1016/j.conb.2014.12.002.

The study of psychiatric disease genes and drugs in zebrafish

Martin Haesemeyer and Alexander F. Schier

Department of Molecular and Cellular Biology, Broad Institute, Center for Brain Science, Harvard Stem Cell Institute, Center for Systems Biology, Harvard University, 16 Divinity Avenue, Room 1027, Cambridge MA 02138

Martin Haesemeyer: haesemeyer@fas.harvard.edu; Alexander F. Schier: schier@fas.harvard.edu

Abstract

Mutations associated with psychiatric disease are being identified, but it remains unclear how the affected genes contribute to disease. Zebrafish is an emerging model to study psychiatric disease genes with a rich repertoire of phenotyping tools. Recent zebrafish research has uncovered potential developmental phenotypes for genes associated with psychiatric disorders, while drug screens have behaviorally characterized small molecules and identified new classes of drugs. Behavioral studies have led to promising models for endophenotypes of psychiatric diseases. While further research is needed to firmly link these models to psychiatric disorders, they are valuable tools for phenotyping genetic mutations and drugs. Recently developed tools in genome editing and *in vivo* imaging promise additional insights into the processes disrupted by mutations in psychiatric disease genes.

Psychiatric diseases such as schizophrenia, depression or autism spectrum disorder (ASD) have debilitating effects on people's lives. It is therefore of great importance to understand the underlying causes of psychiatric diseases and develop effective therapeutics. Human genetics and genomics studies have begun to identify candidate genes that contribute to psychiatric disorders [1–4], but it remains largely unclear how these genes act during normal development and physiology and how gene variants contribute to disease in some individuals but not others. Non-human model organisms provide an opportunity to help identify and determine the function of psychiatric disease genes [5]. Although such model systems will never replicate the full disease phenotype present in humans [6], genetic studies of human disease genes can provide molecular and cellular insights into gene function. In addition, the study of behavioral endophenotypes – focusing on stable phenotypic aspects rather than complex symptoms – can help dissect the development and function of circuit motifs that may be affected in humans [7].

In this review, we will discuss the potential of zebrafish to identify and analyze psychiatric disease genes as well as to discover and characterize drugs that could potentially treat

© 2014 Elsevier Ltd. All rights reserved.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

psychiatric disorders. Although mammalian models are evolutionarily closer to humans and are thus well suited to study the most complex physiological and behavioral aspects of psychiatric disease, zebrafish share many anatomical, physiological and genomic features with humans, have an archetypical vertebrate brain [8–10], and cellular, molecular and functional processes can be studied with ease (Tables 1 and 2). Moreover the powerful combination of high-throughput genetic and high-resolution imaging technologies applicable to this model system have made zebrafish an effective system to study diseases ranging from blood disorders to cancer [11,12]. In the context of human diseases, three general approaches are used in zebrafish. First, zebrafish phenotypes that resemble some aspects of human disease phenotypes can be used to screen for genes that may be partially causal to the disease in humans (zebrafish phenotype > human disease gene). For example, mutations in EGF-CFC protein one-eyed pinhead were found to cause organ laterality defects in zebrafish [13]. This observation then led to the discovery that some human patients with laterality defects harbored mutations in a human orthologue of one-eyed pinhead [14]. Similarly, the discovery of ferroportin as an iron transporter in zebrafish and its involvement in hemochromatosis led to the discovery of human mutations [15,16]. Second, the genetic manipulation and phenotypic study of zebrafish orthologues of human genes can provide fundamental insights into the molecular and cellular roles of disease genes (human disease gene -> zebrafish phenotype). For example, Cadherin 23 had been implicated in human deafness syndromes but it was only through detailed studies in zebrafish that this protein was recognized to be a tip link component required for hair-cell mechanotransduction [17]. In another example, the FTO gene had been implicated in type 2 diabetes in humans, but only studies in zebrafish indicated that the underlying cause is the misregulation of the pancreatic transcription factor IRX3 [18,19]. Third, screens for drugs that induce or suppress specific phenotypes can identify small molecules that might be useful for the treatment of human disease (zebrafish drug phenotype -> human therapeutics). For example, prostaglandin E2 was discovered as an enhancer of haematopoietic stem cell development in zebrafish and is now tested in clinical trials [20]. Drug screens in zebrafish can also identify potent compounds even when the initial phenotypic assays are not directly related to the disease phenotype. For example, the BMP inhibitor dorsomorphin was first discovered as a dorsalizing factor during zebrafish development [21], but it is now a promising lead in the treatment of *fibrodysplasia ossificans progressiva*, a bone disease caused by overactive BMP signaling [22]. In the following sections we discuss how these three approaches can help understand and potentially treat psychiatric disease.

From human gene to zebrafish phenotype

Recent studies have begun to investigate the developmental roles of genes associated with psychiatric disorders in humans using zebrafish as a model system. Those studies have for the most part focused on autism spectrum disorders, which are known to have strong developmental underpinnings [23] and on mutations linked to the risk of developing schizophrenia.

Genes linked to autism

Genome wide association studies often pinpoint large genetic intervals with several candidate genes, creating a major challenge to find the causative gene. Recent studies suggest that the potential contributions of these genes can be systematically assessed in zebrafish. For example, both deletion and duplication of a 593kb spanning region on chromosome 16 has been associated with autism in humans [4]. The region of the 16p11.2 microdeletion contains 29 annotated genes, 24 of which have orthologues in zebrafish [24]. Two studies employed zebrafish to assess the relative importance of these genes in development. Golzio et al. used mRNA injection to overexpress all human genes within the region. Overexpression of KCTD13 led to a reduction in head size, whereas overexpression of other genes in the region did not cause a phenotype. Intriguingly, morpholino knockdown of KCTD13 caused an increase in head size, likely owing to an increase in the number of neurons in the brain [24]. These phenotypes are in agreement with the fact that deletion of 16p11.2 leads to macrocephaly, while duplication of the region is associated with microcephaly [24]. A complementary study by Blaker-Lee et al. used morpholino knockdown of all genes within the region to address their importance, instead of pre-screening by overexpression. This study reported neurodevelopmental defects upon knockdown of KCTD13, including reduced brain ventricle size and defects in responding to touch [25]. However, no change in head size was reported. This phenotypic disagreement could have arisen because different morpholinos were used or because of differences in phenotypic scoring. In addition, knockdown of almost all zebrafish homologs of the 16p11.2 microdeletion resulted in nervous system developmental defects with subtle phenotypic differences [25]. These two studies show how zebrafish can be used to assign specific functions to genes in larger regions associated with a disease, but they also highlight that the discovery of the key genes is strongly dependent on the screening approach.

Autism spectrum disorders display exceptional phenotypic diversity and represent diverse disease subtypes with potentially very different underlying causes. This presents a great challenge for developing treatment strategies, as diagnosis based on symptoms alone may not indicate underlying defects. To address this challenge, a recent study focused on mutations in the gene CHD8 as a potential subtype of autism [26]. The authors not only confirmed linkage of CHD8 truncations to ASD but also showed that most ASD patients with CHD8 mutations display macrocephaly and had gastrointestinal complaints [26]. Strikingly, morpholino knockdown of zebrafish CHD8 induced phenotypic parallels to ASD patients, including an increase in headsize as well as a reduction in gastro-intestinal motility [26]. The macrocephaly phenotype could be replicated using a somatic mutagenesis technique based on CRISPR/Cas9 [26]. This approach leads to mosaicism without knowledge of which cells carry mutant alleles. It will therefore be necessary to generate non-mosaic CHD8 mutants. These mutants can be used to move the phenotypic analysis from coarse defects such as head size to more detailed analyses, ranging from cellular to behavioral phenotypes (Table 1).

Genes linked to schizophrenia

While studies in humans have identified numerous disease-causing gene variants, it is often challenging to determine the biological function of the affected gene and explain how the

variant causes schizophrenia. Studies in zebrafish can reveal the developmental roles of these genes and thereby ultimately aid in understanding disease causality. For example, rare mutations in disrupted-in-schizophrenia-1 (DISC1) have been linked to schizophrenia and other psychiatric disorders through studies of a Scottish pedigree [27,28]. The role of DISC1 has recently been questioned since it was not linked to schizophrenia cases in large-scale GWAS studies [29]. However, it is possible that this disconnect arose because GWAS studies are not ideal to implicate rare mutations such as those reported for DISC1 [30]. Another concern arises from the fact that DISC1 mutations were as strongly linked to major depressive disorder as to schizophrenia in the original pedigree [29]. But even if DISC1 is not strongly linked to schizophrenia, it still serves as an example of how developmental phenotypes of genes implicated in psychiatric disorders can be studied in zebrafish. For example, morpholino-based knockdown of DISC1 impairs neural crest migration and differentiation in larval zebrafish, leading to an expansion of glial populations within the brain [31]. In contrast, another study reported severe defects in brain development and axonal wiring upon knockdown of DISC1 [32]. Future studies, ideally employing DISC1 mutants instead of morpholinos, will be required to untangle these seemingly different phenotypes and determine whether one is the underlying cause of the other. Intriguingly, wild-type human DISC1 but not variants with mutations linked to schizophrenia rescues the neurodevelopmental phenotypes in zebrafish [32]. This result suggests that conservation of molecular function can be exploited in zebrafish to define the activities of alleles associated with disease phenotypes.

Zebrafish behaviors as tools to study disease phenotypes

Zebrafish have a rich behavioral repertoire ranging from simple reflexes to more complex goal directed and social behaviors. Some of these behaviors promise to be directly applicable to the study of endophenotypes of psychiatric disorders. Moreover, even seemingly unrelated behaviors can be used to probe the effect of mutations or drugs on the function of neuronal circuits. This approach is especially valuable where behavioral readout can be combined with functional imaging to study neuronal computation in a behaving animal. We focus on only a few phenotypes but note that there is a very rich repertoire of developmental, physiological and behavioral phenotypes that can be scored in zebrafish (see Table 1).

A zebrafish behavioral model of stress and anxiety disorders

Major depression in humans is often linked to deregulation of the endocrine stress system, particularly the hypothalamus-pituitary-adrenal (HPA) axis [33,34]. In stressful conditions, activation of the HPA axis leads to the release of corticosteroids and other endocrine hormones. Importantly, the production of cortisol provides feedback inhibition on the HPA axis via the glucocorticoid receptor (GR). Lack of this feedback inhibition is often associated with depression, and dysregulation of the HPA axis may be a potential risk factor contributing to the disease [34].

The molecular and functional characteristics of the mammalian HPA axis are highly conserved in zebrafish, [35], making zebrafish a useful model to study stress and associated disorders [36]. For example, zebrafish with a mutation in the glucocorticoid receptor gene

have a hyperactive HPA axis, likely due to the lack of negative feedback usually provided via cortisol through the GR [37]. GR mutant adult fish show increased freezing and less wall exploration when placed in a novel tank [37], a measure of anxiety in zebrafish [38]. These behavioral effects are alleviated both by the selective serotonin reuptake inhibitor and antidepressant fluoxetine, as well as by the anxiolytic diazepam. These results validate the GR mutation as a behavioral model for depression-associated dysregulation of the stress axis and as a potential entry point to screen for drugs with similar effects as classical antidepressants.

The molecular conservation of the HPA axis also makes it possible to gain insight into the molecular genetic regulation of the stress system. Upon sensation of stressful stimuli, specific neurons in the hypothalamus secrete corticotropin-releasing hormone (CRH), which forms the first major step in the induction of the HPA axis. A recent study demonstrated that expression of CRH requires the transcription factor Orthopedia (Otp) [39]. CRH-expressing neurons in the mouse and zebrafish hypothalamus express Otp [39] but *otp* knockout mice die shortly after birth [40], precluding further analysis of its role in the stress system. In contrast, zebrafish have two Orthopedia orthologues, *otpa* and *otpb*. Zebrafish *otpa* mutants are viable but fail to induce CRH expression after physical stress [39]. Moreover, when placed into a novel tank *otpa* mutant fish showed more exploratory behavior than wild-type siblings. This indicates that *otpa* is required to enhance the novelty stress response [39]. Otpa binds to the CRH promoter in zebrafish and mice, indicating that it directly influences its transcription in both species. Otpa also activates transcription of a splicing regulator that seems to be critical in the termination of the stress response [39]. This study demonstrates how zebrafish can be used to obtain new molecular genetic insights into the regulation of a behaviorally relevant system such as the HPA axis.

Pre-pulse inhibition

Patients with schizophrenia often have defects in sensory gating of startle responses [7,41]. This is especially apparent as defects in pre-pulse inhibition (PPI). In PPI a medium intensity stimulus preceding a startling stimulus will normally attenuate the startle response. In patients with schizophrenia, pre-pulse inhibition is often reduced [7,41,42], and defects in PPI are also observed in relatives of schizophrenics [43]. This suggests a genetic link and hence maybe a common circuit mechanism underlying schizophrenia and the reduction in sensory gating. Defects in pre-pulse inhibition are therefore a promising endophenotype for the study of schizophrenia in model systems.

Larval zebrafish respond to strong auditory stimuli with characteristic short-latency escapes. These escapes are inhibited by a pre-pulse given up to 300ms before the startle-inducing stimulus [44]. This interval is in good agreement with human studies [42]. Intriguingly, PPI is mediated by dopamine, as the D1/D2-receptor agonist apomorphine attenuates the inhibition of the startle response [44]. This attenuation is reversed by the antipsychotic haloperidol [44], which is widely used in the treatment of schizophrenia. In addition, it has been shown that serotonin plays an important role in gating the startle circuit [45], making it feasible to interrogate two important neuromodulatory pathways using this behavior.

Shoaling as a model for social behaviors

Psychiatric disease phenotypes are often associated with defects in social behavior, most notably in autism spectrum disorders. From juvenile stages, zebrafish will aggregate into small transient groups, indicative of a social behavior. Fish prefer an environment where they can see conspecifics, arguing for a preference to form social groups [46]. Intriguingly, when presented with a choice, fish will preferentially form shoals with conspecifics that display pigmentation similar to fish in the rearing environment [46]. This suggests that shoaling preference is plastic and controlled by experience much like other social behaviors. Furthermore, drugs that have effects on human social behavior, such as ethanol, nicotine or LSD, also have small but significant effects on zebrafish shoaling [47,48]. These observations raise the possibility of a partial conservation of pathways between human social behaviors and shoaling in zebrafish.

Learning and memory

Learning disabilities have adverse effects on people's life, often accompany psychiatric diseases, and sometimes are associated with the same gene variants [49]. Understanding how neuronal circuits and genetic factors control learning and memory in a healthy brain will be crucial in understanding causes of learning disabilities. Avoidance conditioning is a simple learning paradigm in which juvenile or adult zebrafish learn to associate a visual cue with a noxious stimulus [50,51]. After association, the visual cue will either trigger an escape behavior, indicative of the attempt to avoid a noxious stimulus, or it will trigger a freezing response, indicative of heightened anxiety. Generally, flight responses will dominate over freezing after learning. Ablation of neurons in the zebrafish habenula region of the forebrain, however, will bias learned behavior towards freezing [50], and fish therefore fail to avoid the noxious stimulus [51]. These studies suggest that the habenula plays a role in suppressing innate fear responses in favor of active avoidance. Together with a role of the habenula in modulating anxiety [52], these observations put this evolutionarily highly conserved structure [53] at the center of both innate and learned behaviors in zebrafish. The habenula can therefore serve as a powerful circuit entry point in the study of learning and its interaction with stress and anxiety.

The utility of larval zebrafish for high-throughput drug screens

Due to their small size, larval zebrafish are highly amenable to *in vivo* drug screens in a multiwell format [54]. Two high-throughput behavioral drug screens in larval zebrafish demonstrate the power of this approach for the identification of drugs with human disease relevance.

Rihel et al. screened for drugs that alter rest-wake behavior in larval zebrafish [55]. Larval zebrafish display night-day activity patterns that resemble sleep-wake states. Neuropeptides implicated in regulating human sleep and arousal have strong effects on spontaneous and stimuli-induced locomotion in larval zebrafish [56,57]. This conservation of transmitter systems controlling a behavior similar to human sleep suggests that rest-wake activity in larval zebrafish can be used as a screening tool to identify and study drugs. Indeed, the drug screen by Rihel et al. described hundreds of molecules that altered zebrafish behavior.

Because different drugs caused different behavioral profiles, drugs could be clustered according to shared phenotypes. This approach revealed that drugs in a given cluster often shared biochemical mechanisms or indications in humans. The overlapping phenotypic effects of known drugs in zebrafish and humans indicated a conservation of circuits and transmitter systems, while the analysis of additional drug classes implicated ERG potassium channels and other pathways in rest-wake regulation [55].

Even behaviors that are seemingly unrelated to human disease can be used to profile psychoactive drugs and predict the action of novel compounds. Kokel et al. used a simple locomotion assay in larval zebrafish to behaviorally profile a large library of drugs [58]. The screening assay was based on the photomotor response (PMR), wherein zebrafish embryos respond to a light flash with increased locomotion [58]. Intriguingly, many known psychoactive compounds caused reproducible and distinct effects, and drugs known to have similar targets in humans also gave similar PMR phenotypes [58]. Thus, a simple locomotion based assay can be used to behaviorally profile drugs and help predict which novel compounds may act similar to known psychoactive drugs.

These studies illustrate two approaches for drug discovery in zebrafish. On the one hand simple behaviors that are not directly connected to a psychiatric disease in humans allow the profiling of small molecules and the identification of novel compounds that elicit similar behaviors as well-described drugs. On the other hand, validated behavioral assays that replicate parts of a psychiatric disease can be used to specifically search for novel compounds acting on a given disease phenotype.

Status of the field and Outlook

The application of zebrafish to understand and treat psychiatric diseases is still in its infancy. But the considerable conservation of anatomical, neurochemical and genomic features with humans and the recent establishment of powerful genome editing approaches, imaging technologies and screening tools make the zebrafish well suited for the discovery and characterization of psychiatric genes and drugs. We foresee four major areas of progress.

First, the phenotypic analysis of genes implicated in human disease could help define the molecular and cellular pathways that underlie psychiatric disease. Such studies rely on the high-confidence identification of the human gene variants that contribute to psychiatric disease, a challenge that is just beginning to be met. The analyses of disease genes in zebrafish must rely on the rigorous application of genetic tools. It is disconcerting that morpholino studies that focus on the same gene report different phenotypes and that several morpholino-generated phenotypes have not been reproduced in mutational studies [59]. With the advent of more advanced genome editing technologies in zebrafish [60–63], it is now possible to move beyond morpholinos and use rigorous mutational strategies to disrupt human disease genes in zebrafish.

While the complete zebrafish knock-out of human disease genes might give important insights in the cellular and molecular roles of such genes, some of the alleles generated in zebrafish may need to mirror the alleles found in human patients. This is particularly relevant for disease alleles that are regulatory (change of gene expression), hypomorphic

(partial loss-of-function), antimorphic (dominant negative) or neomorphic (change of function). Such studies will reveal whether the faithful replication of human alleles will have meaningful consequences in zebrafish. The large separation in evolution and genetic background effects could hinder the identification of an obvious phenotype. It will therefore be important to continue to develop phenotyping tools that can detect subtle developmental or behavioral aberrations (see Table 1) rather than use indirect proxies such as head size.

Second, genetic screens for mutations that lead to phenotypes associated with psychiatric disorders might help identify new genes, pathways and mechanisms underlying human disease. The behavioral paradigms presented above provide valuable end-points in such studies. However, it is currently unclear which endophenotypes should be the focus of such screens. For example, a link of human schizophrenia genes to pre-pulse inhibition would go a long way in establishing this zebrafish paradigm as a disease model for schizophrenia. Complex behaviors such as shoaling or learning may allow the isolation of gene variants affecting the higher cognitive functions that are often impaired in psychiatric diseases.

Third, large-scale small molecule screens can help identify psychoactive drugs that lead to new therapeutics. Zebrafish can serve as a step to prescreen large compound libraries *in vivo* to identify new drugs that have the same behavioral effects as already known therapeutics. These candidates can then be tested in models that more closely resemble human disease and thus lead to the identification of new drugs that may have higher efficacy or less side effects than currently used therapeutics. Given appropriate behavioral assays, zebrafish screens can also lead to the identification of completely novel drugs, but it remains unclear how easily the effects of novel drugs discovered in zebrafish can be translated to humans.

Finally, zebrafish might provide circuit- rather than gene-based approaches to understand psychiatric disease. Ultimately, psychiatric diseases are caused by a malfunctioning nervous system. Whole brain imaging may reveal how psychoactive drugs influence circuits during behavior while targeted activation and inhibition of specific classes of neurons or brain regions could induce phenotypes that resemble psychiatric disorders. For example, the habenula is a structure that is highly conserved among vertebrates and has been implicated in psychiatric disorders such as schizophrenia and major depression [53]. Habenular ablation in zebrafish impairs learning, and inactivation of the medial habenula in adult fish leads to heightened anxiety [50–52]. This makes the habenula an interesting candidate to understand circuit dynamics underlying mental disorders, and it will be important in the future to assign specific functions in the modulation of behavior to specific subsets of habenular neurons. Analogously, drug- or gene-induced changes in neural activity might be revealed and even suppressed by optogenetic approaches. For example fluoxetine is able to reduce anxiogenic effects caused by a glucocorticoid receptor mutation in larval zebrafish [64]. It may be possible to use the anxiolytic effect of fluoxetine together with optogenetic suppression or enhancement of the phenotype to screen for neurons controlling anxiety in zebrafish. In combination, these functional approaches may yield a new level of insight into psychiatric disease that so far has been challenging to obtain.

In summary, the field is on the verge of an exciting convergence of the technological progress in zebrafish with the advances in the identification of human gene variants that

increase the risk for psychiatric disease. As more and more disease-associated loci are discovered through human GWAS studies, it will become increasingly important to identify the functional role of associated gene variants. With the advent of new genome editing technologies, zebrafish can provide the necessary throughput and in vivo system for this task. The next ten years will reveal how studies in zebrafish models can help us understand or even treat psychiatric disorders.

Acknowledgments

We thank Florian Merkle, Iris Odstreil and Summer Thyme for critical comments on the manuscript and the Jane Coffin Childs Fund as well as the NIH for funding.

References

1. Consortium C-DGotPG. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet*. 2013; 381:1371–1379. [PubMed: 23453885]
2. Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF, Sklar P. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature*. 2009; 460:748–752. [PubMed: 19571811]
3. Purcell SM, Moran JL, Fromer M, Ruderfer D, Solovieff N, Roussos P, O'Dushlaine C, Chambert K, Bergen SE, Kahler A, et al. A polygenic burden of rare disruptive mutations in schizophrenia. *Nature*. 2014; 506:185–190. [PubMed: 24463508]
4. Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, Saemundsen E, Stefansson H, Ferreira MA, Green T, et al. Association between microdeletion and microduplication at 16p11.2 and autism. *N Engl J Med*. 2008; 358:667–675. [PubMed: 18184952]
5. Gould TD, Gottesman II. Psychiatric endophenotypes and the development of valid animal models. *Genes Brain Behav*. 2006; 5:113–119. [PubMed: 16507002]
6. Hyman SE. How far can mice carry autism research? *Cell*. 2014; 158:13–14. [PubMed: 24995974]
7. Gottesman II, Gould TD. The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry*. 2003; 160:636–645. [PubMed: 12668349]
8. Butler, AB.; Hodos, W. Comparative vertebrate neuroanatomy: evolution and adaptation. 2. Hoboken, N.J: Wiley-Interscience; 2005.
9. Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, Collins JE, Humphray S, McLaren K, Matthews L, et al. The zebrafish reference genome sequence and its relationship to the human genome. *Nature*. 2013; 496:498–503. [PubMed: 23594743]
10. Mueller, T.; Wullimann, MF. Atlas of early zebrafish brain development: a tool for molecular neurogenetics. 1. Amsterdam; Boston, Mass: Elsevier; 2005.
11. Ablain J, Zon LI. Of fish and men: using zebrafish to fight human diseases. *Trends Cell Biol*. 2013; 23:584–586. [PubMed: 24275383]
12. Lieschke GJ, Currie PD. Animal models of human disease: zebrafish swim into view. *Nat Rev Genet*. 2007; 8:353–367. [PubMed: 17440532]
13. Yan YT, Gritsman K, Ding J, Burdine RD, Corrales JD, Price SM, Talbot WS, Schier AF, Shen MM. Conserved requirement for EGF-CFC genes in vertebrate left-right axis formation. *Genes Dev*. 1999; 13:2527–2537. [PubMed: 10521397]
14. Bamford RN, Roessler E, Burdine RD, Saplakoglu U, dela Cruz J, Splitt M, Goodship JA, Towbin J, Bowers P, Ferrero GB, et al. Loss-of-function mutations in the EGF-CFC gene CFC1 are associated with human left-right laterality defects. *Nat Genet*. 2000; 26:365–369. [PubMed: 11062482]
15. Donovan A, Brownlie A, Zhou Y, Shepard J, Pratt SJ, Moynihan J, Paw BH, Drejer A, Barut B, Zapata A, et al. Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. *Nature*. 2000; 403:776–781. [PubMed: 10693807]

16. Njajou OT, Vaessen N, Joosse M, Berghuis B, van Dongen JW, Breuning MH, Snijders PJ, Rutten WP, Sandkuijl LA, Oostra BA, et al. A mutation in SLC11A3 is associated with autosomal dominant hemochromatosis. *Nat Genet.* 2001; 28:213–214. [PubMed: 11431687]
17. Sollner C, Rauch GJ, Siemens J, Geisler R, Schuster SC, Muller U, Nicolson T. Mutations in cadherin 23 affect tip links in zebrafish sensory hair cells. *Nature.* 2004; 428:955–959. [PubMed: 15057246]
18. Ragvin A, Moro E, Fredman D, Navratilova P, Drivenes O, Engstrom PG, Alonso ME, de la Calle Mustienes E, Gomez Skarmeta JL, Tavares MJ, et al. Long-range gene regulation links genomic type 2 diabetes and obesity risk regions to HHEX, SOX4, and IRX3. *Proc Natl Acad Sci U S A.* 2010; 107:775–780. [PubMed: 20080751]
19. Smemo S, Tena JJ, Kim KH, Gamazon ER, Sakabe NJ, Gomez-Marin C, Aneas I, Credidio FL, Sobreira DR, Wasserman NF, et al. Obesity-associated variants within FTO form long-range functional connections with IRX3. *Nature.* 2014; 507:371–375. [PubMed: 24646999]
20. Hagedorn EJ, Durand EM, Fast EM, Zon LI. Getting more for your marrow: Boosting hematopoietic stem cell numbers with PGE. *Exp Cell Res.* 2014
21. Yu PB, Hong CC, Sachidanandan C, Babitt JL, Deng DY, Hoyng SA, Lin HY, Bloch KD, Peterson RT. Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. *Nat Chem Biol.* 2008; 4:33–41. [PubMed: 18026094]
22. Yu PB, Deng DY, Lai CS, Hong CC, Cuny GD, Boussein ML, Hong DW, McManus PM, Katagiri T, Sachidanandan C, et al. BMP type I receptor inhibition reduces heterotopic [corrected] ossification. *Nat Med.* 2008; 14:1363–1369. [PubMed: 19029982]
23. Geschwind DH. Advances in autism. *Annu Rev Med.* 2009; 60:367–380. [PubMed: 19630577]
- 24*. Golzio C, Willer J, Talkowski ME, Oh EC, Taniguchi Y, Jacquemont S, Reymond A, Sun M, Sawa A, Gusella JF, et al. KCTD13 is a major driver of mirrored neuroanatomical phenotypes of the 16p11.2 copy number variant. *Nature.* 2012; 485:363–367. This study set out to assign specific functions to genes in the autism associated 16p11.2 microdeletion. A prescreen in zebrafish using overexpression of genes within the region identified KCTD13 as the only gene inducing a phenotype (in contrast to [25]). Similar to copy-number variants in humans, overexpression and knockdown of KCTD13 had opposing effects on headsize. [PubMed: 22596160]
- 25*. Blaker-Lee A, Gupta S, McCammon JM, De Rienzo G, Sive H. Zebrafish homologs of genes within 16p11.2, a genomic region associated with brain disorders, are active during brain development, and include two deletion dosage sensor genes. *Dis Model Mech.* 2012; 5:834–851. This paper used zebrafish to screen specific genes within the autism associated 16p11.2 genomic region for developmental phenotypes. Using morpholino-based knockdown the study identified partially overlapping developmental and behavioral phenotypes for almost all genes within the region (in contrast to [24]). [PubMed: 22566537]
- 26*. Bernier R, Golzio C, Xiong B, Stessman HA, Coe BP, Penn O, Witherspoon K, Gerdt J, Baker C, Vulto-van Silfhout AT, et al. Disruptive CHD8 Mutations Define a Subtype of Autism Early in Development. *Cell.* 2014; 158:263–276. This is an example of how zebrafish can be used as a model to identify developmental phenotypes for genes associated with disease risk in humans. The authors found that ASD patients with CHD8 mutations have distinct clinical typology, including an enrichment of gastrointestinal complaints. Morpholino-based knockdown of CHD8 in zebrafish caused developmental phenotypes that led to an increase in head-size and a reduction in gut motility. [PubMed: 24998929]
27. Millar JK, Wilson-Annan JC, Anderson S, Christie S, Taylor MS, Semple CA, Devon RS, St Clair DM, Muir WJ, Blackwood DH, et al. Disruption of two novel genes by a translocation cosegregating with schizophrenia. *Hum Mol Genet.* 2000; 9:1415–1423. [PubMed: 10814723]
28. Blackwood DH, Fordyce A, Walker MT, St Clair DM, Porteous DJ, Muir WJ. Schizophrenia and affective disorders--cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: clinical and P300 findings in a family. *Am J Hum Genet.* 2001; 69:428–433. [PubMed: 11443544]
29. Sullivan PF. Questions about DISC1 as a genetic risk factor for schizophrenia. *Mol Psychiatry.* 2013; 18:1050–1052. [PubMed: 24056909]

30. Porteous DJ, Thomson PA, Millar JK, Evans KL, Hennah W, Soares DC, McCarthy S, McCombie WR, Clapcote SJ, Korth C, et al. DISC1 as a genetic risk factor for schizophrenia and related major mental illness: response to Sullivan. *Mol Psychiatry*. 2014; 19:141–143. [PubMed: 24457522]
31. Drerup CM, Wiora HM, Topczewski J, Morris JA. Disc1 regulates foxd3 and sox10 expression, affecting neural crest migration and differentiation. *Development*. 2009; 136:2623–2632. [PubMed: 19570850]
32. Singh KK, De Rienzo G, Drane L, Mao Y, Flood Z, Madison J, Ferreira M, Bergen S, King C, Sklar P, et al. Common DISC1 polymorphisms disrupt Wnt/GSK3beta signaling and brain development. *Neuron*. 2011; 72:545–558. [PubMed: 22099458]
33. Holsboer F. The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology*. 2000; 23:477–501. [PubMed: 11027914]
34. Pariante CM, Lightman SL. The HPA axis in major depression: classical theories and new developments. *Trends Neurosci*. 2008; 31:464–468. [PubMed: 18675469]
35. Alsop D, Vijayan MM. Development of the corticosteroid stress axis and receptor expression in zebrafish. *Am J Physiol Regul Integr Comp Physiol*. 2008; 294:R711–719. [PubMed: 18077507]
36. Steenbergen PJ, Richardson MK, Champagne DL. The use of the zebrafish model in stress research. *Prog Neuropsychopharmacol Biol Psychiatry*. 2011; 35:1432–1451. [PubMed: 20971150]
- 37*. Ziv L, Muto A, Schoonheim PJ, Meijsing SH, Strasser D, Ingraham HA, Schaaf MJ, Yamamoto KR, Baier H. An affective disorder in zebrafish with mutation of the glucocorticoid receptor. *Mol Psychiatry*. 2013; 18:681–691. This study established a zebrafish glucocorticoid receptor (GR) mutant as a physiological and behavioral model of dysregulation of the major stress axis. GR mutants failed to terminate the stress response and showed signs of heightened anxiety. These behavioral effects could be alleviated by the antidepressant fluoxetine and the anxiolytic diazepam, indicating a conservation of transmitter systems controlling anxiety in zebrafish and humans. [PubMed: 22641177]
38. Levin ED, Bencan Z, Cerutti DT. Anxiolytic effects of nicotine in zebrafish. *Physiol Behav*. 2007; 90:54–58. [PubMed: 17049956]
- 39*. Amir-Zilberstein L, Blechman J, Sztainberg Y, Norton WH, Reuveny A, Borodovsky N, Tahor M, Bonkowsky JL, Bally-Cuif L, Chen A, et al. Homeodomain protein otp and activity-dependent splicing modulate neuronal adaptation to stress. *Neuron*. 2012; 73:279–291. This study used zebrafish to identify orthopedia (otp) as a major regulator of CRH expression, the first step in the stress response. Otp also regulates the expression of the splicing regulator a2bp1, which in turn is important for the downregulation of the stress response. [PubMed: 22284183]
40. Acampora D, Postiglione MP, Avantsgiato V, Di Bonito M, Vaccarino FM, Michaud J, Simeone A. Progressive impairment of developing neuroendocrine cell lineages in the hypothalamus of mice lacking the Orthopedia gene. *Genes Dev*. 1999; 13:2787–2800. [PubMed: 10557207]
41. Braff DL, Geyer MA, Swerdlow NR. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berl)*. 2001; 156:234–258. [PubMed: 11549226]
42. Braff D, Stone C, Callaway E, Geyer M, Glick I, Bali L. Prestimulus effects on human startle reflex in normals and schizophrenics. *Psychophysiology*. 1978; 15:339–343. [PubMed: 693742]
43. Cadenhead KS, Swerdlow NR, Shafer KM, Diaz M, Braff DL. Modulation of the startle response and startle laterality in relatives of schizophrenic patients and in subjects with schizotypal personality disorder: evidence of inhibitory deficits. *Am J Psychiatry*. 2000; 157:1660–1668. [PubMed: 11007721]
44. Burgess HA, Granato M. Sensorimotor gating in larval zebrafish. *J Neurosci*. 2007; 27:4984–4994. [PubMed: 17475807]
45. Curtin PC, Medan V, Neumeister H, Bronson DR, Preuss T. The 5-HT5A receptor regulates excitability in the auditory startle circuit: functional implications for sensorimotor gating. *J Neurosci*. 2013; 33:10011–10020. [PubMed: 23761896]
46. Engeszer RE, Barbiano LA, Ryan MJ, Parichy DM. Timing and plasticity of shoaling behaviour in the zebrafish, *Danio rerio*. *Anim Behav*. 2007; 74:1269–1275. [PubMed: 18978932]

47. Green J, Collins C, Kyzar EJ, Pham M, Roth A, Gaikwad S, Cachat J, Stewart AM, Landsman S, Grieco F, et al. Automated high-throughput neurophenotyping of zebrafish social behavior. *J Neurosci Methods*. 2012; 210:266–271. [PubMed: 22884772]
48. Miller N, Greene K, Dydinski A, Gerlai R. Effects of nicotine and alcohol on zebrafish (*Danio rerio*) shoaling. *Behav Brain Res*. 2013; 240:192–196. [PubMed: 23219966]
49. Gillberg C, Soderstrom H. Learning disability. *Lancet*. 2003; 362:811–821. [PubMed: 13678878]
50. Agetsuma M, Aizawa H, Aoki T, Nakayama R, Takahoko M, Goto M, Sassa T, Amo R, Shiraki T, Kawakami K, et al. The habenula is crucial for experience-dependent modification of fear responses in zebrafish. *Nat Neurosci*. 2010; 13:1354–1356. [PubMed: 20935642]
51. Lee A, Mathuru AS, Teh C, Kibat C, Korzh V, Penney TB, Jesuthasan S. The habenula prevents helpless behavior in larval zebrafish. *Curr Biol*. 2010; 20:2211–2216. [PubMed: 21145744]
52. Mathuru AS, Jesuthasan S. The medial habenula as a regulator of anxiety in adult zebrafish. *Front Neural Circuits*. 2013; 7:99. [PubMed: 23750127]
53. Hikosaka O. The habenula: from stress evasion to value-based decision-making. *Nat Rev Neurosci*. 2010; 11:503–513. [PubMed: 20559337]
54. Rihel J, Schier AF. Behavioral screening for neuroactive drugs in zebrafish. *Dev Neurobiol*. 2012; 72:373–385. [PubMed: 21567979]
55. Rihel J, Prober DA, Arvanites A, Lam K, Zimmerman S, Jang S, Haggarty SJ, Kokel D, Rubin LL, Peterson RT, et al. Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. *Science*. 2010; 327:348–351. [PubMed: 20075256]
56. Prober DA, Rihel J, Onah AA, Sung RJ, Schier AF. Hypocretin/orexin overexpression induces an insomnia-like phenotype in zebrafish. *J Neurosci*. 2006; 26:13400–13410. [PubMed: 17182791]
- 57*. Woods IG, Schoppik D, Shi VJ, Zimmerman S, Coleman HA, Greenwood J, Soucy ER, Schier AF. Neuropeptidergic signaling partitions arousal behaviors in zebrafish. *J Neurosci*. 2014; 34:3142–3160. This study used neuropeptide overexpression to study their roles in zebrafish arousal behaviors. Different peptides caused diverse behavioral effects and revealed that arousal can be partitioned into independent behavioral components. [PubMed: 24573274]
58. Kokel D, Bryan J, Laggner C, White R, Cheung CY, Mateus R, Healey D, Kim S, Werdich AA, Haggarty SJ, et al. Rapid behavior-based identification of neuroactive small molecules in the zebrafish. *Nat Chem Biol*. 2010; 6:231–237. [PubMed: 20081854]
59. Schulte-Merker S, Stainier DY. Out with the old, in with the new: reassessing morpholino knockdowns in light of genome editing technology. *Development*. 2014; 141:3103–3104. [PubMed: 25100652]
- 60*. Auer TO, Duroure K, De Cian A, Concordet JP, Del Bene F. Highly efficient CRISPR/Cas9-mediated knock-in in zebrafish by homology-independent DNA repair. *Genome Res*. 2014; 24:142–153. This study described an efficient method to perform knock-ins of large DNA fragments in zebrafish using the CRISPR/Cas9 system. [PubMed: 24179142]
- 61*. Gagnon JA, Valen E, Thyme SB, Huang P, Ahkmetova L, Pauli A, Montague TG, Zimmerman S, Richter C, Schier AF. Efficient mutagenesis by Cas9 protein-mediated oligonucleotide insertion and large-scale assessment of single-guide RNAs. *PLoS One*. 2014; 9:e98186. This study probed the rules of genome modifications by CRISPR/Cas9 on a large scale and described detailed protocols for effective guide RNA design and production in zebrafish. [PubMed: 24873830]
- 62*. Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, Peterson RT, Yeh JR, Joung JK. Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nat Biotechnol*. 2013; 31:227–229. This study applied the bacterial CRISPR/Cas9 system for genome modification in zebrafish for the first time. The big advantage of CRISPR/Cas9 over other genome editing tools such as TALE- or Zinc-finger-nucleases is that it does not require the cloning of custom DNA-binding protein domains but relies on guide RNAs for site-specific targeting. [PubMed: 23360964]
63. Zu Y, Tong X, Wang Z, Liu D, Pan R, Li Z, Hu Y, Luo Z, Huang P, Wu Q, et al. TALEN-mediated precise genome modification by homologous recombination in zebrafish. *Nat Methods*. 2013; 10:329–331. [PubMed: 23435258]

64. Griffiths BB, Schoonheim PJ, Ziv L, Voelker L, Baier H, Gahtan E. A zebrafish model of glucocorticoid resistance shows serotonergic modulation of the stress response. *Front Behav Neurosci.* 2012; 6:68. [PubMed: 23087630]
65. Müller P, Rogers KW, Jordan BM, Lee JS, Robson D, Ramanathan S, Schier AF. Differential diffusivity of Nodal and Lefty underlies a reaction-diffusion patterning system. *Science.* 2012; 336:721–724. [PubMed: 22499809]
66. Keller PJ, Schmidt AD, Santella A, Khairy K, Bao Z, Wittbrodt J, Stelzer EH. Fast, high-contrast imaging of animal development with scanned light sheet-based structured-illumination microscopy. *Nat Methods.* 2010; 7:637–642. [PubMed: 20601950]
67. Kinkhabwala A, Riley M, Koyama M, Monen J, Satou C, Kimura Y, Higashijima S, Fetcho J. A structural and functional ground plan for neurons in the hindbrain of zebrafish. *Proc Natl Acad Sci U S A.* 2011; 108:1164–1169. [PubMed: 21199947]
68. Huang P, Xiong F, Megason SG, Schier AF. Attenuation of Notch and Hedgehog signaling is required for fate specification in the spinal cord. *PLoS Genet.* 2012; 8:e1002762. [PubMed: 22685423]
69. Xiong F, Tentner AR, Huang P, Gelas A, Mosaliganti KR, Souhait L, Rannou N, Swinburne IA, Obholzer ND, Cowgill PD, et al. Specified neural progenitors sort to form sharp domains after noisy Shh signaling. *Cell.* 2013; 153:550–561. [PubMed: 23622240]
70. Bianco IH, Carl M, Russell C, Clarke JD, Wilson SW. Brain asymmetry is encoded at the level of axon terminal morphology. *Neural Dev.* 2008; 3:9. [PubMed: 18377638]
71. Pan YA, Livet J, Sanes JR, Lichtman JW, Schier AF. Multicolor Brainbow imaging in zebrafish. *Cold Spring Harb Protoc.* 2011; 2011. doi:10.1101/005546.
72. Koyama M, Kinkhabwala A, Satou C, Higashijima S, Fetcho J. Mapping a sensory-motor network onto a structural and functional ground plan in the hindbrain. *Proc Natl Acad Sci U S A.* 2011; 108:1170–1175. [PubMed: 21199937]
- 73*. Ahrens MB, Li JM, Orger MB, Robson DN, Schier AF, Engert F, Portugues R. Brain-wide neuronal dynamics during motor adaptation in zebrafish. *Nature.* 2012; 485:471–477. This study is the first example of brain-wide imaging in larval zebrafish during behavior and uncovered important neuronal signals during motor learning. [PubMed: 22622571]
- 74*. Ahrens MB, Orger MB, Robson DN, Li JM, Keller PJ. Whole-brain functional imaging at cellular resolution using light-sheet microscopy. *Nat Methods.* 2013; 10:413–420. Together with [75] this study introduced whole brain imaging at fast timescales in zebrafish using light-sheet microscopy. Due to much higher acquisition speeds compared to traditional point-scanning methods, this technique allows to obtain more functional signals at the same instant in time. [PubMed: 23524393]
- 75*. Panier T, Romano SA, Olive R, Pietri T, Sumbre G, Candelier R, Debregeas G. Fast functional imaging of multiple brain regions in intact zebrafish larvae using selective plane illumination microscopy. *Front Neural Circuits.* 2013; 7:65. Together with [74] this study introduces whole brain imaging at fast timescales in zebrafish using light-sheet microscopy. Due to much higher acquisition speeds compared to traditional point-scanning methods, this technique allows to obtain more functional signals at the same instant in time. [PubMed: 23576959]
- 76*. Portugues R, Feierstein CE, Engert F, Orger MB. Whole-brain activity maps reveal stereotyped, distributed networks for visuomotor behavior. *Neuron.* 2014; 81:1328–1343. This study is the first example of whole brain imaging at cellular resolution during zebrafish behavior. Access to complete activity maps of many individual fish during behavior together with 3D-image registration allowed the comparison of neuronal circuit activity across fish. [PubMed: 24656252]
77. Orger MB, Kampff AR, Severi KE, Bollmann JH, Engert F. Control of visually guided behavior by distinct populations of spinal projection neurons. *Nat Neurosci.* 2008; 11:327–333. [PubMed: 18264094]
78. Bianco IH, Ma LH, Schoppik D, Robson DN, Orger MB, Beck JC, Li JM, Schier AF, Engert F, Baker R. The tangential nucleus controls a gravito-inertial vestibulo-ocular reflex. *Curr Biol.* 2012; 22:1285–1295. [PubMed: 22704987]
79. Douglass AD, Kraves S, Deisseroth K, Schier AF, Engert F. Escape behavior elicited by single, channelrhodopsin-2-evoked spikes in zebrafish somatosensory neurons. *Curr Biol.* 2008; 18:1133–1137. [PubMed: 18682213]

80. Wyart C, Del Bene F, Warp E, Scott EK, Trauner D, Baier H, Isacoff EY. Optogenetic dissection of a behavioural module in the vertebrate spinal cord. *Nature*. 2009; 461:407–410. [PubMed: 19759620]
81. Arrenberg AB, Del Bene F, Baier H. Optical control of zebrafish behavior with halorhodopsin. *Proc Natl Acad Sci U S A*. 2009; 106:17968–17973. [PubMed: 19805086]
82. Kubo F, Hablitzel B, Dal Maschio M, Driever W, Baier H, Arrenberg AB. Functional architecture of an optic flow-responsive area that drives horizontal eye movements in zebrafish. *Neuron*. 2014; 81:1344–1359. [PubMed: 24656253]
83. Portugues R, Engert F. Adaptive locomotor behavior in larval zebrafish. *Front Syst Neurosci*. 2011; 5:72. [PubMed: 21909325]

Highlights

- Zebrafish can be used to identify cellular and molecular roles of genes associated with psychiatric diseases
- Zebrafish behavioral assays that are related to aspects of psychiatric disorders have been developed
- These behavioral assays can provide valuable tools to screen for new mutations that give rise to disease related phenotypes
- High-throughput drug screens together with behavioral phenotyping discovered novel compounds with similar effects as validated psychoactive drugs

Table 1

Analysis tools in zebrafish – from single cells to systems

Analysis domain	Process	References
Early morphogenesis	Morphogen diffusion	Mueller et al., 2012 [65]
	Whole embryo single cell tracking	Keller et al., 2010 [66]
Nervous system development	Fate mapping	Kinkhabwala et al., 2011 [67] Huang et al., 2012 [68] Xiong et al., 2013 [69]
	Single cell morphology	Bianco et al., 2008 [70] Pan et al., 2011 [71]
	Single cell electrophysiology	Koyama et al., 2011 [72]
Nervous system function	Whole brain functional imaging	Ahrens et al., 2012 [73] Ahrens et al., 2013 [74] Panier et al., 2013 [75] Portugues et al., 2014 [76]
	Single-cell ablation	Orger et al., 2008 [77] Bianco et al., 2012 [78]
	Optogenetic stimulation	Douglass et al., 2008 [79] Wyart et al., 2009 [80]
	Optogenetic silencing	Arrenberg et al., 2009 [81]
	Pre-pulse inhibition	Burgess et al., 2007 [44]
Behavior	Sleep	Prober et al., 2006 [56] Rihel et al., 2010 [55]
	Optokinetic reflex	Kubo et al., 2014 [82] Portugues et al., 2014 [76]
	Motor learning	Portugues et al., 2011 [83] Ahrens et al., 2012 [73]
	Associative learning	Agetsuma et al., 2010 [50] Lee et al., 2010 [51]

Table 2

Comparison of advantages of mouse and zebrafish as psychiatric disease models

Mouse	
Closer evolutionary proximity to humans	Closer resemblance to human alleles and behavioral phenotypes
	Effects of psychoactive drugs on mouse behaviors may be better predictors of their effects in humans.
Electrophysiological approaches	Multi-electrode recordings in behaving animals give insights into physiological abnormalities.
Cell culture approaches	Generation of different neuronal cell types <i>in vitro</i> facilitates disease modeling.
Zebrafish	
External development	All developmental stages are fully accessible, facilitating direct observation of developmental and cellular phenotypes.
Cost-effective husbandry and breeding	Hundreds of mutants can be generated with relative ease, allowing the study of disease-associated mutations at medium to high throughput.
	<i>In-vivo</i> drug-screens can be conducted at large scales.
Compact nervous system in transparent larvae	Whole brain imaging approaches enable functional phenotyping without <i>a-priori</i> knowledge about the brain regions affected by a mutation or drug.