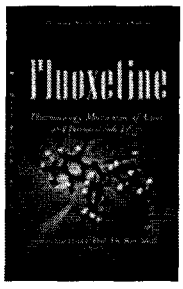

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Fluoxetine: Pharmacology, Mechanisms of Action and Potential Side Effects

Editors: Graziano Pinna (University of Illinois at Chicago, Psychiatric Institute, Chicago, Illinois, USA)

Book Description:

Fluoxetine, best known by the trade name Prozac®, unlike other psychotropic drugs whose effects were serendipitously stumbled upon, was the first developed for a precise mechanism of action, that is, the ability to selectively inhibit serotonin reuptake, based upon the theory that increasing the availability of serotonin would treat major depression. Once approved by the FDA in 1987, fluoxetine quickly became the most prescribed psychotropic drug worldwide and its success in improving mood disorders has triggered the development of a large number of congener molecules, commonly known as SSRIs after their purported mechanism of action.

However, a quarter of a century after its development, the idea that fluoxetine asserts its positive behavioral effect through inhibition of serotonergic reuptake is not firmly established. This book reviews several preclinical and clinical reports suggesting that the pharmacological effects of fluoxetine may be mediated by means other than the regulation of serotonin, including the regulation of gene expression, modifying epigenetic mechanisms as well as modifying microRNAs. One of the most prominent mechanisms for the therapeutic relevance of fluoxetine relates to influencing neuroplasticity by enhacing neurotropic factors, including BDNF signaling and altering adult neurogenesis. The ability of fluoxetine to rapidly increase neurosteroid levels accounts for the fast anxiolytic effects of this drug. Fluoxetine action at sigma-1 receptor or modulating glutamatergic neurotransmission as well as the combination of fluoxetine with other psychotropic drugs is discussed in relation to its therapeutic effects.

While fluoxetine was primarily prescribed as an antidepressant, this drug currently represents a treatment of choice for a broad spectrum of psychiatric disorders, including post-traumatic stress disorder and a range of anxiety disorders. This drug even possesses analgesic actions and is a valuable therapy for stroke. This book also highlights emerging evidence on the gender-specific effects of fluoxetine, its potential adverse features, including its addiction liability in combination with psychostimulants, and the impact of perinatal fluoxetine exposure.

Chapter 14

FLUOXETINE: PHARMACOLOGICAL ANALYSIS OF DEPRESSION-LIKE RESPONSES IN ZEBRAFISH

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ABSTRACT

Due to their high throughput value, genetic tractability, physiological homology to humans, low cost, and quick reproductive cycle, zebrafish (Danio rerio) have emerged as a promising new model species for studying various central nervous system disorders. In this chapter, we discuss the current state of utilizing zebrafish to study depression, and outline further directions of investigation, using this model organism, to understand the pathogenesis and develop innovative therapeutic approaches for depression.

Keywords: Fluoxetine; Anxiety/depression; Model development; Behavioral tests

INTRODUCTION

Nearly one half-century ago several classes of medications, discovered by serendipity, were introduced for the treatment of depression. These medications revolutionized our approach to mood disorders and helped launch the modern era of psychiatry. However, our progress since those serendipitous discoveries has been rather disappointing. We still do not understand with absolute certainty how those medications produce their desired clinical effects. We have not introduced newer medications with fundamentally different mechanisms-of-action. We have not identified the genetic and neurobiological mechanisms underlying depression, nor do we understand the mechanisms by which nongenetic factors influence these disorders. We have only a rudimentary understanding of the circuits in the brain responsible for the normal regulation of mood and affect and of those circuits that function abnormally in mood disorders. In approaching these gaps in our knowledge, there are three primary areas that need to be addressed: development of animal models, identification of genetic determinants, and discovery of novel targets/biomarkers of

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depression. This chapter discusses how zebrafish (Danio rerio) may be utilized in the modeling and analysis of the mechanisms of depression. Furthermore, this chapter argues that the foundation of research into the mechanisms of depression is that behavioral paradigms, as they allow the quantification of functional changes in the brain induced by mutations or drugs (fluoxetine), will facilitate the discovery of underlying mechanisms and drug targets. The chapter also provides a detailed description of the behavioral responses and makes recommendations for further development of these methods.

Most of the antidepressants available today inhibit the reuptake or breakdown of serotonin, noradrenaline, or both in the brain. However, elevation of the extracellular concentration of these monoamines, which occurs rapidly, does not explain the time lag of several weeks to months that is required before a therapeutic response is achieved (Berton and Nestler 2006; Wong and Licinio 2004). Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed drugs for the treatment of depression. Among SSRIs, fluoxetine is widely used to treat depression, and its biochemical and pharmacological properties have been studied extensively in animals and humans (Wong et al. 2005).

Zebrafish are rapidly becoming a promising model organism for experimental studies of affective disorders (Best 2008, Flint 2008, Kyzar 2012). Nevertheless, while zebrafish are becoming a prevalent model in the field of biological psychiatry, their application as a robust translational model is still very much in its infancy. This species demonstrates the potential to be an exceptional animal for investigating experimental, genetic, and pharmacological models of neurobehavioral disorders, such as anxiety/depression (Dooley K 2000, Sprague J 2001, Zon L 2005, Blaser R 2006, Alsop D 2008, Flint 2008, Alsop D 2008 , Mathur P 2010, Norton W 2010). As a result of the past three decades of intensive investigation with zebrafish, this species has become geneticists' favorite model organisms (Dooley K 2000). The accumulated genetic knowledge about, and the genetic methods specifically developed for the zebrafish now make this species particularly attractive for several research fields. One of these fields is behavioral neuroscience. Zebrafish models strike an optimal balance between system complexity and practical simplicity, possessing brain anatomy, physiology, and genome very similar to those of other vertebrates including mammals (Lele Z 1996, Dooley K 2000, Wullimann M 2000, Moorman 2001, Shin J 2002, Ward A 2002, McGrath P 2008). Furthermore, they are small, easy and cheap to maintain in the laboratory, and are highly amenable to high-throughput screening (e.g., forward genetic or drug screens). The latter is particularly noteworthy for the purposes of unraveling the genetic, and in general the biological, mechanisms of complex brain functions and the disorders of these functions. High-throughput screens may have the ability to identify a significant proportion of the potentially large number of molecular players involved in these functions (Norton W 2010, Blackburn J 2011).

Anxiety/depression are common, serious brain disorders (Kessler R 2005). Numerous studies have examined the biological mechanisms of anxiety/depression, and a considerable amount of effort has been invested in the development of pharmacological treatments (P. 1984, Willner 1990, C. 1998, Leonard 1998, Geyer M 2002, Kato T 2007, Flint 2008, Egan R 2009). For preclinical research, most of these studies have used rodents. Since a large amount of data has been accumulated on rodent species, it may seem logical to think that building upon this well-laid foundation is the only way to proceed. The abandonment of rodent research is certainly not likely or recommended; however, utilization of another vertebrate,

zebrafish, appears to be a fruitful direction to pursue namely because they are robust, small, reproduce quickly, possess evolutionarily conserved traits.

Development of Models

Notwithstanding our increased understanding of both the pathophysiology and treatment of anxiety/depression, it remains highly prevalent; affecting 6.7 percent of adult Americans in a given year (Kessler R 2005). Moreover, there are no validated, diagnostically useful biological tests for depression that reliably predict a response to well-established and effective treatments for depression. Similarly, there are no biomarkers, such as expression of a gene, that reliably change as a function of treatment response to antidepressants or psychotherapy.

While there is little doubt that numerous neurotransmitter systems are pathologically involved in the etiology of anxiety/depression, no single neurotransmitter systems appear to be solely responsible. This is not surprising given the panoply of symptoms that comprise the depressive syndrome: depressed mood, loss of interest in usual activities, inability to experience pleasure, impaired concentration, disturbed sleep, decreased appetite, and suicidality. A more recent conceptual approach to the biology of anxiety/depression is to consider it a systems-level 'spectrum' disorder involving several critical brain regions and pathways involving these regions. A comprehensive understanding of the genetic and environmental contributions to anxiety/depression and its associated neurobiology is required before scientifically-based rational new treatment strategies are likely to be developed.

The current view of the etiology of anxiety/depression is based on gene-environment interactions, with a focus on the three major monoamine systems—serotonin (5-hydroxytryptamine, 5HT), norepinephrine (NE), and dopamine (DA). The emerging new tools of molecular neurobiology and functional brain imaging have provided additional support for the involvement of these three systems. In contrast with previous views (C. 1998), considerable evidence now supports a preeminent role for central nervous system DA circuits (Dunlop B 2007), with many investigators suggesting that the now well-documented suboptimal therapeutic responses to SSRIs and selective serotonin-norepinephrine reuptake inhibitors (SNRIs) may be due, in part, to their relative lack of effect on brain DA circuits.

It is exceedingly difficult to develop an animal model that perfectly reproduces the symptoms of anxiety/depression (Willner 1990, Weiss 1995, Leonard 1998). Animals lack self-consciousness, self-reflection, and consideration; moreover, hallmarks of the disorder such as depressed mood, low self-esteem, or suicidality are hardly accessible in non-humans. However, anxiety/depression, as with other mental disorders, consists of endophenotypes (Hasler 2004) that can be reproduced independently and evaluated in animals. An ideal animal model offers an opportunity to understand molecular, genetic, and epigenetic factors that may lead to depression. By using animal models, the underlying molecular alterations and the causal relationship between genetic or environmental alterations and depression can be examined, which affords insight into the pathology of anxiety/depression. In addition, animal models of anxiety/depression are indispensable for identifying novel therapies for depression. For medical disorders of unclear pathophysiology or genetic etiology, such as depression, the emerging approach to developing an animal model is to model a single symptom or endophenotype of the disorder, rather than attempting to create a full phenotypic recreation of anxiety/depression. For example, anxiety/depression symptomology is often

characterized by lack of activity or motivation to be active. In modeling behaviors, in general, the behavioral measures should satisfy the criteria of being robust and reliable, having strong predictive validity to be initially used as a "new model" (Geyer M 1995, Geyer M 2002).

While several groups have actively modeled anxiety in zebrafish (and many of those tests may offer clues into the mechanisms of depression), very few studies have attempted to model depression *per se* in zebrafish. The following endophenotypes have been described in rodents; some of the below tests can be adapted, or serve as inspiration for new tests to be developed, using zebrafish. *Anhedonia*: The loss of interest is a core symptom of depression. Anhedonia in rodents can be assessed by sucrose preference or by intracranial self-stimulation. *Behavioral despair*: Behavioral despair may be assessed with tests such as the forced-swimming test or the tail suspension test. *Changes in appetite or weight gain*: Depression is often associated with changes in appetite or weight gain, which is easily measured in rodents. *Neuroanatomy*: Depressed subjects display decreased hippocampal volume and rodents exposed to chronic stress or excess glucocorticoids exhibit similar signs of hippocampal loss of neurons and dendritic atrophy. *Neuroendocrine disturbances*: Disturbances of the hypothalamic–pituitary–adrenal axis (HPA) are one of the most consistent symptoms in major depression. The functionality of the HPA can be assessed by dexamethasone suppression test. *Alterations in sleep architecture*: Disturbances in the circadian rhythm and especially in the sleep architecture are often observed in depression. In rodents, it is accessible via electroencephalography (EEG). *Anxiety-related behavior*: Anxiety is a symptom with high prevalence in depression. Therefore, animal models of depression often display altered anxiety-related behavior.

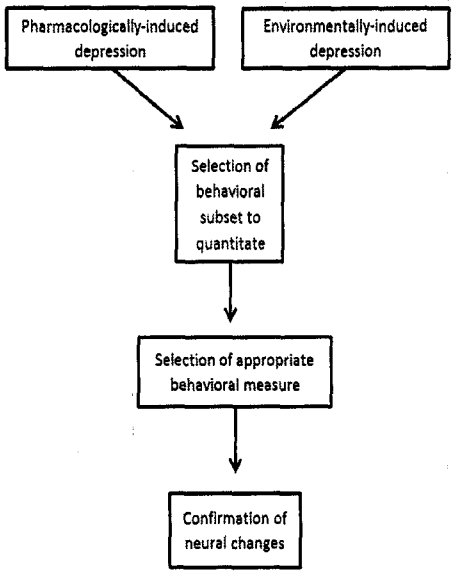

Figure 1. General approach to model development relevant to zebrafish anxiety/depression-like behavior.

Classic Behavioral Tests

There are several ways one can induce and study the effects of depression-related behaviors. Novelty has long been known to induce anxiety responses in a variety of species including humans. For example, the "open field task" has been extensively used with rodents (Crusio E 1986, Prut L 2003) and other animals including fish (Csányi V 1988, Egan R 2009). In this task, the subject is exposed to an unfamiliar environment. The response to this novel environment is believed to arise as a result of a compromise between opposing forces or tendencies; exploration, which is believed to be associated with active responses, and anxiety, which is often associated with passive responses. Exploratory activity is considered adaptive as it may lead to finding food, mates and escape routes, while passive anxiety-induced responses (immobility/freezing) are argued to reduce predation risk (Crusio E 1986). The adaptive aspect of these responses may seem speculative, but quantitative genetic analyses have confirmed ambidirectional selection forces underlying open field behavior. That is, in the evolutionary past of mice/rats, individuals that performed at intermediate levels (not too active but not too passive either) had been favored (Crusio E 1986), a finding that extends to other vertebrates including fish (Gerlai R 1990). This could prove as an extremely valuable metric for depressive behavior in zebrafish and offer possible utility in identifying new genetic lines.

It is likely that the evolutionary past of zebrafish is similar to mice/rats in that this species too has been under ambidirectional selection with regard to novelty induced behavioral responses. Therefore, exposing zebrafish to a novel environment is expected to induce moderate levels of anxiety. Importantly, behavioral experimentation almost always includes at least some level of handling of animals by humans, which is also expected to induce anxiety. Novelty induced anxiety responses have been analyzed in zebrafish by (Levin E 2007), who demonstrated an initially low level of exploratory activity of zebrafish that gradually increased with time. Levin also described a "diving" response, i.e., increased amount of time spent on the bottom of the test tank, a response that slowly habituated as the fish adapted to their novel environment. (Egan R 2009) also reported similar findings. Furthermore, (Levin E 2007) showed nicotine had anxiolytic properties as this drug reduced novelty induced fear responses.

Importantly, a decrease in serotonergic activity is associated with depression. In experimental studies, reduced brain serotonergic activity due to social isolation has been known for decades (Garattini S 1967). Specifically, rodents show hyperactive and aggressive behavior during long-term social isolation, which can be blocked with anti-depressant treatment (Garzon J 1981). These social isolation paradigms based on serotonin deficiency are used as experimental depression models in rodents (Leonard 1998). Similar tests may be useful experimentally for zebrafish. However, with all behavioral tests and endpoints, one must exercise extreme care and ensure there is some ability to provide a dissection between anxiety and depression (Table 1).

Pharmacological Models of Anxiety/Depression-like Responses

Some approaches that can be employed to model depression, are currently being used in our lab (Pittman J. 2012). One such model involves the administration of one drug followed by the administration of another that will elicit conflicting effects. For example, administration of psychostimulants, such as amphetamine, leading to hyperactivity, that can

then be used to test the efficacy of anti-manic treatments, such as Valproate. Furthermore, behavioral sensitization by repeated administration of psychostimulants can also be used as a model of bipolar disorder (Kato T 2007). Since repeated exposure to cocaine can induce a "cycling" in a variety of neurochemical and physiological systems (Antelman S 1998), it may be possible to evoke bipolar-like behavior in zebrafish, for example, by using a combination of cocaine and anti-psychotic agents.

Another approach that can be employed to induce anxiety/depressive-like behavior is withdrawal from an anxiolytic agent, such as ethanol; this methodology involves chronic administration (3+weeks) of high doses (1-3%) of ethanol, and at least 7 days post-withdrawal before behavioral symptoms are readily evident. Additionally, these depressive-like behaviors can be reversed with fluoxetine. We have had particular success with this approach in our lab using some of the behavioral tests (Figure 2). In addition, quantitative changes in immunoreactive neurons are also quite evident following this course of treatment with ethanol, and mirrors many of the neurochemical hallmarks of clinical depression (Figure 3)(Pittman J. 2012).

Table 1. Tests that can be used to study animal depression-related behavior

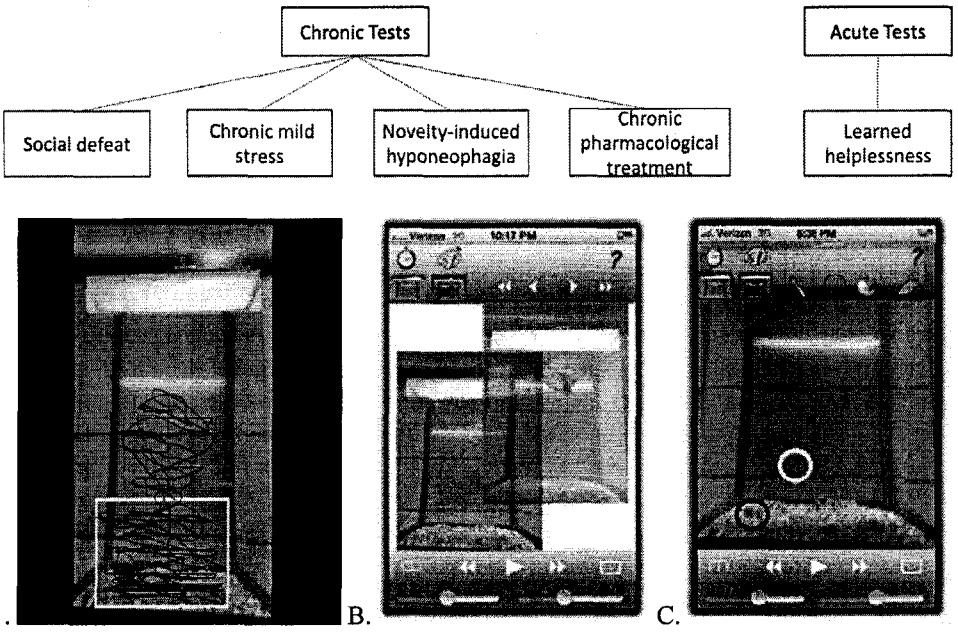

Figure 2. (A) Locomotion trajectories during the Novel Tank Diving Test of fish exposed to fluoxetine (100 µg/L). Swimming path tracings generated by iPhone App (SwingReader Golf Lite™). (B) Image frames where the corresponding images of 2 zebrafish were processed in overlay. (C) Image frames where the corresponding images of 2 zebrafish were identified, tagged, and processed in overlay from (B).

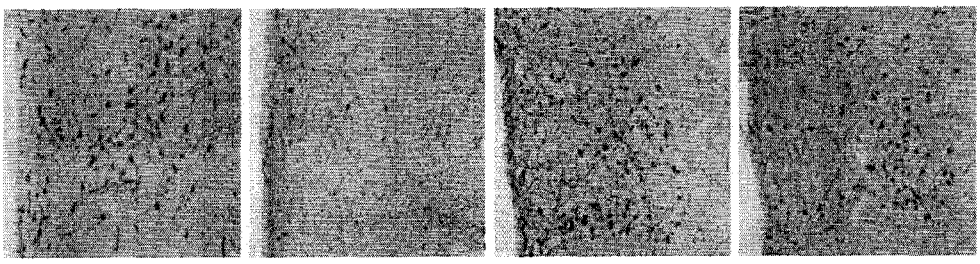

Figure 3. Representative images of coronal sections through the telencephalon (hippocampus) showing an example of immunopositive staining of serotonin terminals. (A) control, (B) ethanol withdrawal, (C) ethanol, (D) fluoxetine. Images (100x).

The motivation for the continued search for improved drugs to treat depression is not only to improve the quality of life of those suffering from it, but also to aid in our understanding of how depression develops, and what biological mechanisms may underlie this disorder cluster. Another reason is that the currently available, however numerous, drugs are often not efficacious or do not work for all patients. One way zebrafish may be beneficial for such research is by speeding up the discovery of the biological mechanisms responsible for the symptoms of depression. This may be achieved using, for example, forward genetic screens that identify mutations leading to the isolation of underlying genes. Another completely different approach has been to search for compounds, or "small molecules", which may alter expression–like symptoms. It is thus important to consider what is known about the psychopharmacological properties of zebrafish in the context of depression. For example, can one consistently detect the efficacy of "gold standard" drugs for depression using zebrafish? That is to say, does the zebrafish model have predictive validity? Predictive validity is an important question for the use of novel model organisms. The principal theme with regard to the translational relevance of laboratory model organisms concerns the notion "evolutionary homology", i.e., conservation of biological function across previously utilized species (e.g., rodents), the novel laboratory species (e.g., zebrafish), and humans.

Zebrafish have been used very infrequently in psychopharmacological analyses, nevertheless, the few studies that have been completed suggest a possibly bright future for drug development with the use of zebrafish. Alcohol (ethanol) is one of the best studied drugs in zebrafish research. For example, the effect of developmental alcohol exposure has been shown to be strain dependent (Loucks E 2004), early embryonic alcohol exposure has been found to exert significant behavioral effects in the adult (Fernandes Y 2009), adaptation (tolerance) after chronic alcohol exposure as well as alcohol withdrawal induced behavioral responses were all demonstrated (Gerlai R 1990, Gerlai R 2000, Gerlai R 2009), and numerous changes induced by acute alcohol administration have also been revealed (Gerlai R 2000). Importantly, alcohol has both anxiolytic (for the effects of lower doses of alcohol in zebrafish see (Gerlai R 2000), also see (Egan R 2009) as well as anxiogenic properties (for the effects of prolonged exposure to alcohol and during withdrawal in zebrafish see (Gerlai R 2009), also see (Egan R 2009) depending on concentration and mode or regime of its administration. Other drugs of abuse have also been shown, to exert significant behavioral effects in zebrafish. For example, the rewarding properties of cocaine have been shown and mutants with altered cocaine reinforced place preference have already been identified in

forward genetic screens (Darland T 2001). The reinforcing properties of drugs of abuse have also been analyzed (Ninkovic J 2006). Drugs of abuse, similarly to alcohol, often have anxiety/depression altering properties depending on concentration and dosing regimen employed. For example, cocaine withdrawal induces anxiety and depressive responses in zebrafish (Lopez-Patino M 2008).

Some classical anti-anxiety drugs have also been tested using zebrafish, e.g., fluromethylhistidine exhibited an anxiolytic profile (Peitsaro N 2003), diazepam reversed cocaine withdrawal induced anxiety, and the benzodiazepine inverse agonist FG-7142 induced anxiety in zebrafish (Lopez-Patino M 2008). Also, acute administration of caffeine, known to induce anxiety in humans (Childs E 2008) and rodents (El Yacoubi M 2000), also led to increased anxiety responses; reduced frequency of visits to the upper water layer and increased erratic movements in zebrafish (Egan R 2009).

Levels of stress hormones have also been analyzed in zebrafish (Alsop D 2008) and numerous similarities between zebrafish and human stress responses have been revealed, which strengthen the translational relevance of zebrafish in depression research. For example, the sight of a predator elevates cortisol levels in zebrafish (Barcellos G 2007) (also see Egan et al. 2009, Cachat et al. 2010). It is important to note that cortisol, as in zebrafish, is also the primary stress hormone of the hypothalamic-pituitary-adrenal (HPA) axis in human but not in rodents (using corticosterone instead). At the Society for Neuroscience meeting in San Diego (2010), Dr. Herwig Baier (University of California, San Fransico) presented some interesting findings related to the HPA axis (Baier 2010). Baier and his team found that disrupting the stress response in zebrafish can generate behavioral phenotypes that resemble behaviors characteristic of depression. His work suggests that depression could be linked to an individual's ability to cope with stress. The zebrafish displaying depression-like behaviors carried a mutation in the glucocorticoid receptor gene, which is involved in stress management. However, the depression-like behaviors were ameliorated when the fish were given fluoxetine (Prozac; a selective serotonin reuptake inhibitor (SSRI)). New therapies might be able to play into the activity of the glucocorticoid receptor, and promoting its activity instead of blocking it. Treatment with the widely prescribed antidepressant Prozac in zebrafish has been shown to reduce anxiety responses, with more time spent in the top portion of a novel tank and also performing fewer erratic movements, also accompanied by reduced whole-body cortisol levels (Egan R 2009), generally paralleling the responses seen in rodents (Dulawa S 2004).

Kalueff's group has recently reported depression-like motor retardation in adult zebrafish several days after an exposure to reserpine (Kyzar 2012) – a dopamine-depleting agent known to evoke depression like responses in rodents and trigger clinical depression in humans. However, with the use of all the above pharmacological treatments, one must exercise extreme care and ensure there is some ability to provide a dissection between anxiety and depression endpoints, especially given a high degree of comorbidity of anxiety with depression clinically. How will this be accomplished? Through careful selection of both the pharmacological agent used to induce depression-like symptoms, employing robust behavioral tests (much development is needed in this area), and finally confirmation via quantitative changes in neural circuitry involved in depression.

CONCLUSION

A significant difficulty with using zebrafish is that the behavior of this species is not well characterized. While there is an increasing number of behavioral studies published on zebrafish, compared to classical laboratory study species such as the rat, mouse, or even the fruit fly, zebrafish behavioral research is very much still in its infancy (Weiss 1995). Without robust behavioral tests, and without thorough understanding of the behavioral features of zebrafish, it is not possible to utilize behavioral phenotyping of mutation or drug effects, and how these manipulations may influence brain function becomes exceedingly difficult to investigate. Notably, the majority of zebrafish behavioral publications have appeared only recently, demonstrating a clear upsurge of interest in this species, as behavioral neuroscientists and behavioral geneticists have discovered novel ways to use zebrafish models.

It is challenging to predict how beneficial zebrafish may become in modeling and analysis of the biological mechanisms of human depression. At this juncture, however, it seems that the main components, necessary for such research to be successful in the future, already exist. While only distantly related to humans, the zebrafish has already proven its translational relevance. However, perhaps the most important advantage of this species as a laboratory tool may be best described with one word: numbers. Complex biological phenomena are associated with large numbers of mechanisms. These may be discovered using broad screens, genetic, or pharmacological tools. Zebrafish have been shown to be ideal for large-scale screens due to several of its features, but principally to the fact that a large amount of these fish can be produced fast and can be maintained and now tested efficiently in the laboratory.

Given the complexity of the mechanisms of depression, one may assume the need to identify a large number of molecular players, i.e., genes and their protein products and the biochemical interactions between the proteins. It can be argued that this complexity may be best tackled, at least initially, using large scale screens for mutations and drugs. These screens are the key to the identification of potential targets and leads that may subsequently be followed up on by more targeted hypothesis driven analyses. It is important to note that I am not advocating the screening approach as the only possible or only potentially fruitful one. There are a large number of unknown mechanisms waiting to be discovered and their discovery may be significantly facilitated by "blind", i.e., unbiased, screening applications. This is exactly where zebrafish have a major advantage over other, more traditional laboratory organisms. It is imperative that additional novel behavioral endpoints and observational methodologies, such as automated video-tracking systems, strengthen the utility of zebrafish for use as an animal model for depression research. Using biomolecular markers, such as gene expression, immunohistochemistry etc., to parallel zebrafish physiology with behavioral data gives us another essential research direction. Lastly, expanding the area of zebrafish research by including cross-domain modeling, for example drug withdrawal/ depression, as is currently done in my laboratory, may well lead to new translational models using a combination of both larval and adult zebrafish.

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