Pharmacological modulation of anxiety-like phenotypes in adult zebrafish behavioral models

Adam Stewart a, Nadine Wu a, Jonathan Cachat a,b, Peter Hart a, Siddharth Gaikwad a, Keith Wong a, Eli Utterback a, Thomas Gilder a, Evan Kyzar a, Alan Newman a, Dillon Carlos a, Katie Chang a, Molly Hook a, Catherine Rhynes a, Michael Caffery a, Mitchell Greenberg a, James Zadina a,b,c, Allan V. Kalueff a,b,⁎

a Department of Pharmacology and Zebrafish Neuroscience Research Consortium, Tulane University Medical School, 1430 Tulane Avenue, New Orleans, LA 70112, USA
b Neuroscience Program, Tulane University Uptown Campus, 2007 Stern Hall, New Orleans, LA 70118, USA
c Southeast LA Veterans Health Care System (SLVHCS) and Department of Medicine, Tulane University Medical School, 1430 Tulane Avenue, New Orleans, LA 70112, USA

Abstract

Zebrafish (Danio rerio) are becoming increasingly popular in neurobehavioral research. Here, we summarize recent data on behavioral responses of adult zebrafish to a wide spectrum of putative anxiolytic and anxiogenic agents. Using the novel tank test as a sensitive and efficient behavioral assay, zebrafish anxiety-like behavior can be bi-directionally modulated by drugs affecting the gamma-aminobutyric acid, monoaminergic, cholinergic, glutamatergic and opioidergic systems. Complementing human and rodent data, zebrafish drug-evoked phenotypes obtained in this test support this species as a useful model for neurobehavioral and psychopharmacological research.

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1. Introduction

Anxiety is a common neurobehavioral disorder with multiple genetic and environmental determinants (Bishop, 2007; Landgraf and Wigger, 2002; Olivier et al., 1998; Suveg et al., 2010). Experimental animal models of anxiety have been successfully used in rodents, based on their behavioral responses to novelty (Belzung and Agmo, 1997b; Kurt et al., 2000; Ribeiro and De Lima, 1998). Similar novelty-based paradigms have recently been developed for zebrafish (Danio rerio) (Blaser et al., 2010; Cachat et al., 2010b; Champagne et al., 2010; Gerlai, 2009; Maximino et al., 2010c; Stewart et al., 2010c) to assess their behavioral phenotypes (Canavello et al., 2010; Egan et al., 2009, Gerlai, 2005; Stewart et al., 2010b).

Novelty is thought to be the key anxiogenic factor in rodent exploration-based paradigms (File, 2001; Kim et al., 2005; Powell et al., 2004). Since it appears to play a similar role in zebrafish tests (Cachat et al., 2010b; Egan et al., 2009), this paper will limit its focus to novelty-evoked anxiety-like phenotypes. However, other factors (such as predator avoidance/escape (Gallup and Suarez, 1980; Suarez and Gallup, 1982a,b), defense behavior (Blanchard et al., 1991, 1998b, 1999; Griebel et al., 1995), risk assessment (Martin and Réale, 2008; Ohl et al., 2001) or the conflict between the motivations to explore and avoid (File, 2001; McNaughton and Corr, 2004; Montgomery, 1955; Montgomery and Monkmann, 1955)) contribute to animals’ behavioral responses, and merit further scrutiny in zebrafish models.

Mounting evidence demonstrates the sensitivity of zebrafish behavior to pharmacological manipulations, including anxiolytic and anxiogenic drugs (Table 1) or withdrawal from cocaine (Lopez-Patino et al., 2008; Lopez Patino et al., 2008), ethanol (Lack et al., 2007), morphine, diazepam (Wong et al., 2010a) and chlordiazepoxide (Stewart et al., 2011). To demonstrate the utility of zebrafish models for anxiety research, we will evaluate their responses to a wide spectrum of psychotropic drugs, paralleling these findings with rodent and human evidence.

Importantly, both larval and adult models are widely used in psychopharmacological screening in zebrafish (Chakraborty et al., 2009; Darland and Dowling, 2001; Gerlai et al., 2006; Linker et al., 2010; Rihel et al., 2010; Rubinstein, 2006). The strength of larval models is in their high-throughput nature, ease of genetic manipulations, and simple, well-defined behavioral endpoints (Best and Alderton, 2008;
Summary of published data on pharmacological manipulations of anxiety-like behavior in adult zebrafish.

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Lockwood et al., 2004; Renier et al., 2007; Rubinstein, 2006). However, larval zebrafish possess certain translational limitations for neurobehavioral research, being less complex behaviorally and morphologically, and not always translating drug-evoked behavioral and spinal responses into brain phenotypes (e.g., Airhart et al., 2007). At the same time, there is a growing recognition of opportunities offered by adult zebrafish models, whose strengths include relevance of adult fish physiology to human brain disorders, well-developed motor, sensory and endocrine systems, high sensitivity to environmental challenges, and a wider spectrum of behavioral phenotypes (Burne et al., 2011; Cachat et al., 2010d; Egan et al., 2009; Grossman et al., 2010; Norton and Bally-Cuif, 2010; Stewart et al., 2010b; Webb et al., 2009). Therefore, our paper will focus on adult zebrafish models and their developing utility to study pharmacogenic anxiety.

In addition to various drugs summarized in Table 1, several compounds were tested in adult zebrafish in our laboratory, including pentylenetetrazole (PTZ), pentobarbital, cocaine, tranylcypromine (TCP), fluoxetine, lysergic acid diethylamide (LSD), morphine, and naloxone (Figs. 1–4). PTZ is a blocker of the gamma-aminobutyric acid (GABA) A receptor channel, and is often used to induce pharmacogenic anxiety in rodent studies (de Angelis, 1992; Kayir and Uzbay, 2006). The barbiturate pentobarbital is an anxiolytic drug facilitating GABA-ergic neurotransmission (Wong et al., 2010b). Cocaine, TCP, and fluoxetine modulate central monamines, such as serotonin, by blocking their reuptake (cocaine, fluoxetine) or degradation (TCP) (Airhart et al., 2007; Jie et al., 2009; Lopez-Patino et al., 2008). LSD is a potent hallucigen that acts via several serotonin receptors (Backstrom et al., 1999; Wing et al., 1990). Morphine is the
Fig. 1. Behavioral effects of acute 30-min pentobarbital (5–20 mg/L) exposure on zebrafish behavior in the novel tank test. A one-way ANOVA test (factor: dose) revealed that the drug significantly affects top transitions (F(3, 37) = 3.5, P < 0.05) and the time spent in top (F(3, 37) = 3.5, P < 0.05) in adult wild type (short-fin) zebrafish. Data are presented as mean ± SEM (n = 8–10 per group), *P < 0.05 vs. control; post-hoc Tukey test for significant ANOVA data.

Fig. 2. Behavioral effects of acute 20-min morphine (A) and naloxone (B) exposure on zebrafish behavior in the novel tank test. A one-way ANOVA test (factor: dose) revealed that morphine (1–5 mg/L) significantly affects the latency to enter the top (F(3, 51) = 2.9, P < 0.005) and the number of top transitions (F(3, 51) = 2.8, P < 0.005). Naloxone (0.5–5 mg/L) significantly affected time spent in top (F(3, 59) = 3.2, P < 0.05) and the number of erratic movements (F(3, 59) = 4.6, P < 0.005) in adult wild type (short-fin) zebrafish. Data are presented as mean ± SEM (n = 13–16 per group), *P < 0.05, ***P < 0.005 vs. control; post-hoc Tukey test for significant ANOVA data.
prototypical opioid receptor agonist, whereas its competitive antagonist naloxone has been used to define various effects mediated by endogenous opioids (Brownstein, 1993; Sawynok et al., 1979). The drug doses and pretreatment times were chosen based on pilot studies with a wide range of doses and treatment times (Figs. 1–4).

In addition to acute exposure, zebrafish anxiety behavior can be modulated by chronic drug administration (Table 1), as well as by withdrawal. Recently reviewed in-depth (Stewart et al., 2011), these models will not be discussed here. Finally, zebrafish behavioral neuroscience is a relatively young field, and continues to adapt rodent paradigms, such as open field, light–dark box, startle, shoaling, and predator exposure tests (Champagne et al., 2010; Dlugos and Rabin, 2003; Grossman et al., 2010; Levin et al., 2006, 2007; Maximino et al., 2010a, 2010c; Stewart et al., 2010b). Our paper will focus on the model that is currently most widely used in adult zebrafish anxiety research—the novel tank test (Cachat et al., 2010a; Egan et al., 2009; Levin et al., 2007; Sackerman et al., 2010; Wong et al., 2010a).

2. Analysis of zebrafish anxiety-like behavior

The novel tank test (also known in the literature as the novel tank diving test) is based on the tendency of zebrafish to seek protection in an unfamiliar environment by diving and remaining at the bottom (geotaxis) while they are acclimated to the novel environment (Cachat et al., 2010b; Egan et al., 2009; Stewart et al., 2010b; Wong et al., 2010a). Adult zebrafish used in behavioral research are generally obtained from various vendors, or raised in-house in the animal facilities. For example, zebrafish used in our studies (Figs. 1–4) were of wild type short-fin strain, 5–7 month-old, 2–3 cm long, and ~50:50 male:female ratio. They were housed in groups (15–20 fish per tank) in 40-L glass tanks filled with filtered facility water for at least 20 days prior to the novel tank testing (room and water temperature was maintained at 25–27 °C, and water pH at 7.0–7.5).

Following a 1-h acclimation to the testing room (Cachat et al., 2010b), zebrafish are typically placed individually in a 1.5-L trapezoidal tank (e.g., 15 height × 28 top × 22 bottom × 7 cm width; Aquatic Habitats, Apopka, FL) maximally filled with water (Egan et al., 2009; Levin et al., 2007). The novel tank rests on a level, stable surface and is divided into three (Cachat et al., 2010a) or two (Egan et al., 2009; Wong et al., 2010a) equal virtual horizontal sections, marked by a dividing line on the outside walls of the tank. If testing continues over a period of several days, the apparatus remains in the same location with uniform consistent lighting conditions (Cachat et al., 2010b). Fish are also tested during the same time frame each day (e.g.,
10.00 to 16.00), to avoid circadian variation in locomotor activity and hormonal secretion (Cachat et al., 2010a; Grossman et al., 2010; Wong et al., 2010a; also see Cachat et al., 2010b for a detailed review). The following endpoints are typically recorded in the novel tank test for 5-6 min: the latency to reach the upper portion of the tank (s), time spent in the upper portion of the tank (s), number of transitions (entries) into the upper portion of the tank, number of erratic movements, number of freezing bouts and time spent freezing (s). Erratic movements represent sudden changes in direction or velocity and repeated rapid darting behaviors. A bout of freezing is defined as a total absence of movement, except for the gills and eyes, for 2 s or longer. A significant decrease in exploration (i.e., longer latency to reach the top, fewer entries to the top, longer and more frequent freezing) together with elevated erratic movements and freezing generally reflect high stress and anxiety in this model (Barcellos et al., 2007; Cachat et al., 2010b; Egan et al., 2009; Levin et al., 2007). Automated video-tracking further complements manual observation of zebrafish behavior (Bencan et al., 2009; Gerlai, 2005; Gerlai et al., 2009; Grossman et al., 2010; Levin et al., 2007). Trials can be recorded to a computer via hardware video-camera, and subsequently analyzed using video-tracking software (e.g., Ethovision XT7, Noldus IT, Netherlands) to generate additional endpoints, such as distance traveled or velocity (Cachat et al., 2010d).

For acute drug exposure, fish are generally transferred from home tanks to exposure beakers for a specified pretreatment time prior to novel tank testing (Cachat et al., 2010b). However, because the zebrafish is a relatively new model organism, effective concentrations for many psychotropic compounds are unknown. Since the primary method for zebrafish drug exposure—immersion (water bath application)—differs from rodent models (generally exposed via injection [13]), relating an injected drug dose to an immersed drug dose is a difficult task (Stewart et al., 2011). However, while finding effective doses and optimal pretreatment times requires numerous pilot experiments, the growing body of zebrafish evidence (Table 1, Figs. 1–4) continues to narrow this knowledge gap (Cachat et al., 2010b).

Immersion, widely used in zebrafish behavioral research (Cachat et al., 2010a; Egan et al., 2009; Grossman et al., 2010; Wong et al., 2010b), is preferred over injection techniques (which lead to pain and can confound behavioral data (Cachat et al., 2010b; Stewart et al., 2010a)), and is particularly suitable for chronic drug treatment. However, administration of certain drugs may affect water pH, oxygen exchange in the gills or swim bladder physiology (Bailey et al., 1996; 2006).
Finney et al., 2006; Nilsson and Fange, 1967; Stray-Pedersen, 1970), leading to potential confounds in data interpretation. Thus, it may be necessary to examine various additional anxiety-related phenotypes (e.g., c-fos expression, endocrine responses, escape reactions and non-vertical scototoxic/thigmotactic behaviors; Champagne et al., 2010; Maximino et al., 2010a,c), assess possible spinal and peripheral drug effects (e.g., irritation to the gills, eyes or skin), and perform chemical analyses of drug concentrations in the brain (Nakanishi et al., 2002; Stewart et al., 2010a; Storey, 2005). In any case, a careful analysis of such potential factors, and putting them in a context of known effects of different drugs in other non-fish models, is key for correct data interpretation in this model.

3. Pharmacological modulation of zebrafish anxiety

3.1. GABA-ergic system

Central GABA is a key regulator of clinical (Kalueff and Nutt, 1996; Plïc and Nowak, 2005; Rupprecht and Zwanzger, 2003; Zwanzger et al., 2009) and experimental anxiety (Frankowski et al., 2007; Gilhotra and Dhangra, 2010; Mombereau et al., 2004). Zebrafish have a well-described GABA-ergic system (Kim et al., 2010; MacDonald et al., 2010; Mueller and Wullimann, 2009) which, while not identical to human, functions in the neuronal pathways similarly to its role in the mammalian brain (Panula et al., 2010; Panula et al., 2006). Like in rodents (de Angelis, 1992; Kayir and Uzbay, 2006), inhibition of the zebrafish GABA-ergic system by PTZ leads to robust anxiety behavior (Wong et al., 2010a). For example, acute PTZ exposure (900 mg/L for 10 min; n=10 per group) reduced top transitions (5.1±0.88; P<0.005, U-test) and tends to increase erratic behavior (13.5±3.3; P=0.05–0.09, U-test) vs. controls (14.4±2.3 and 6.6±2.4, respectively). Together with the anxiogenic action of a benzodiazepine receptor inverse agonist FG-7142 on zebrafish (Table 1), this indicates that inhibition of the GABA-ergic system in fish has a similar anxiogenic effect as in other vertebrate species, including humans.

Interestingly, pentobarbital administration evokes sedation in zebrafish (Fig. 1), consistent with its effects in humans and animals (Abruźni, 1964; Atkins et al., 2000) and similar to effects of some other GABA-ergic enhancing drugs (e.g., chlordiazepoxide) on zebrafish (Table 1). Recently suggested as a good model for studying GABA-ergic sedative agents, zebrafish possess multiple high-affinity sites and robust genomic/proteomic responses to these drugs (Renier et al., 2007). Together with anxiolytic effects also found in zebrafish for GABA-enhancing drugs, such as diazepam and ethanol (Table 1), this confirms zebrafish as a model bi-directionally sensitive to GABA-active anxiotropic drugs.

3.2. Opioidergic system

The opioidergic system plays an important role in the modulation of human (Castilla-Cortazar et al., 1998; Colasanti et al., 2010; Sher, 1998) and animal (Colasanti et al., 2010; Wilson and Junor, 2008; Zarrindast et al., 2008a; Zhang, 1997) anxiety. Similar to mammals, zebrafish possess a functional opioidergic system, including both opioid peptides and their receptors (Gonzalez-Nunez and Rodriguez, 2009; Stevens, 2009; Sundstrom et al., 2010). Supporting the utility of zebrafish in opioid research, recent behavioral studies have confirmed their sensitivity to the rewarding properties of morphine (Bretaud et al., 2007; Lai et al., 2006). Our experiments (Fig. 2A) showed that zebrafish are also sensitive to the non-rewarding anxiolytic action of morphine (Fig. 2A), similar to that reported in rodents (Kalveti et al., 2006; Shin et al., 2003; Zhang and Schultes, 2008), primates (Kalveti et al., 1988; Winslow et al., 2007) and humans (Koran et al., 2005). Although increased exploration in zebrafish can also be explained by hyperlocomotion (reported for morphine in rodents; Kalveti et al., 2006; Shin et al., 2003), a marked reduction in erratic movements (Fig. 2A) is consistent with the overall anxiolytic nature of these responses.

In contrast, acute administration of opioid antagonist naloxone induced anxiety-like behaviors in zebrafish (Fig. 2B), accompanied by restlessness with frequent short hyperactivity bouts (data not shown). While mouse naloxone data show either no effects (Belzung and Agmo, 1997a,b; Ribeiro and De Lima, 1998) or paradoxical anxiolysis (Onaivi and Martin, 1989; Rodgers et al., 2006), this drug does not affect anxiety in primates (Kalveti et al., 1988) and, to the best of our knowledge, has no clinical effects on anxiety. Notably, several clinical studies have reported anxiogenic/panicogenic effects of naltrexone, another opioid receptor antagonist (Esquivel et al., 2009; Kozak et al., 2007; Marenmanni et al., 1998). Our naloxone data (Fig. 2B) suggests that opioid antagonists may trigger anxiety in zebrafish, most likely by inhibiting their naturally occurring “anti-anxiety” opioid ligands.

It should not be surprising that the effects of opioid ligands, such as naloxone, may be more complex in the zebrafish than in mammals. For example, it has been suggested that two rounds of whole genome duplication (2R) occurred in early vertebrate evolution, and only the genome of teleost fishes doubled again (3R) (Sundstrom, Dregro). While duplicate genes for dynorphin and the mu or kappa opioid receptors may have degenerated or adapted to other functions, zebrafish appear to have two versions of proenkephalin, pro- opioids, proenkephalin, pronociceptin (Sundstrom, Dregro) and the delta opioid receptor (Gonzalez-Nunez and Rodriguez, 2009). The active opioid peptides produced from the precursors are similar in sequence to those of mammals, but are likely to be in varying relative concentrations. The presence of two delta receptors may be particularly interesting in relation to anxiety. For example, delta receptor- and proenkephalin-knockout mice show increased anxiety (Filliol et al., 2000; Ragnauth et al., 2001; Roberts et al., 2001), implying that delta receptor agonists may be anxiolytic. This may explain some of the drug-induced behaviors in zebrafish (Fig. 2B), which, given their elaborate opioidergic system, may be particularly sensitive to antagonists like naloxone. Thus, zebrafish may provide a useful model to study both opioid receptor-mediated anxiety and withdrawal-induced anxiety (Colasanti et al., 2010). However, further studies utilizing various zebrafish paradigms and other opioid agonists or antagonists are needed to better understand the complex modulation of anxiety by opioidergic agents.

3.3. Serotonergic system

Serotonergic mechanisms are strongly implicated in human (Charney et al., 1990; Deakin, 1998; Eison, 1990; Hoes, 1982) and animal anxiety (Handley and McBlane, 1993; Handley et al., 1993; Heisler et al., 2007). Since selective serotonin reuptake inhibitors (SSRIs) are potent modulators of brain serotonin (Essler et al., 2007; Goldstein and Goodnick, 1998), behavioral effects of fluoxetine on zebrafish merit further scrutiny. Zebrafish possess a well-developed serotonergic system (Stewart et al., 2010b) which makes them an ideal model for such analyses. Although not anatomically and genetically conserved, many serotonin receptors have similar expression patterns, binding, and signaling properties as in mammals (Panula et al., 2010). Generally paralleling rodent and clinical literature on SSRIs, robust anxiolytic action of chronic fluoxetine has been reported in zebrafish (Egan et al., 2009; Stewart et al., 2010b). In contrast, acute SSRI treatment has been reported to evoke anxiety in humans (Belzung et al., 2001; Enginar et al., 2008; Goldstein and Goodnick, 1998) and rodents (Bagdy et al., 2001; Draper et al., 2007; Kurt et al., 2000; Silva et al., 1999). Acute fluoxetine did not affect zebrafish behavior (Fig. 3A), and citalopram was anxiolytic in this model (Sackerman et al., 2010). However, further studies utilizing various zebrafish paradigms and other opioid agonists or antagonists are needed to better understand the complex modulation of anxiety by opioidergic agents.
A serotonergic 5-HT_{1A} agonist buspirone dose-dependently increases time in top of the novel tank test (Bencan et al., 2009), which is consistent with well-known anxiolytic effects of this agent in humans and rodents. Olanzapine (an antipsychotic drug with affinity for 5-HT_{2A/C} and 5-HT_{3} receptors) evokes similar responses in zebrafish (Seib et al., 2010), paralleling its clinical (Freeman et al., 2009; Maina et al., 2008) and preclinical (Mead et al., 2008; Sun et al., 2010) anti-anxiety effects. LSD, recently tested in several zebrafish paradigms or both (Grossman et al., 2010), increases top exploration and reduces freezing in the novel tank test (Fig. 3B), resembling the second (positive) phase of the drug’s well-known biphasic action on rodents and humans (Adams and Geyer, 1982, 1985; Gupta, 1971; Krebs-Thomson and Geyer, 1996; Marona-Lewicka et al., 2005; Mittman and Geyer, 1991; Palenicek et al., 2010; Uyeno and Benson, 1965). While it is unclear whether the behavioral effects of LSD in zebrafish are hallucinogenic, anxiolytic, or both (Grossman et al., 2010), the lack of confounding anxiety (typical for the initial “anxiety” phase of LSD action in rodents and humans) in these fish models is beneficial. Furthermore, ethograms-based analyses have also been applied to zebrafish models, assessing frequencies and transitions between different behaviors to reveal drug-induced alterations in the overall sequential patterning of their novel tank activity (Cachat et al., 2010b,c; Grossman et al., 2010).

In contrast, cocaine dose-dependently inhibited zebrafish behavior, evoking longer freezing and fewer top transitions (Fig. 4A). This response parallels cocaine’s known anxiogenic profile in rodents and humans (Blanchard and Blanchard, 1999; Blanchard et al., 1998a, 1999; Costall et al., 1989; Daza-Losada et al., 2009; Fontana and Commissaris, 1989; Salas-Ramirez et al., 2010; Simon et al., 1994; Sobrian et al., 2003), but is not consistent with previous zebrafish studies showing the lack of anxiety in a wide range of systemic doses (Lopez-Patio et al., 2008; Lopez-Patio et al., 2008). It is possible that the inbred AB zebrafish strain (hyperactive in anxiety-evoking situations Norton and Bally-Cuif, 2010) used in these studies was less sensitive to the anxiogenic effects, compared to the outbred wild type short-fin strain used here (Fig. 4A). A similar situation has been reported in rodents, where cocaine was anxiogenic in non-anxious strains, but failed to affect the behavior of selectively-bred anxious rats (Rogero and Takahashi, 1992). Likewise, zebrafish strains may be differentially sensitive to cocaine (similar to their strain-specific sensitivity to ethanol Drugs and Rabin, 2003) or treatments (0.0045–45 mg/L cocaine for 75 min (Lopez-Patio et al., 2008) vs. 1–25 mg/L for 20 min here).

Furthermore, we also examined the behavioral effects of inhibition of monoamine oxidase (MAO), whose inhibitors (MAOIs) are clinically effective against various anxiety disorders (Ballenger, 1999; Mallinger et al., 2009). In rodents, MAOIs reduce anxiety- and depression-like behavior chronically (Crawley, 1985; Maki et al., 2000; Takamori et al., 2001) but yield conflicting results after acute administration, including both a lack of effects (Griebel et al., 1998, 1997; Holmes and Rodgers, 2003; Lecci et al., 1990) and anxiolysis (de Angelis, 1996; Freund et al., 1979; Maki et al., 2000). Reducing anxiety in rodents (de Angelis, 1996; Freund et al., 1979; Maki et al., 2000; Negishi et al., 2004), a non-selective irreversible MAO TCP produced similar anxiolytic-like responses in zebrafish (Fig. 4B; albeit causing behavioral inhibition at high doses). While these findings parallel clinical and rodent data, further research using various serotonergic drugs will provide more insights on their behavioral effects in zebrafish. For example, as zebrafish possess only one isoform of MAO, it is interesting to establish whether MAOIs modulate their serotonergic and noradrenergic systems (e.g., affecting anxiety) or act on the dopaminergic system (e.g., producing motor activation).

3.4. Cholinergic system

The cholinergic system is emerging as another target for pharmacological modulation of zebrafish anxiety, since N-cholinergic agonist nicotine elicits consistent and very robust anxiolytic responses in the novel tank test (Bencan and Levin, 2008; Levin et al., 2007; Stewart et al., 2011) (Table 1). Although 100 mg/L produces the most reliable anxiolytic effects (Bencan and Levin, 2008), they are dose-dependent (Levin et al., 2006, 2007) and parallel clinical (Picciotto et al., 2002) and rodent data (Cohen et al., 2009) for this drug. Interestingly, co-administration of nicotine with methylphenidate attenuates the anxiolytic response in zebrafish, increasing bottom dwelling and reducing activity (Bencan and Levin, 2008). Since MLA is an antagonist for the N-cholinergic receptor, the novel tank test may be useful in screening the effects of various cholinergic compounds on zebrafish anxiety.

3.5. Other systems

In addition to geotaxis, adult zebrafish show overt thigmotaxis in novel environments (Champagne et al., 2010; Maximino et al., 2010b), resembling anxiety-like peripheral locomotion in a rodent open field test. Rodent thigmotaxis is sensitive to anxiogenic and anxiolytic drugs (Choleris et al., 2001; Simon et al., 1994), and similar modulation exists for adult zebrafish behavior. For example, zebrafish spend more time in the center of the open field test after a single injection of α-fluoromethylhistidine — an inhibitor of histidine decarboxylase (Peitsaro et al., 2003). These findings strongly implicate central histamine in the regulation of anxiety in zebrafish, which possess a well-developed histaminergic system with a conserved innervation pattern (Coffiel and Mattioli, 2009; Kaslin and Panula, 2001; Panula et al., 2010; Peitsaro et al., 2007), and three histamine receptors that parallel the H1, H2 and H3 receptors of the mammalian brain (Panula et al., 2010). Given the important role of histamine and its receptors in clinical and animal anxiety-related states (Dere et al., 2010; Zarrindast et al., 2006, 2008b), zebrafish are likely to represent useful screens for anxiotrophic histaminergic drugs.

The central glutamatergic system has also been linked to anxiety in humans (Mathew et al., 2008; Nair and Singh Ajit, 2008) and rodents (Blanchard et al., 1992; Moraes et al., 2008). Since glutamatergic mechanisms play an important role in the zebrafish brain (e.g., Edwards and Michel, 2002), recent behavioral studies have exposed zebrafish to several glutamatergic drugs. For example, N-methyl d-aspartate (NMDA) receptor antagonist ketamine produces robust behavioral activation in adult zebrafish (Zakhary et al., 2011), strikingly paralleling the drug’s hyperlocomotory effects in rodents (da Silva et al., 2010; Irifune et al., 1998). Although ketamine has anxiolytic-like action on clinical (Irwin and Iglewicz, 2010) and animal (Engin et al., 2009; Pietersen et al., 2006; Sufka et al., 2009) anxiety, anxiogenic effects were also reported in rodents (da Silva et al., 2010). Therefore, this aspect of ketamine’s behavioral pharmacology remains to be explored in zebrafish in detail. Interestingly, a similar profile was reported for another NMDA antagonist, MK-801, reducing anxiety in both zebrafish (Seib et al., 2010) (Table 1) and rodents (Blanchard et al., 1992; Soderpalm et al., 1995).

Finally, a growing body of literature confirms the role of adenosine and its receptors in anxiety pathogenesis (Correa and Font, 2008; Kulkarni et al., 2007). Adenosine has an inhibitory effect on the brain, and exerts robust anxiolysis in rodents (Kulkarni et al., 2007). In contrast, its non-selective antagonist caffeine acts as an anxiogenic agent, as shown in clinical (Childs et al., 2008; Lara, 2010), rodent (Bradley et al., 2010; Kulkarni et al., 2007) and zebrafish studies (Egan et al., 2009) (Table 1).

4. Concluding remarks

Overall, this paper provided an updated summary of pharmacogenetic modulation of adult zebrafish anxiety. In addition to GABAergic, serotonergic, histaminergic, cholinergic and opioidergic systems, the role of other neurotransmitters continue to emerge in
zebrafish models. This paper also raises several other important questions. For example, while adult zebrafish anxiety is sensitive to many classes of traditional psychotropic drugs (Table 1, Figs. 1–4), modern biological psychiatry requires new models to be able to identify novel drugs (Bergner et al., 2009; Kalueff et al., 2007; LaPorte et al., 2010). Therefore, in addition to further validating zebrafish paradigms using agents with known psychopharmacology, these models may also help identify potential new classes of psychotropic drugs.

As already mentioned, species differences in neurobiology and pharmacology may be crucial in some aspects of drug effects. However, in many cases (Table 1, Figs. 1–4) the observed phenotypes parallel animal and clinical evidence, thereby supporting the validity and translatability of adult zebrafish models. Zebrafish models are also important from an evolutionary perspective, allowing identification of common conserved pathways and circuits involved in anxiety regulation.

A common problem with most exploration-based models is their sensitivity to variations in other domains, such as cognitive functions, locomotor activity and arousal. For example, similar to rodent models, zebrafish hypometacactivity may easily be misinterpreted as anxiety or increased habituation (Kalueff and Murphy, 2007; Stewart et al., 2010c; Wong et al., 2010a). Therefore, it is important to detect drug effects and discriminate between classes of effects, also recognizing that zebrafish phenotypes may be more complex than currently understood. For example, while non-competitive NMDA receptor antagonists ketamine or MK-801 evoke anxiolytic-like responses in zebrafish, their reversal by antipsychotics implies an additional, psychotomimetic-like profile (Seibt et al., 2010). Therefore, further domain-specific research may be needed, with agents that produce hyperlocomotion (e.g., amphetamine) used to dissect novelty-evoked anxiety (vs. activity-related) phenotypes. Again, sophisticated video-tracking tools will be particularly useful for this, as selected computer-generated endpoints (e.g., distance travelled or velocity) can reliably characterize activity-related responses (Cachat et al., 2010b).

From past literature (Egan et al., 2009; Norton and Bally-Cuif, 2010; Speedie and Gerlai, 2008) we know that zebrafish display robust stress-related behaviors. However, it is unclear whether they display common stress-evoked behaviors, or different (e.g., anxiety vs. fear) behaviors in different situations. While interest in zebrafish models is rapidly growing, the entire catalog of zebrafish behaviors remains unclear, and we do not know when and where these behaviors occur within the zebrafish locomotor path. As all traditional zebrafish paradigms are based on fish location and velocity in 2D coordinates, recent data obtained in our laboratory (Cachat et al., 2010b,c; Grossman et al., 2010) suggests that analysis of 3D swimming trajectories may reveal new endpoints sensitive to stress, providing a promising data-mining approach to detect and interpret drug-evoked behavioral responses in these fish.

In summary, complex zebrafish behavioral responses to pharmacological modulation (Table 1, Figs. 1–4) support their utility as a new model organism for anxiety research. As novel zebrafish paradigms continue to be developed, the field may benefit from creatively using this new model species for further conceptual and methodological progress (Egan et al., 2010; Kalueff et al., 2007; LaPorte et al., 2010). And while these fish with “small brains” may take a while to generate “big waves” (Burne et al., 2011; Gerlai, 2009), comprehensive characterization of zebrafish drug-evoked anxiety phenotypes is a step in the right direction.

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