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

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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.

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 Monkman, 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;

Abbreviations: GABA, gamma-aminobutyric acid; LSD, lysergic acid diethylamide; MAO, monoamine oxidase; MAOIs, monoamine oxidase inhibitors; MLA, methyllycaconitine; NMDA, N-methyl D-aspartate; PTZ, pentylenetetrazole; TCP, tranylcypromine; SSRIs, selective serotonin reuptake inhibitors.

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Table 1
Summary of published data on pharmacological manipulations of anxiety-like behavior in adult zebrafish.

Drugs	Effective doses and treatment details	Behavioral test (zebrafish strain)	Behavioral effects	References
Anxiolytic compounds				
Gamma-aminobutyric acid (GABA)-ergic drugs				
Ethanol	0.5 and 1% immersed for 60 min	Predator exposure (wild type)	Acute: lower avoidance	Gerlai et al. (2006)
	0.25, 0.5 and 1% immersed for 120 min (acute) or 1–2 weeks (chronic)	Open field (wild type)	Acute: reduced startle and shoaling; chronic: reduced shoaling (0.5%)	(Dlugos and Rabin, 2003)
	0.2–0.3% immersed for 5 min (acute) or 1–2 weeks (chronic)	Novel tank (wild type)	Acute and chronic: shorter latency to top, more top entries and time spent	(Egan et al., 2009; Wong et al., 2010a; Stewart et al., 2011) (Wong et al., 2010a)
	0.5% immersed for 3–4 min	Light–dark plus maze (AB, WIK, GloFish)	More total arm entries and time spent in white arm	Sackerman et al. (2010)
Chlordiazepoxide	5, 10 and 20 mg/L immersed for 3 min	Novel tank (wild type)	Sedation and slower swimming	Bencan et al. (2009)
	25 mg/L immersed for 3–4 min	Light–dark plus maze (AB, WIK, GloFish)	More white arm entries and time spent	Sackerman et al. (2010)
Diazepam	1.5 and 5 mg/L immersed for 3 min	Novel tank (wild type)	Reduced bottom dwelling	Bencan et al. (2009)
Serotonergic drugs				
Buspirone	6.25 and 50 mg/L immersed for 3 min	Novel tank (wild type)	Reduced diving and bottom dwelling	Bencan et al. (2009)
Citalopram	100 mg/L immersed in water for 3–4 min	Novel tank (AB, WIK, GloFish)	More time in top	Sackerman et al. (2010)
Desipramine	25 mg/L immersed for 3–4 min	Novel tank (AB, WIK, GloFish)	More time in top	Sackerman et al. (2010)
Fluoxetine	100 μg/L immersed for 2 weeks	Novel tank (wild type)	More top entries and time in top, less freezing and erratic movements	Wong et al. (2010a)
Lysergic acid diethylamide (LSD)	250 μg/L immersed for 20–50 min	Novel tank, open field (wild type)	Increased top dwelling, more time in top, reduced freezing, mild increase in light behavior	Grossman et al. (2010)
Olanzapine	3.12 mg/L immersed for 15 and 30 min	Novel tank (wild type)	More time in top, increased overall and top swimming	Seibt et al. (2010)
Cholinergic drugs				
Nicotine	50 and 100 mg/L immersed for 3 min	Novel tank (wild type)	More time in top	Levin et al. (2007)
	25 mg/L immersed for 3–4 min	Light–dark plus-maze (AB, WIK, GloFish)	Longer freezing duration in center (AB strain)	Sackerman et al. (2010)
	50 mg/L immersed for 3–4 min	Novel tank (AB, WIK, GloFish)	More time in top	Sackerman et al. (2010)
	10 mg/L immersed for 5 min	Novel tank (wild type)	Shorter latency to top, more time in top	Stewart et al. (2011)
Histaminergic drugs				
α-Fluoro-methyl-histidine	100 mg/kg injected systemically 24 h prior to testing	Open field (AB, wild type)	Increased center swimming	Peitsaro et al., (2003)
Glutamatergic drugs				
MK-801	6.74 mg/L immersed for 30 or 60 min	Novel tank (wild type)	More time in top, increased overall and top swimming	Seibt et al., (2010)
Anxiogenic compounds				
Adenosinergic system				
Caffeine	100 mg/L immersed for 15 min	Novel tank (wild type)	Longer latency to top, fewer transitions, more erratic movements	Egan et al. (2009)
GABA-ergic drugs				
FG-7142	0.12, 0.17 and 0.23 mg/L immersed for 75 min	Open field (AB)	Overall hyperlocomotion, increased thigmotaxis	(Lopez-Patino et al., 2008; Lopez Patino et al., 2008)

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 hallucinogen that acts via several serotonin receptors (Backstrom et al., 1999; Wing et al., 1990). Morphine is the

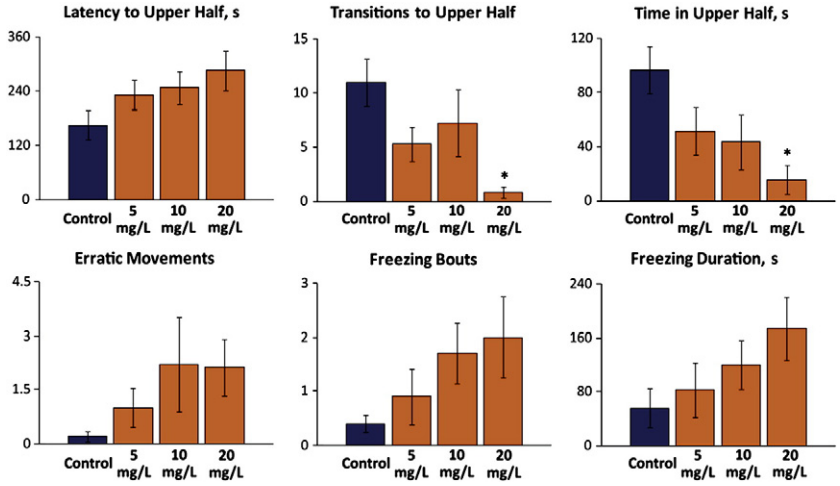

Fig. 1. Behavioral effects of acute 30-min pentobarbital (5–20 mg/L) exposure on zebrafish behavior in the novel tank test. A one-way ANOVA test (factor:dose) revealed that the drug significantly affects top transitions ($F(3, 37) = 3.5$, $P<0.05$) and the time spent in top ($F(3, 37) = 3.5$, $P<0.05$) 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.

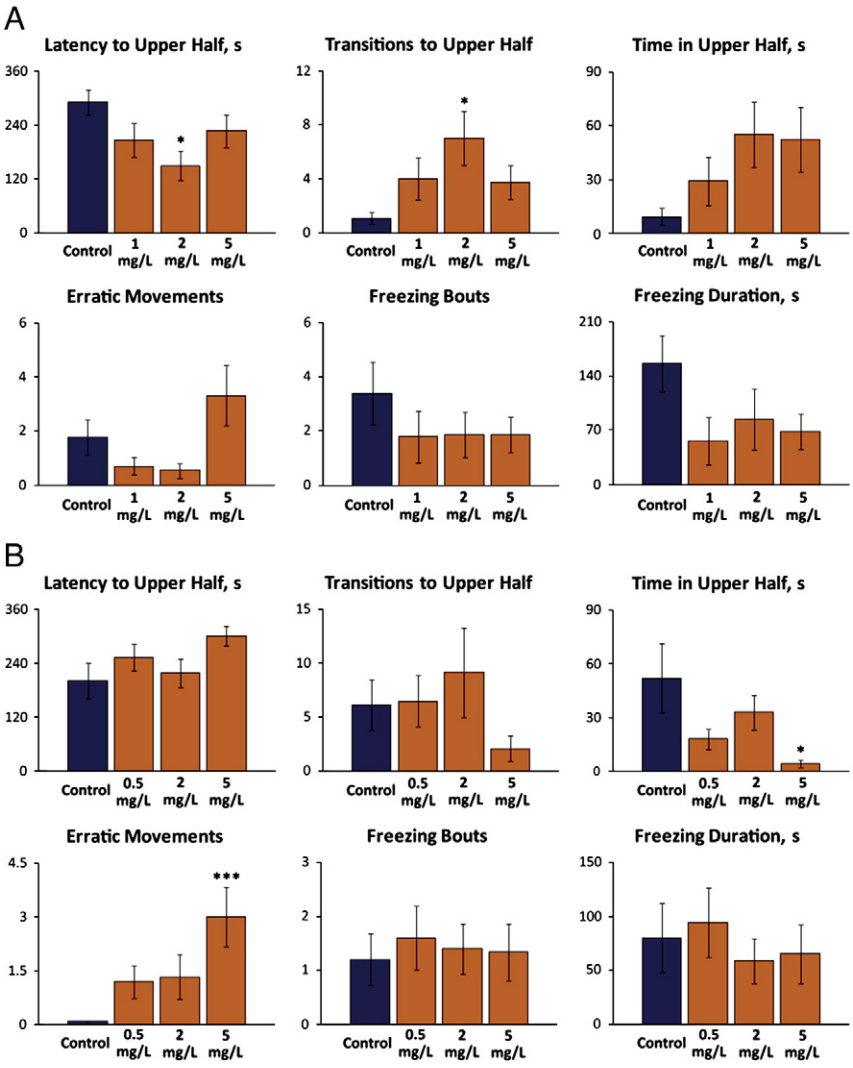

Fig. 2. Behavioral effects of acute 20-min morphine (A) and naloxone (B) exposure on zebrafish behavior in the novel tank test. A one-way ANOVA test (factor: dose) revealed that morphine (1–5 mg/L) significantly affects the latency to enter the top ($F(3, 51) = 2.9$, $P<0.005$) and the number of top 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.

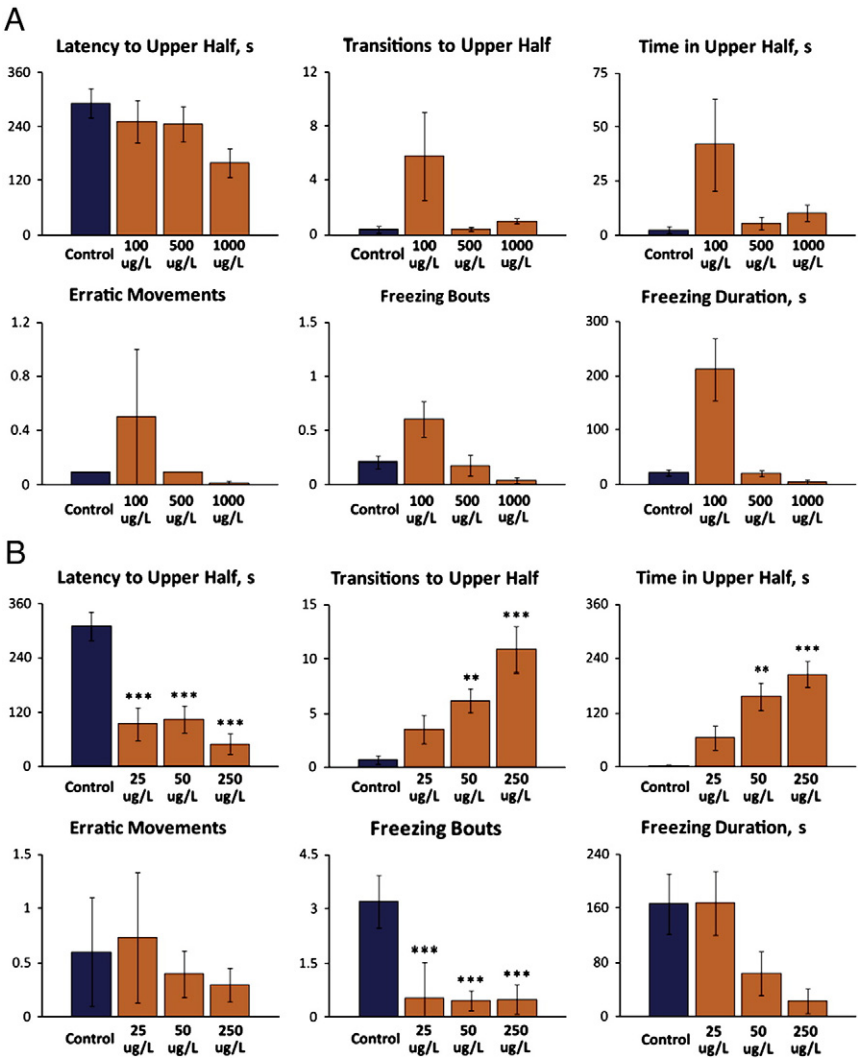

Fig. 3. Behavioral effects of acute 20-min fluoxetine (A) and lysergic acid diethylamide (LSD; B) exposure on zebrafish behavior in the novel tank test. A one-way ANOVA test (factor: dose) revealed that fluoxetine (100–1000 μg/L) did not affect zebrafish behavior, whereas LSD (25–250 μg/L) significantly affected the latency to enter the top ($F(3, 49) = 10.3$, $P<0.005$), number of top transitions ($F(3, 49) = 8.7$, $P<0.005$), time spent in top ($F(3, 49) = 9.7$, $P<0.005$), and freezing bouts ($F(3, 49) = 13.8$, $P<0.005$) in adult wild type (short-fin) zebrafish. Data are presented as mean ± SEM ($n = 10–16$ per group), **$P<0.01$, ***$P<0.005$ 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 (Levin et al., 2006, 2007) 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.,

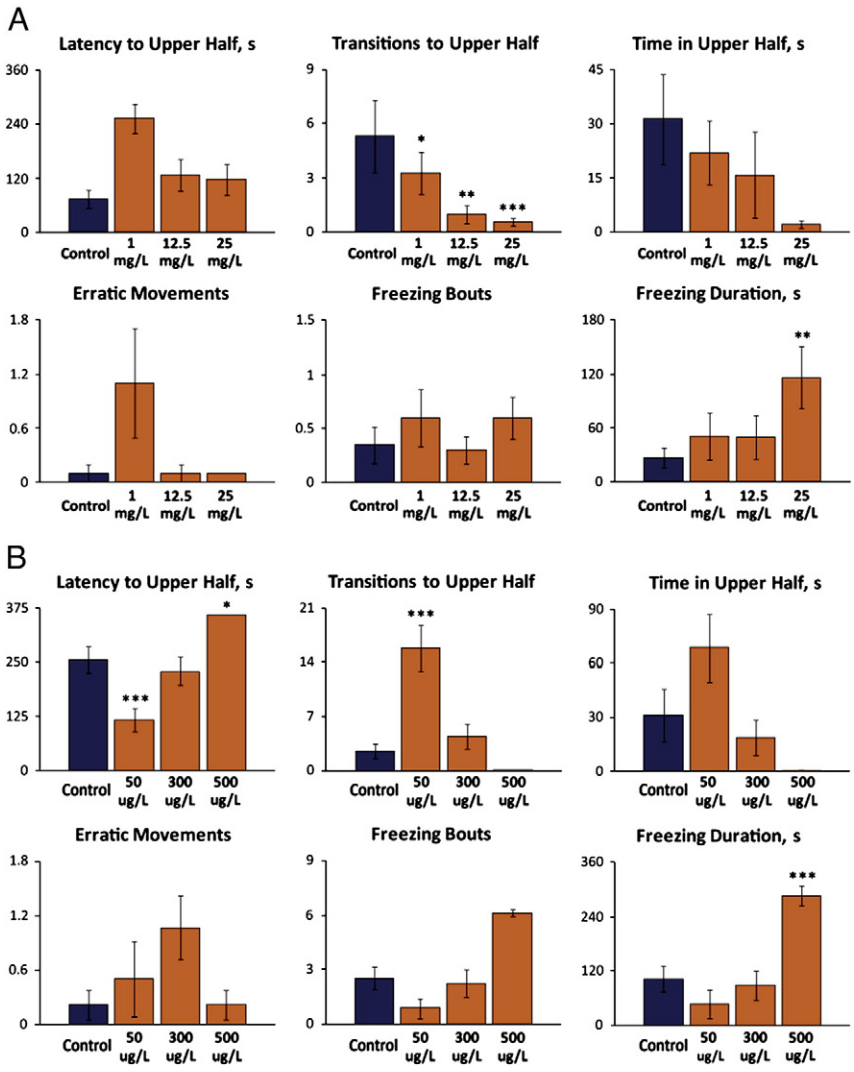

Fig. 4. Behavioral effects of acute 20-min cocaine (A) and tranylcypromine (TCP; B) exposure on zebrafish behavior in the novel tank test. A one-way ANOVA test (factor: dose) revealed that cocaine (1–25 mg/L) significantly affects the number of top transitions ($F(3, 39) = 5.9$, $P<0.005$) and freezing duration ($F(3, 39) = 5.7$, $P<0.005$). TCP exposure (50–500 μg/L) significantly affected the latency to enter the top ($F(3, 53) = 13.8$, $P<0.005$), number of top transitions ($F(3, 53) = 16.4$, $P<0.005$), and freezing duration ($F(3, 53) = 14.1$, $P<0.005$) in adult wild type (short-fin) zebrafish. Data are presented as mean ± SEM (n = 10–14 per group), *$P<0.05$, **$P<0.01$, ***$P<0.005$ vs. control; post-hoc Tukey test for significant ANOVA data.

10.00–16.00), 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;

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 scototaxic/thigmotaxic 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; Pilc and Nowak, 2005; Rupprecht and Zwanzger, 2003; Zwanzger et al., 2009) and experimental anxiety (Frankowska et al., 2007; Gilhotra and Dhingra, 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 (Abruzzi, 1964; Atkins et al., 2000) and similar to effects of some other GABA-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; Lau et al., 2006). Our experiments (Fig. 2A) showed that zebrafish are also sensitive to the non-rewarding *anxiolytic* action of morphines (Fig. 2A), similar to that reported in rodents (Kahveci et al., 2006; Shin et al., 2003; Zhang and Schulteis, 2008), primates (Kalin 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; Kahveci 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 (Kalin 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; Maremmani 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, Dreborg). 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-opiomelanocortin, pronociceptin (Sundstrom, Dreborg) 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 anxiolysis 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 (Esler 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; Drapier 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). While the lack of zebrafish anxiety following acute fluoxetine (Fig. 3A) contradicts clinical and rodent findings, acute SSRIs may exert complex behavioral profiles, including anxiolysis (Hascoet et al., 2000; Lightowler et al., 1994; Molewijk et al., 1995; Varty et al., 2002). Furthermore, the lack of anxiogenic effects of acute SSRI may also be due to permeability to serotonin of the blood-brain barrier in teleosts (Khan and Deschaux, 1997), counterbalancing potentially anxiogenic effects of the sharp elevation of brain serotonin caused by these drugs.

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 (Seibt et al., 2010), paralleling its clinical (Freeman et al., 2009; Maina et al., 2008) and pre-clinical (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-Patino et al., 2008; Lopez Patino 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 (Rogerio and Takahashi, 1992). Likewise, zebrafish strains may be differentially sensitive to cocaine (similar to their strain-specific sensitivity to ethanol Dlugos and Rabin, 2003)) or treatments (0.0045–45 mg/L cocaine for 75 min (Lopez-Patino 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 MAOI 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 methyllycaconitine (MLA) 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 (Cofiel 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 anxiotropic 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 (Seibt 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 pharmacogenic 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 hypoactivity 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 locomotory 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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References

Abruzzi WA. A long release dose form of pentobarbital compared with meprobamate in the management of anxiety states. Clin Med Northfield Il 1964;71:1231–4.
Adams LM, Geyer MA. LSD-induced alterations of locomotor patterns and exploration in rats. Psychopharmacol Berl 1982;77:179–85.
Adams LM, Geyer MA. A proposed animal model for hallucinogens based on LSD's effects on patterns of exploration in rats. Behav Neurosci 1985;99:881–900.
Airhart MJ, Lee DH, Wilson TD, Miller BE, Miller MN, Skalko RG. Movement disorders and neurochemical changes in zebrafish larvae after bath exposure to fluoxetine (PROZAC). Neurotoxicol Teratol 2007;29:652–64.
Atkins AL, Rustay NR, Crabbe JC. Anxiety and sensitivity to ethanol and pentobarbital in alcohol withdrawal seizure-prone and withdrawal seizure-resistant mice. Alcohol Clin Exp Res 2000;24:1743–9.
Backstrom JR, Chang MS, Chu H, Niswender CM, Sanders-Bush E. Agonist-directed signaling of serotonin 5-HT2C receptors: differences between serotonin and lysergic acid diethylamide (LSD). Neuropsychopharmacology 1999;21:77S–81S.
Bagdy G, Graf M, Anheuer ZE, Modos EA, Kantor S. Anxiety-like effects induced by acute fluoxetine, sertraline or m-CPP treatment are reversed by pretreatment with the 5-HT2C receptor antagonist SB-242084 but not the 5-HT1A receptor antagonist WAY-100635. Int J Neuropsychopharmacol 2001;4:399–408.
Bailey GS, Williams DE, Hendricks JD. Fish models for environmental carcinogenesis: the rainbow trout. Environ Health Perspect 1996;104(Suppl 1):5-21.
Ballenger JC. Current treatments of the anxiety disorders in adults. Biol Psychiatry 1999;46:1579–94.
Barcellos LJG, Ritter F, Kreutz LC, Quevedo RM, da Silva LB, Bedin AC, et al. Whole-body cortisol increases after direct and visual contact with a predator in zebrafish, *Danio rerio*. Aquaculture 2007;272:774–8.
Belzung C, Agmo A. Naloxone blocks anxiolytic-like effects of benzodiazepines in Swiss but not in Balb/c mice. Psychopharmacology 1997b;132:195–201.
Belzung C, Agmo A. Naloxone potentiates the effects of subeffective doses of anxiolytic agents in mice. Eur J Pharmacol 1997a;323:133–6.
Belzung C, Le Guisquet AM, Barreau S, Calatayud F. An investigation of the mechanisms responsible for acute fluoxetine-induced anxiogenic-like effects in mice. Behav Pharmacol 2001;12:151–62.
Bencan Z, Levin ED. The role of alpha7 and alpha4beta2 nicotinic receptors in the nicotine-induced anxiolytic effect in zebrafish. Physiol Behav 2008;95:408–12.
Bencan Z, Sledge D, Levin ED. Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety. Pharmacol Biochem Behav 2009;94:75–80.
Bergner C, Egan C, Hart P, Cachat J, Canavello P, Kalueff AV. Mutant and transgenic zebrafish in modeling neurobehavioral disorders. In: Kalueff AV, Bergner C, editors. Transgenic and mutant models of brain disorders. New York: Humana Press; 2009.
Best JD, Alderton WK. Zebrafish: an in vivo model for the study of neurological diseases. Neuropsychiatr Dis Treat 2008;4:567–76.
Bishop SJ. Neurocognitive mechanisms of anxiety: an integrative account. Trends Cogn Sci 2007;11:307–16.
Blanchard DC, Blanchard RJ. Cocaine potentiates defensive behaviors related to fear and anxiety. Neurosci Biobehav Rev 1999;23:981–91.
Blanchard DC, Blanchard RJ, Carobrez Ade P, Veniegas R, Rodgers RJ, Shepherd JK. MK-801 produces a reduction in anxiety-related antipredator defensiveness in male and female rats and a gender-dependent increase in locomotor behavior. Psychopharmacol Berl 1992;108:352–62.
Blanchard DC, Weatherspoon A, Shepherd J, Rodgers RJ, Weiss SM, Blanchard RJ. “Paradoxical” effects of morphine on antipredator defense reactions in wild and laboratory rats. Pharmacol Biochem Behav 1991;40:819–28.
Blanchard RJ, Hebert MA, Dulloog L, Kaawaloa N, Nishimura O, Blanchard DC. Acute cocaine effects on stereotypy and defense: an ethoexperimental approach. Neurosci Biobehav Rev 1998a;23:179–88.
Blanchard RJ, Hebert MA, Ferrari PF, Palanza P, Figueira R, Blanchard DC, et al. Defensive behaviors in wild and laboratory (Swiss) mice: the mouse defense test battery. Physiol Behav 1998b;65:201–9.
Blanchard RJ, Kaawaloa JN, Hebert MA, Blanchard DC. Cocaine produces panic-like flight responses in mice in the mouse defense test battery. Pharmacol Biochem Behav 1999;64:523–8.
Blaser RE, Chadwick L, McGinnis GC. Behavioral measures of anxiety in zebrafish (*Danio rerio*). Behav Brain Res 2010;208:56–62.
Bradley BF, Bridges NJ, Starkey NJ, Brown SL, Lea RW. Anxiolytic and anxiogenic drug effects on male and female gerbils in the black–white box. Behav Brain Res 2010;216(1):285–92.
Bretaud S, Li Q, Lockwood BL, Kobayashi K, Lin E, Guo S. A choice behavior for morphine reveals experience-dependent drug preference and underlying neural substrates in developing larval zebrafish. Neuroscience 2007;146:1109–16.
Brownstein MJ. A brief history of opiates, opioid peptides, and opioid receptors. Proc Natl Acad Sci USA 1993;90:5391–3.
Burne T, Scott E, van Swinderen B, Hilliard M, Reinhard J, Claudianos C, et al. Big ideas for small brains: what can psychiatry learn from worms, flies, bees and fish? Mol Psychiatry 2011;16:7-16.
Cachat J, Canavello P, Elegante M, Bartels B, Hart P, Bergner C, et al. Modeling withdrawal syndrome in zebrafish. Behav Brain Res 2010a;208:371–6.

Cachat J, Stewart A, Grossman L, Gaikwad S, Kadri F, Min Chung K, et al. Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. Nat Prot 2010d;5:1786–99.
Cachat J, Stewart A, Utterback E, Gaikwad S, Hook M, Rhymes K, et al. Deconstructing adult zebrafish behavior with swim trace visualizations. In: Kalueff AV, Cachat J, editors. Zebrafish neurobehavioral protocols. New York: Humana Press; 2010b.
Cachat JM, Canavello PR, Elegante MF, Bartels BK, Elkhayat SI, Hart PC, et al. Modeling stress and anxiety in zebrafish. In: Kalueff AV, Cachat J, editors. Zebrafish models in neurobehavioral research. New York: Humana Press; 2010c.
Canavello PR, Cachat JM, Beeson E, Laffoon AL, Grimes C, Haymore W, et al. Measuring endocrine (cortisol) responses of zebrafish to stress. In: Kalueff AV, Cachat J, editors. Zebrafish neurobehavioral protocols. New York: Humana Press; 2010.
Castilla-Cortazar I, Castilla A, Gurpegui M. Opioid peptides and immunodysfunction in patients with major depression and anxiety disorders. J Physiol Biochem 1998;54: 203–15.
Chakraborty C, Hsu CH, Wen ZH, Lin CS, Agoramoorthy G. Zebrafish: a complete animal model for in vivo drug discovery and development. Curr Drug Metab 2009;10: 116–24.
Champagne DL, Hoefnagels CC, de Kloet RE, Richardson MK. Translating rodent behavioral repertoire to zebrafish (*Danio rerio*): relevance for stress research. Behav Brain Res. 2010;214(2):332–42.
Charney DS, Woods SW, Krystal JH, Heninger GR. Serotonin function and human anxiety disorders. Ann NY Acad Sci 1990;600:558–72 discussion 72–3.
Childs E, Hohoff C, Deckert J, Xu K, Badner J, de Wit H. Association between ADORA2A and DRD2 polymorphisms and caffeine-induced anxiety. Neuropsychopharmacology 2008;33:2791–800.
Choleris E, Thomas AW, Kavaliers M, Prato FS. A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neurosci Biobehav Rev 2001;25:235–60.
Cofiel LP, Mattioli R. L-histidine enhances learning in stressed zebrafish. Braz J Med Biol Res 2009;42:128–34.
Cohen A, Young RW, Velazquez MA, Groysman M, Noorbehesht K, Ben-Shahar OM, et al. Anxiolytic effects of nicotine in a rodent test of approach–avoidance conflict. Psychopharmacol Berl 2009;204:541–9.
Colasanti A, Rabiner EA, Lingford-Hughes A, Nutt DJ. Opioids and anxiety. J Psychopharmacol in press. doi:10.1177/0269881110367726.
Correa M, Font L. Is there a major role for adenosine A2A receptors in anxiety? Front Biosci 2008;13:4058–70.
Costall B, Kelly ME, Naylor RJ, Onaivi ES. The actions of nicotine and cocaine in a mouse model of anxiety. Pharmacol Biochem Behav 1989;33:197–203.
Crawley JN. A monoamine oxidase inhibitor reverses the 'separation syndrome' in a new hamster separation model of depression. Eur J Pharmacol 1985;112:129–33.
da Silva FC, do Carmo de Oliveira Cito M, da Silva MI, Moura BA, de Aquino Neto MR, Feitosa ML. Behavioral alterations and pro-oxidant effect of a single ketamine administration to mice. Brain Res Bull 2010;83:9-15.
Darland T, Dowling JE. Behavioral screening for cocaine sensitivity in mutagenized zebrafish. Proc Natl Acad Sci USA 2001;98:11691–6.
Daza-Losada M, Rodriguez-Arias M, Maldonado C, Aguilar MA, Guerri C, Minarro J. Acute behavioural and neurotoxic effects of MDMA plus cocaine in adolescent mice. Neurotoxicol Teratol 2009;31:49–59.
de Angelis L. The anxiogenic-like effects of pentylenetetrazole in mice treated chronically with carbamazepine or valproate. Meth Find Exp Clin Pharmacol 1992;14:767–71.
de Angelis L. Experimental anxiety and antidepressant drugs: the effects of moclobemide, a selective reversible MAO-A inhibitor, fluoxetine and imipramine in mice. Naunyn Schmiedebergs Arch Pharmacol 1996;354:379–83.
Deakin JF. The role of serotonin in panic, anxiety and depression. Int Clin Psychopharmacol 1998;13(Suppl 4):S1–5.
Dere E, Zlomuzica A, de Souza Silva MA, Ruocco LA, Sadile AG, Huston JP. Neuronal histamine and the interplay of memory, reinforcement and emotions. Behav Brain Res 2010;215(2):209–20.
Dlugos CA, Rabin RA. Ethanol effects on three strains of zebrafish: model system for genetic investigations. Pharmacol Biochem Behav 2003;74:471–80.
Drapier D, Bentue-Ferrer D, Laviolle B, Millet B, Allain H, Bourin M, et al. Effects of acute fluoxetine, paroxetine and desipramine on rats tested on the elevated plus-maze. Behav Brain Res 2007;176:202–9.
Edwards JG, Michel WC. Odor-stimulated glutamatergic neurotransmission in the zebrafish olfactory bulb. J Comp Neurol 2002;454:294–309.
Egan R, Smolinsky A, Bergner C, LaPorte J, Hart P, Kalueff A. Hybridizing experimental paradigms to increase high throughput of neurobehavioral data. In: Warnick J, Kalueff A, editors. Translational neuroscience in animal research: advancements, challenges, and research ethics. Hauppauge: Nova Publishers; 2010.
Egan RJ, Bergner CL, Hart PC, Cachat JM, Canavello PR, Elegante MF, et al. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behav Brain Res 2009;205:38–44.
Eison MS. Serotonin: a common neurobiologic substrate in anxiety and depression. J Clin Psychopharmacol 1990;10:26S–30S.
Engin E, Treit D, Dickson CT. Anxiolytic- and antidepressant-like properties of ketamine in behavioral and neurophysiological animal models. Neuroscience 2009;161: 359–69.
Enginar N, Hatipoglu I, Firtina M. Evaluation of the acute effects of amitriptyline and fluoxetine on anxiety using grooming analysis algorithm in rats. Pharmacol Biochem Behav 2008;89:450–5.
Esler M, Lambert E, Alvarenga M, Socratous F, Richards J, Barton D, et al. Increased brain serotonin turnover in panic disorder patients in the absence of a panic attack: reduction by a selective serotonin reuptake inhibitor. Stress 2007;10: 295–304.
Esquivel G, Fernández-Torre O, Schruers KR, Wijnhoven LL, Griez EJ. The effects of opioid receptor blockade on experimental panic provocation with CO2. J Psychopharmacol 2009;23:975–8.
File SE. Factors controlling measures of anxiety and responses to novelty in the mouse. Behav Brain Res 2001;125:151–7.
Filliol D, Ghozland S, Chluba J, Martin M, Matthes HW, Simonin F, et al. Mice deficient for delta- and mu-opioid receptors exhibit opposing alterations of emotional responses. Nat Genet 2000;25:195–200.
Finney JL, Robertson GN, McGee CA, Smith FM, Croll RP. Structure and autonomic innervation of the swim bladder in the zebrafish (*Danio rerio*). J Comp Neurol 2006;495:587–606.
Fontana DJ, Commissaris RL. Effects of cocaine on conflict behavior in the rat. Life Sci 1989;45:819–27.
Frankowska M, Filip M, Przegalinski E. Effects of GABAB receptor ligands in animal tests of depression and anxiety. Pharmacol Rep 2007;59:645–55.
Freeman DJ, DiPaula BA, Love RC. Intramuscular haloperidol versus intramuscular olanzapine for treatment of acute agitation: a cost-minimization study. Pharmacotherapy 2009;29:930–6.
Freund JL, Freund D, Hoffmann R, Glanzmann P, Kahlau F. The open-field, non-stressed behavior of rats under the acute and chronic effect of imipramine and tranylcypromine, depending on the individual reaction type (emotional and non-emotional). Arzneimittelforschung 1979;29:1150–4.
Gallup GG, Suarez SD. An ethological analysis of open-field behaviour in chickens. Anim Behav 1980;28:368–78.
Gerlai R. Event recording and video-tracking: towards the development of high throughput zebrafish screens. Proc 5th Conference on Methods in Behav Res. Wageningen, The Netherlands; 2005.
Gerlai R. Zebrafish antipredatory responses: a future for translational research? Behav Brain Res 2009;207:223–31.
Gerlai R, Fernandes Y, Pereira T. Zebrafish (*Danio rerio*) responds to the animated image of a predator: towards the development of an automated aversive task. Behav Brain Res 2009;201:318–24.
Gerlai R, Lee V, Blaser R. Effects of acute and chronic ethanol exposure on the behavior of adult zebrafish (*Danio rerio*). Pharmacol Biochem Behav 2006;85:752–61.
Gilhotra N, Dhingra D. GABAergic and nitriergic modulation by curcumin for its antianxiety-like activity in mice. Brain Res 2010;1352:167–75.
Goldstein BJ, Goodnick PJ. Selective serotonin reuptake inhibitors in the treatment of affective disorders—III. Tolerability, safety and pharmacoeconomics. J Psychopharmacol 1998;12:S55–87.
Gonzalez-Nunez V, Rodriguez RE. The zebrafish: a model to study the endogenous mechanisms of pain. ILAR J 2009;50:373–86.
Griebel G, Blanchard DC, Jung A, Lee JC, Masuda CK, Blanchard RJ. Further evidence that the mouse defense test battery is useful for screening anxiolytic and panicolytic drugs: effects of acute and chronic treatment with alprazolam. Neuropharmacology 1995;34:1625–33.
Griebel G, Curet O, Perrault G, Sanger DJ. Behavioral effects of phenelzine in an experimental model for screening anxiolytic and anti-panic drugs: correlation with changes in monoamine-oxidase activity and monoamine levels. Neuropharmacology 1998;37:927–35.
Griebel G, Perrault G, Sanger DJ. Behavioural profiles of the reversible monoamine-oxidase-A inhibitors befloxatone and moclobemide in an experimental model for screening anxiolytic and anti-panic drugs. Psychopharmacol Berl 1997;131: 180–6.
Grossman L, Utterback E, Stewart A, Gaikwad S, Chung K, Suciu C, Wong K, Elegante M, Elkhayat S, Tan J, Gilder T, Wu N, DiLeo J, Cachat J, Kalueff AV. Characterization of behavioral and endocrine effects of LSD on zebrafish. Behav Brain Res 2010;214(2): 277–84.
Gupta. An examination of the effect of central nervous system stimulant and anti-depressant drugs on open field performance in rats. Eur J Pharmacol 1971:341–6.
Handley SL, McBlane JW. Serotonin mechanisms in animal models of anxiety. Braz J Med Biol Res 1993;26:1-13.
Handley SL, McBlane JW, Critchley MA, Njung'e K. Multiple serotonin mechanisms in animal models of anxiety: environmental, emotional and cognitive factors. Behav Brain Res 1993;58:203–10.
Hascoet M, Bourin M, Colombel MC, Fiocco AJ, Baker GB. Anxiolytic-like effects of antidepressants after acute administration in a four-plate test in mice. Pharmacol Biochem Behav 2000;65:339–44.
Heisler LK, Zhou L, Bajwa P, Hsu J, Tecott LH. Serotonin 5-HT(2C) receptors regulate anxiety-like behavior. Genes Brain Behav 2007;6:491–6.
Hoes MJ. Monoamines in Psychiatry: the role of serotonin in depression, anxiety and stress. Acta Psychiatr Belg 1982;82:287–309.
Holmes A, Rodgers RJ. Prior exposure to the elevated plus-maze sensitizes mice to the acute behavioral effects of fluoxetine and phenelzine. Eur J Pharmacol 2003;459: 221–30.
Irifune M, Sato T, Kamata Y, Nishikawa T, Nomoto M, Fukuda T, et al. Inhibition by diazepam of ketamine-induced hyperlocomotion and dopamine turnover in mice. Can J Anaesth 1998;45:471–8.
Irwin SA, Iglewicz A. Oral ketamine for the rapid treatment of depression and anxiety in patients receiving hospice care. J Palliat Med 2010;13:903–8.
Jie Z, Li T, Jia-Yun H, Qiu J, Ping-Yao Z, Houyan S. Trans-2-phenylcyclopropylamine induces nerve cells apoptosis in zebrafish mediated by depression of LSD1 activity. Brain Res Bull 2009;80:79–84.
Kahveci N, Gulec G, Ozluk K. Effects of intracerebroventricularly-injected morphine on anxiety, memory retrieval and locomotor activity in rats: involvement of vasopressinergic system and nitric oxide pathway. Pharmacol Biochem Behav 2006;85:859–67.

Kalin NH, Shelton SE, Barksdale CM. Opiate modulation of separation-induced distress in non-human primates. Brain Res 1988;440:285–92.
Kalueff A, Nutt DJ. Role of GABA in memory and anxiety. Depress Anxiety 1996;4: 100–10.
Kalueff AV, Murphy DL. The importance of cognitive phenotypes in experimental modeling of animal anxiety and depression. Neural Plast 2007;2007:52087.
Kalueff AV, Wheaton M, Murphy DL. What's wrong with my mouse model? Advances and strategies in animal modeling of anxiety and depression. Behav Brain Res 2007;179:1-18.
Kaslin J, Panula P. Comparative anatomy of the histaminergic and other aminergic systems in zebrafish (*Danio rerio*). J Comp Neurol 2001;440:342–77.
Kayir H, Uzbay IT. Nicotine antagonizes caffeine- but not pentylenetetrazole-induced anxiogenic effect in mice. Psychopharmacol Berl 2006;184:464–9.
Khan N, Deschaux P. Role of serotonin in fish immunomodulation. J Exp Biol 1997;200: 1833–8.
Kim D, Chae S, Lee J, Yang H, Shin HS. Variations in the behaviors to novel objects among five inbred strains of mice. Genes Brain Behav 2005;4:302–6.
Kim YH, Lee Y, Lee K, Lee T, Kim YJ, Lee CJ. Reduced neuronal proliferation by proconvulsant drugs in the developing zebrafish brain. Neurotoxicol Teratol. 2010;32(5):551–7.
Koran LM, Aboujaoude E, Bullock KD, Franz B, Gamel N, Elliott M. Double-blind treatment with oral morphine in treatment-resistant obsessive–compulsive disorder. J Clin Psychiatry 2005;66:353–9.
Kozak AT, Spates CR, McChargue DE, Bailey KC, Schneider KL, Liepman MR. Naltrexone renders one-session exposure therapy less effective: a controlled pilot study. J Anxiety Disord 2007;21:142–52.
Krebs-Thomson K, Geyer MA. The role of 5-HT(1A) receptors in the locomotor-suppressant effects of LSD: WAY-100635 studies of 8-OH-DPAT, DOI and LSD in rats. Behav Pharmacol 1996;7:551–9.
Kulkarni SK, Singh K, Bishnoi M. Involvement of adenosinergic receptors in anxiety related behaviours. Indian J Exp Biol 2007;45:439–43.
Kurt M, Arik AC, Celik S. The effects of sertraline and fluoxetine on anxiety in the elevated plus-maze test in mice. J Basic Clin Physiol Pharmacol 2000;11:173–80.
Lack AK, Diaz MR, Chappell A, DuBois DW, McCool BA. Chronic ethanol and withdrawal differentially modulate pre- and postsynaptic function at glutamatergic synapses in rat basolateral amygdala. J Neurophysiol 2007;98:3185–96.
Landgraf R, Wigger A. High vs low anxiety-related behavior rats: an animal model of extremes in trait anxiety. Behav Genet 2002;32:301–14.
LaPorte JL, Egan RJ, Hart PC, Bergner CL, Cachat JM, Canavello PR, et al. Qui non proficit, deficit: experimental models for 'integrative' research of affective disorders. J Affect Disord 2010;121:1–9.
Lara DR. Caffeine, mental health, and psychiatric disorders. J Alzheimers Dis 2010;20 (Suppl 1):S239–48.
Lau B, Bretaud S, Huang Y, Lin E, Guo S. Dissociation of food and opiate preference by a genetic mutation in zebrafish. Genes Brain Behav 2006;5:497–505.
Lecci A, Borsini F, Volterra G, Meli A. Pharmacological validation of a novel animal model of anticipatory anxiety in mice. Psychopharmacol Berl 1990;101:255–61.
Levin ED, Bencan Z, Cerutti DT. Assessing stress in zebrafish: anxiolytic effects of nicotine. Neurotoxicol Teratol 2006;28:709–10.
Levin ED, Bencan Z, Cerutti DT. Anxiolytic effects of nicotine in zebrafish. Physiol Behav 2007;90:54–8.
Lightowler S, Kennett GA, Williamson lJ, Blackburn TP, Tulloch IF. Anxiolytic-like effect of paroxetine in a rat social interaction test. Pharmacol Biochem Behav 1994;49: 281–5.
Linker A, Stewart A, Gaikwad S, Cachat J, Elegante M, Kalueff AV, et al. Assessing the maximal predictive validity for neuropharmacological anxiety screening assays using zebrafish. In: Kalueff AV, Cachat J, editors. Zebrafish neurobehavioral protocols. New York: Humana Press; 2010.
Lockwood B, Bjerke S, Kobayashi K, Guo S. Acute effects of alcohol on larval zebrafish: a genetic system for large-scale screening. Pharmacol Biochem Behav 2004;77: 647–54.
Lopez-Patino MA, Yu L, Cabral H, Zhdanova IV. Anxiogenic effects of cocaine withdrawal in zebrafish. Physiol Behav 2008;93:160–71.
Lopez Patino MA, Yu L, Yamamoto BK, Zhdanova IV. Gender differences in zebrafish responses to cocaine withdrawal. Physiol Behav 2008;95:36–47.
MacDonald RB, Debiais-Thubaud M, Talbot JC, Ekker M. The relationship between dlx and gad1 expression indicates highly conserved genetic pathways in the zebrafish forebrain. Dev Dyn. 2010;239(8):2298–306.
Maina G, Pessina E, Albert U, Bogetto F. 8-week, single-blind, randomized trial comparing risperidone versus olanzapine augmentation of serotonin reuptake inhibitors in treatment-resistant obsessive–compulsive disorder. Eur Neuropsychopharmacol 2008;18:364–72.
Maki Y, Inoue T, Izumi T, Muraki I, Ito K, Kitaichi Y, et al. Monoamine oxidase inhibitors reduce conditioned fear stress-induced freezing behavior in rats. Eur J Pharmacol 2000;406:411–8.
Mallinger AG, Frank E, Thase ME, Barwell MM, Diazgranados N, Luckenbaugh DA, et al. Revisiting the effectiveness of standard antidepressants in bipolar disorder: are monoamine oxidase inhibitors superior? Psychopharmacol Bull 2009;42: 64–74.
Maremmani I, Marini G, Fornai F. Naltrexone-induced panic attacks. Am J Psychiatry 1998;155:447.
Marona-Lewicka D, Thisted RA, Nichols DE. Distinct temporal phases in the behavioral pharmacology of LSD: dopamine D2 receptor-mediated effects in the rat and implications for psychosis. Psychopharmacol Berl 2005;180:427–35.
Martin JGA, Réale D. Temperament, risk assessment and habituation to novelty in eastern chipmunks, *Tamias striatus*. Anim Behav 2008;75:309–18.
Mathew SJ, Price RB, Charney DS. Recent advances in the neurobiology of anxiety disorders: implications for novel therapeutics. Am J Med Genet C Semin Med Genet 2008;148C:89–98.
Maximino C, de Brito TM, Colmanetti R, Pontes AA, de Castro HM, de Lacerda RI, et al. Parametric analyses of anxiety in zebrafish scototaxis. Behav Brain Res 2010a;210: 1–7.
Maximino C, de Brito TM, da Silva Batista AW, Herculano AM, Morato S, Gouveia Jr A. Measuring anxiety in zebrafish: a critical review. Behav Brain Res 2010b;214: 157–71.
Maximino C, de Brito TM, Dias CA, Gouveia Jr A, Morato S. Scototaxis as anxiety-like behavior in fish. Nat Protoc 2010c;5:209–16.
McNaughton N, Corr PJ. A two-dimensional neuropsychology of defense: fear/anxiety and defensive distance. Neurosci Biobehav Rev 2004;28:285–305.
Mead A, Li M, Kapur S. Clozapine and olanzapine exhibit an intrinsic anxiolytic property in two conditioned fear paradigms: contrast with haloperidol and chlordiazepoxide. Pharmacol Biochem Behav 2008;90:551–62.
Mittman SM, Geyer MA. Dissociation of multiple effects of acute LSD on exploratory behavior in rats by ritanserin and propranolol. Psychopharmacol Berl 1991;105: 69–76.
Molewijk HE, van der Poel AM, Mos J, van der Heyden JA, Olivier B. Conditioned ultrasonic distress vocalizations in adult male rats as a behavioural paradigm for screening anti-panic drugs. Psychopharmacol Berl 1995;117:32–40.
Mombereau C, Kaupmann K, Froestl W, Sansig G, van der Putten H, Cryan JF. Genetic and pharmacological evidence of a role for GABA(B) receptors in the modulation of anxiety- and antidepressant-like behavior. Neuropsychopharmacology 2004;29:1050–62.
Montgomery KC. The relation between fear induced by novel stimulation and exploratory behavior. J Comp Physiol Psychol 1955;48:254–60.
Montgomery KC, Monkman JA. The relation between fear and exploratory behavior. J Comp Physiol Psychol 1955;48:132–6.
Moraes CL, Bertoglio LJ, Carobrez AP. Interplay between glutamate and serotonin within the dorsal periaqueductal gray modulates anxiety-related behavior of rats exposed to the elevated plus-maze. Behav Brain Res 2008;194:181–6.
Mueller T, Wullimann MF. An evolutionary interpretation of teleostean forebrain anatomy. Brain Behav Evol 2009;74:30–42.
Nair J, Singh Ajit S. The role of the glutamatergic system in posttraumatic stress disorder. CNS Spectr 2008;13:585–91.
Nakanishi T, Kiryu I, Ototake M. Development of a new vaccine delivery method for fish: percutaneous administration by immersion with application of a multiple puncture instrument. Vaccine 2002;20:3764–9.
Negishi T, Kawasaki K, Suzaki S, Maeda H, Ishii Y, Kyuwa S, et al. Behavioral alterations in response to fear-provoking stimuli and tranylcypromine induced by perinatal exposure to bisphenol A and nonylphenol in male rats. Environ Health Perspect 2004;112:1159–64.
Nilsson S, Fange R. Adrenergic receptors in the swimbladder and gut of a teleost (*Anguilla anguilla*). Comp Biochem Physiol 1967;23:661–4.
Norton W, Bally-Cuif L. Adult zebrafish as a model organism for behavioural genetics. BMC Neurosci 2010;11:90.
Ohl F, Toschi N, Wigger A, Henniger MS, Landgraf R. Dimensions of emotionality in a rat model of innate anxiety. Behav Neurosci 2001;115:429–36.
Olivier B, Molewijk HE, van der Heyden JA, van Oorschot R, Ronken E, Mos J, et al. Ultrasonic vocalizations in rat pups: effects of serotonergic ligands. Neurosci Biobehav Rev 1998;23:215–27.
Onaivi ES, Martin BR. Neuropharmacological and physiological validation of a computer-controlled two-compartment black and white box for the assessment of anxiety. Prog Neuropsychopharmacol Biol Psychiatry 1989;13:963–76.
Palenicek T, Hlinak Z, Bubenikova-Valesova V, Novak T, Horacek J. Sex differences in the effects of N, N-diethyllysergamide (LSD) on behavioural activity and prepulse inhibition. Prog Neuropsychopharmacol Biol Psychiatry 2010;34:588–96.
Panula P, Chen YC, Priyadarshini M, Kudo S, Semenova S, Sundvik M, et al. The comparative neuroanatomy and neurochemistry of zebrafish CNS systems of relevance to human neuropsychiatric diseases. Neurobiol Dis 2010;40(1):46–57.
Panula P, Sallinen V, Sundvik M, Kolehmainen J, Torkko V, Tiittula A, et al. Modulatory neurotransmitter systems and behavior: towards zebrafish models of neurodegenerative diseases. Zebrafish 2006;3:235–47.
Peitsaro N, Kaslin J, Anichtchik OV, Panula P. Modulation of the histaminergic system and behaviour by alpha-fluoromethylhistidine in zebrafish. J Neurochem 2003;86: 432–41.
Peitsaro N, Sundvik M, Anichtchik OV, Kaslin J, Panula P. Identification of zebrafish histamine H1, H2 and H3 receptors and effects of histaminergic ligands on behavior. Biochem Pharmacol 2007;73:1205–14.
Picciotto MR, Brunzell DH, Caldarone BJ. Effect of nicotine and nicotinic receptors on anxiety and depression. NeuroReport 2002;13:1097–106.
Pietersen CY, Bosker FJ, Postema F, Fokkema DS, Korf J, den Boer JA. Ketamine administration disturbs behavioural and distributed neural correlates of fear conditioning in the rat. Prog Neuropsychopharmacol Biol Psychiatry 2006;30:1209–18.
Pilc A, Nowak G. GABAergic hypotheses of anxiety and depression: focus on GABA-B receptors. Drugs Today Barc 2005;41:755–66.
Powell SB, Geyer MA, Gallagher D, Paulus MP. The balance between approach and avoidance behaviors in a novel object exploration paradigm in mice. Behav Brain Res 2004;152:341–9.
Ragnauth A, Schuller A, Morgan M, Chan J, Ogawa S, Pintar J, et al. Female preproenkephalin-knockout mice display altered emotional responses. Proc Natl Acad Sci USA 2001;98:1958–63.
Renier C, Faraco JH, Bourgin P, Motley T, Bonaventure P, Rosa F, et al. Genomic and functional conservation of sedative-hypnotic targets in the zebrafish. Pharmacogenet Genomics 2007;17:237–53.

Ribeiro SJ, De Lima TC. Naloxone-induced changes in tachykinin NK3 receptor modulation of experimental anxiety in mice. Neurosci Lett 1998;258:155–8.
Rihel J, Prober DA, Arvanites A, Lam K, Zimmerman S, Jang S, et al. Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. Science 2010;327: 348–51.
Roberts AJ, Gold LH, Polis I, McDonald JS, Filliol D, Kieffer BL, et al. Increased ethanol self-administration in delta-opioid receptor knockout mice. Alcohol Clin Exp Res 2001;25:1249–56.
Rodgers RJ, Augar R, Berryman N, Hansom CJ, O'Mahony ML, Palmer RM, et al. Atypical anxiolytic-like response to naloxone in benzodiazepine-resistant 129S2/SvHsd mice: role of opioid receptor subtypes. Psychopharmacol Berl 2006;187:345–55.
Rogerio R, Takahashi RN. Anxiogenic properties of cocaine in the rat evaluated with the elevated plus-maze. Pharmacol Biochem Behav 1992;43:631–3.
Rubinstein AL. Zebrafish assays for drug toxicity screening. Expert Opin Drug Metab Toxicol 2006;2:231–40.
Rupprecht R, Zwanzger P. Significance of GABAA receptors for the pathophysiology and therapy of panic disorders. Nervenarzt 2003;74:543–51.
Sackerman J, Donegan JJ, Cunningham CS, Nguyen NN, Lawless K, Long A, et al. Zebrafish behavior in novel environments: effects of acute exposure to anxiolytic compounds and choice of *Danio rerio* line. Int J Comp Psychol 2010;23:43–61.
Salas-Ramirez KY, Frankfurt M, Alexander A, Luine VN, Friedman E. Prenatal cocaine exposure increases anxiety, impairs cognitive function and increases dendritic spine density in adult rats: influence of sex. Neuroscience 2010;169:1287–95.
Sawynok J, Pinsky C, LaBella FS. On the specificity of naloxone as an opiate antagonist. Life Sci 1979;25:1621–32.
Seibt KJ, Oliveira Rda L, Zimmermann FF, Capiotti KM, Bogo MR, Ghisleni G, et al. Antipsychotic drugs prevent the motor hyperactivity induced by psychotomimetic MK-801 in zebrafish (*Danio rerio*). Behav Brain Res 2010;214:417–22.
Sher L. The role of the endogenous opioid system in the pathogenesis of anxiety disorders. Med Hypotheses 1998;50:473–4.
Shin IC, Kim HC, Swanson J, Hong JT, Oh KW. Anxiolytic effects of acute morphine can be modulated by nitric oxide systems. Pharmacology 2003;68:183–9.
Silva MT, Alves CR, Santarem EM. Anxiogenic-like effect of acute and chronic fluoxetine on rats tested on the elevated plus-maze. Braz J Med Biol Res 1999;32:333–9.
Simon P, Dupuis R, Costentin J. Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. Behav Brain Res 1994;61:59–64.
Sobrian SK, Marr L, Ressman K. Prenatal cocaine and/or nicotine exposure produces depression and anxiety in aging rats. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:501–18.
Soderpalm AK, Blomqvist O, Engel JA, Soderpalm B. Characterization of the anticonflict effect of MK-801, a non-competitive NMDA antagonist. Pharmacol Toxicol 1995;76:122–7.
Speedie N, Gerlai R. Alarm substance induced behavioral responses in zebrafish (*Danio rerio*). Behav Brain Res 2008;188:168–77.
Stevens CW. The evolution of vertebrate opioid receptors. Front Biosci 2009;14: 1247–69.
Stewart A, Cachat J, Suciu C, Gaikwad S, Utterback E, DiLeo J, et al. Intraperitoneal injection as a method of psychotropic drug delivery in adult zebrafish. In: Kalueff AV, Cachat J, editors. Zebrafish neurobehavioral protocols. New York: Humana Press; 2010a.
Stewart A, Kadri F, DiLeo J, Chung K, Cachat J, Goodspeed J, et al. The developing utility of zebrafish in modeling neurobehavioral disorders. Int J Comp Psychol 2010b;23: 104–21.
Stewart A, Maximino C, de Brito TM, Herculano AM, Gouveia A, Morato S, et al. Neurophenotyping of adult zebrafish using the light/dark box paradigm. In: Kalueff AV, Cachat J, editors. Zebrafish neurobehavioral protocols. New York: Humana Press; 2010c.
Stewart A, Wong K, Cachat J, Gaikwad S, Kyzar E, Wu N. Zebrafish models to study drug abuse-related phenotypes. Revs in Neurosci 2011;22:95-105.
Storey S. Challenges with the development and approval of pharmaceuticals for fish. AAPS J 2005;7:E335–43.
Stray-Pedersen S. Vascular responses induced by drugs and by vagal stimulation in the swimbladder of the eel, *Anguilla vulgaris*. Comp Gen Pharmacol 1970;1:358–64.
Suarez SD, Gallup GG. Open-field behavior in chickens: the experimenter is a predator. J Comp Physiol Psych 1982a;96:432–9.
Suarez SD, Gallup GG. Open-field behavior in guinea pigs: developmental and adaptive considerations. Behav Process 1982b;7:267–74.
Sufka KJ, Warnick JE, Pulaski CN, Slauson SR, Kim YB, Rimoldi JM. Antidepressant efficacy screening of novel targets in the chick anxiety-depression model. Behav Pharmacol 2009;20:146–54.
Sun T, He W, Hu G, Li M. Anxiolytic-like property of risperidone and olanzapine as examined in multiple measures of fear in rats. Pharmacol Biochem Behav 2010;95: 298–307.
Sundstrom G, Dreborg S, Larhammar D. Concomitant duplications of opioid peptide and receptor genes before the origin of jawed vertebrates. PLoS One 2010;5(5):e10512.
Suveg C, Morelen D, Brewer GA, Thomassin K. The emotion dysregulation model of anxiety: a preliminary path analytic examination. J Anxiety Disord 2010;24(8): 924–30.
Takamori K, Yoshida S, Okuyama S. Repeated treatment with imipramine, fluvoxamine and tranylcypromine decreases the number of escape failures by activating dopaminergic systems in a rat learned helplessness test. Life Sci 2001;69:1919–26.
Uyeno ET, Benson WM. Effects of lysergic acid diethylamide on attack behavior of male albino mice. Psychopharmacologia 1965;7:20–6.
Varty GB, Morgan CA, Cohen-Williams ME, Coffin VL, Carey GJ. The gerbil elevated plus-maze I: behavioral characterization and pharmacological validation. Neuropsychopharmacology 2002;27:357–70.
Webb KJ, Norton WH, Trumbach D, Meijer AH, Ninkovic J, Topp S, et al. Zebrafish reward mutants reveal novel transcripts mediating the behavioral effects of amphetamine. Genome Biol 2009;10:R81.
Wilson MA, Junor L. The role of amygdalar mu-opioid receptors in anxiety-related responses in two rat models. Neuropsychopharmacology 2008;33:2957–68.
Wing LL, Tapson GS, Geyer MA. 5HT-2 mediation of acute behavioral effects of hallucinogens in rats. Psychopharmacol Berl 1990;100:417–25.
Winslow JT, Noble PL, Davis M. Modulation of fear-potentiated startle and vocalizations in juvenile rhesus monkeys by morphine, diazepam, and buspirone. Biol Psychiatry 2007;61:389–95.
Wong K, Elegante M, Bartels B, Elkhayat S, Tien D, Roy S, et al. Analyzing habituation responses to novelty in zebrafish (*Danio rerio*). Behav Brain Res 2010a;208:450–7.
Wong K, Stewart A, Gilder T, Wu N, Frank K, Gaikwad S, et al. Modeling seizure-related behavioral and endocrine phenotypes in adult zebrafish. Brain Res 2010b;1348:209–15.
Zakhary SM, Ayubcha D, Ansari F, Kamran K, Karim M, Leheste JR, et al. A behavioral and molecular analysis of ketamine in zebrafish. Synapse 2011;65(2):160–7.
Zarrindast MR, Babapoor-Farrokhran S, Rezayof A. Involvement of opioidergic system of the ventral hippocampus, the nucleus accumbens or the central amygdala in anxiety-related behavior. Life Sci 2008a;82:1175–81.
Zarrindast MR, Torabi M, Rostami P, Fazli-Tabaei S. The effects of histaminergic agents in the dorsal hippocampus of rats in the elevated plus-maze test of anxiety. Pharmacol Biochem Behav 2006;85:500–6.
Zarrindast MR, Valizadegan F, Rostami P, Rezayof A. Histaminergic system of the lateral septum in the modulation of anxiety-like behaviour in rats. Eur J Pharmacol 2008b;583:108–14.
Zhang HT. Regulation of the central opioidergic nervous system on the emotional state of anxiety and its possible mechanisms. Sheng Li Ke Xue Jin Zhan 1997;28:41–4.
Zhang Z, Schulteis G. Withdrawal from acute morphine dependence is accompanied by increased anxiety-like behavior in the elevated plus maze. Pharmacol Biochem Behav 2008;89:392–403.
Zwanzger P, Eser D, Nothdurfter C, Baghai TC, Moller HJ, Padberg F, et al. Effects of the GABA-reuptake inhibitor tiagabine on panic and anxiety in patients with panic disorder. Pharmacopsychiatry 2009;42:266–9.