

Zebrafish models to study drug abuse-related phenotypes

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Abstract

Mounting evidence implicates the zebrafish (*Danio rerio*) as a promising model species for reward and addiction research. Modeling drug abuse-related behavior in both adult and larval zebrafish produced a wealth of clinically translatable data, also demonstrating their sensitivity to various drugs of abuse and the ability to develop tolerance. Several studies have also applied withdrawal paradigms to model the adverse effects of drug abuse in zebrafish. In this review, we summarize recent findings of a wide spectrum of zebrafish drug abuse-related behavioral and physiological phenotypes, discuss the existing challenges, and outline potential future directions of research in this field.

Keywords: anxiety; drug abuse; cortisol; stress tolerance; withdrawal; zebrafish.

Introduction

Drug abuse and addiction are serious mental health and societal problems (Larson and Bammer, 1996; Banken, 2004; Aceijas et al., 2006; Brady et al., 2008). They represent complex brain disorders with multiple symptoms (Figure 1) caused by both genetic and environmental factors (Brunette et al., 2003; Sareen et al., 2004; Busto et al., 2010; Cheung et al., 2010; Hamilton, 2010). Various experimental (animal) models have been introduced to target different aspects of drug abuse (Brady, 1991; Markou et al., 1993; Crabbe et al., 1994; Friedman and Eisenstein, 2004; Jentsch, 2008).

Zebrafish (*Danio rerio*) continue to emerge as a new promising model for reward and addiction research (Gerlai et al., 2000; Ninkovic and Bally-Cuif, 2006; Webb et al.,

2009; Cachat et al., 2010). Dopaminergic projections to zebrafish forebrain parallel the mesolimbic system (implicated in drug addiction in mammals; Rink and Wullimann, 2002), representing a pathway that is highly conserved and develops at early ontogenesis (Boehmler et al., 2004). Various behavioral paradigms have been particularly useful in zebrafish addiction research (Table 1). For example, conditioned place preference (CPP) and similar models reveal strong rewarding properties of different drugs in zebrafish (Kily et al., 2008; Webb et al., 2009). Genetic factors also contribute to zebrafish behavioral responses (Egan et al., 2009), demonstrating the link between individual genes and reward phenotypes (Ninkovic et al., 2006; Webb et al., 2009).

Modeling zebrafish behavior traditionally utilizes both adults and younger animals, including ‘larvae’ and older, free-feeding ‘fry’. Although the distinction between larvae and fry is important, for the purposes of this article we will apply the term ‘larvae’ to both these stages. Overall, zebrafish larvae display robust drug-evoked neurobehavioral phenotypes (Best and Alderton, 2008) and offer the ability to study multiple animals simultaneously within a high-throughput battery (Best and Alderton, 2008; Best et al., 2008; Creton, 2009). However, larval models do not always display the complex behavior of their adult counterparts and lack fully established mediatory and endocrine systems (Kimmel et al., 1995), neural circuits (Kastenhuber et al., 2010), and neuromuscular systems (Dou et al., 2008). Therefore, both models should be used complementarily to study drug abuse-related neurobehavioral domains (Figure 1).

Particular focus must be given to selecting the drug concentrations for zebrafish studies. First, background literature is lacking for many psychotropic drugs, because the zebrafish is a new model in behavioral pharmacology (Zon and Peterson, 2005; Rubinstein, 2006; Liang, 2009). Second, although zebrafish possess all major ‘mammalian’ neurotransmitters, peptides, and hormones (Egan et al., 2009; Mueller and Neuhaus, 2010), species differences in animal physiology play a role. For example, unlike humans and rodents, zebrafish have two forms of the serotonin transporter (SERT A and B) (Wang et al., 2006; Norton et al., 2008; Severinsen et al., 2008). Furthermore, the blood-brain barrier of teleost fish is less exclusive than in mammals, enabling serotonin to pass through it and affect both central and peripheral mechanisms (Genot et al., 1981; Khan and Deschaux, 1997; Stoddard et al., 2003). Thus, zebrafish might be differentially sensitive to various serotonergic drugs, compared to other model species. Third, discrepancies are probable within different zebrafish studies, because it is difficult to translate drug concentrations from larval into adult fish models. Finally, because the

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Figure 1 Multiple inter-related domains of drug abuse.

Note that all these behavioral domains are highly relevant to zebrafish models of drug abuse described here.

method of drug treatment in most zebrafish studies is immersion (instead of injection, as in other species), the effective drug concentrations can be based on different pharmacokinetics than in rodents or humans. However, this does not necessarily represent a flaw of a model. For example, water immersion can be advantageous for some drugs (e.g., that are quickly metabolized or require prolonged treatment) and does not involve injection stress that could confound behavioral data.

Sensitivity of zebrafish to drugs of abuse

Animal sensitivity to acute and chronic drugs of abuse (including both reward-related and other behavioral effects) is an important phenotype (Figure 1) (Gerlai et al., 2000, 2006; Ninkovic and Bally-Cuif, 2006; Cachat et al., 2010) which correlates with the potential of drug abuse. The sensitivity to drugs of abuse revealed strong genetic determinants of both increased (Martin et al., 2000; Michna et al., 2001; Lindemann et al., 2008) and decreased (Trigo et al., 2007; Krall et al., 2008; Thomsen et al., 2009) risks of drug abuse. Both animal and clinical models reveal striking parallels in their sensitivity to cocaine (Reichel and Bevins, 2010), amphetamine (Mathews et al., 2010), benzodiazepines (Straub et al., 2010), ethanol (Heilig et al., 2010), nicotine (Jackson et al., 2009), opiates (Solecki et al., 2009), and other psychotropic compounds (Melichar et al., 2001; Passie et al., 2008). Zebrafish models are also sensitive to a wide range of psychotropic compounds (Table 1). Although these responses will be only briefly

discussed here, they generally parallel rodent and clinical observations, thereby confirming the translational value of zebrafish models.

Larval zebrafish display an overt morphine preference, which is reduced by pretreatment with naloxone and abolished by blocking dopamine signaling with D1 antagonists (Bretaud et al., 2007). Adult zebrafish also show a strong dose-dependent preference for morphine (Lau et al., 2006), which strikingly resembles rodent responses to this drug (Barr et al., 1985; Sala et al., 1992; Garcia-Lecumberri et al., 2010).

Zebrafish models have been extensively used to study the effects of ethanol. In larvae, acute exposure to ethanol evokes hyperlocomotion at lower doses and a hypolocomotory effect at higher doses (Lockwood et al., 2004). Strain differences in larval ethanol responses have also been reported (Loucks and Carvan, 2004; Lockwood et al., 2004). A similar U-shaped dose-response curve has been observed in adult zebrafish (Gerlai et al., 2000), also showing strain-dependent variations in their responses to ethanol (Gerlai et al., 2008), as well as reduced shoaling and increased aggressiveness (Echevarria et al., 2010). By contrast, chronic ethanol treatment has an anxiolytic effect on zebrafish behavior (Egan et al., 2009) (Figure 2A), also altering the expression of multiple brain genes, some of which are implicated in addiction (Kily et al., 2008) and similarly affected by ethanol in mammals (Sircar and Sircar, 2006; Heilig et al., 2010).

Nicotine exposure produces strong effects on zebrafish place preference and learning (Levin and Chen, 2004; Kily et al., 2008). Although learning and memory are not

Table 1 Sensitivity of zebrafish models to selected drugs of abuse (reported positive findings: A, adult zebrafish models; L, larval zebrafish models).

Domains	Drugs							References	
	Morphine	Ethanol	Nicotine	Cocaine	Caffeine	Amphetamine	Benzodiazepines		LSD ^a
Reward properties	A L	A	A	A	A	A			Darland and Dowling, 2001; Kily et al., 2006; Lau et al., 2006; Ninkovic and Bally-Cuif, 2006; Bretaud et al., 2007; Kily et al., 2008
Acute behavioral effects	A L	A L	A L	A L	A	A L	A	A	Gerlai et al., 2000; Darland and Dowling, 2001; Loucks and Carvan, 2004; Lockwood et al., 2004; Lau et al., 2006; Bretaud et al., 2007; Parker and Connaughton, 2007; Lopez-Patino et al., 2008a; Bencan et al., 2009; Egan et al., 2009; Grossman et al., 2010; Irons et al., 2010; Sackerman et al., 2010; Wong et al., 2010; Lopez-Patino et al., 2008a; Egan et al., 2009; Cachat et al., 2010
Chronic behavioral effects	A	A	A	A			A		Dlugos and Rabin, 2003; Gerlai et al., 2006; Kily et al., 2008; Cachat et al., 2010
Developing tolerance	A	A	A						Cachat et al., 2010
Acute (single) withdrawal	A	A	A	A	A	A	A	^a	Lopez-Patino et al., 2008a; Egan et al., 2009; Gerlai et al., 2009; Cachat et al., 2010
Repeated withdrawal	A	A	A				A	^a	Cachat et al., 2010, Figure 5

^aLysergic acid diethylamide (note that its acute or repeated withdrawal in our studies with adult zebrafish failed to evoke overt anxiety, which is in line with clinically known weak withdrawal-evoking potential of this drug).

specifically addressed here, the established effects of drugs of abuse that affect cognitive functions give further validity to the zebrafish model of drug abuse (Gerlai et al., 2006; Ninkovic and Bally-Cuif, 2006; Lopez-Patino et al., 2008; Grossman et al., 2010). Chronic nicotine exposure in larval zebrafish leads to reduced swimming and impairs their startle response (Parker and Connaughton, 2007). In adult zebrafish, acute administration of nicotine has an anxiolytic-like effect (Levin et al., 2006, 2007) (also see Figure 2B) similar to its effect in humans and rodents (Jackson et al., 2009).

Although zebrafish show a clear preference for cocaine in the CPP paradigm, there are also several strains with a decreased sensitivity in this model (Darland and Dowling, 2001; Lopez-Patino et al., 2008). Overall, zebrafish CPP models display a substantial similarity to rodent cocaine CPP studies (Dietz et al., 2007). Adult zebrafish treated acutely with mild doses of cocaine display arousal (e.g., circling, fin extension), increased aggressiveness, and decreased visual sensitivity (Darland and Dowling, 2001). However, higher concentrations of cocaine reduce fish responses (Darland and Dowling, 2001) in spite of the high brain cocaine levels (Lopez-Patino et al., 2008).

The sensitivity of larval zebrafish to amphetamine (Irons et al., 2010) generally parallels a similar locomotor response observed in mammals. Whereas low concentrations of amphetamine increase activity, higher concentrations of this drug markedly reduce zebrafish locomotion (Ninkovic and Bally-Cuif, 2006; Webb et al., 2009). The rewarding properties of amphetamine have been reported in adult zebrafish in the CPP test (Ninkovic and Bally-Cuif, 2006) and also parallel those seen in rodents (Mathews et al., 2010).

Benzodiazepines are known to activate the reward system in rodents (Straub et al., 2010) and have also been tested in zebrafish models. For example, both chlordiazepoxide (CDP) and diazepam display anxiolytic-like effects in adult zebrafish. Although CDP increases exploratory behavior in the light/dark box paradigm, it does not affect vertical localization in the novel tank test (Bencan et al., 2009; Sackerman et al., 2010). In contrast, diazepam increases exploration in the novel tank, exhibiting a biphasic response for low to moderate doses (Bencan et al., 2009).

Unlike their extensive use in rodent research, hallucinogenic drugs have only recently been tested in zebrafish. For example, salvinorin A, one of the most potent hallucinogens, exhibits rewarding properties in the CPP, accelerates zebrafish swimming in acute low doses, and reduces locomotion (evoking low-velocity 'trance-like' state) at high doses (Braida et al., 2007). Recently resurrected interest in psychedelic drug research (Dyck, 2005; Passie et al., 2008; Gonzalez-Maeso and Sealfon, 2009) guided our group's interest to testing these drugs in zebrafish. Similar to other fish species' responses to lysergic acid diethylamide (LSD) (Keller and Umbreit, 1956; Arbit, 1957; Trout, 1957; Chessick et al., 1964), adult zebrafish produced significantly shorter latency to enter the top and fewer freezing bouts (Grossman et al., 2010). LSD also caused significantly more top transitions and time spent in top, as well as elevated

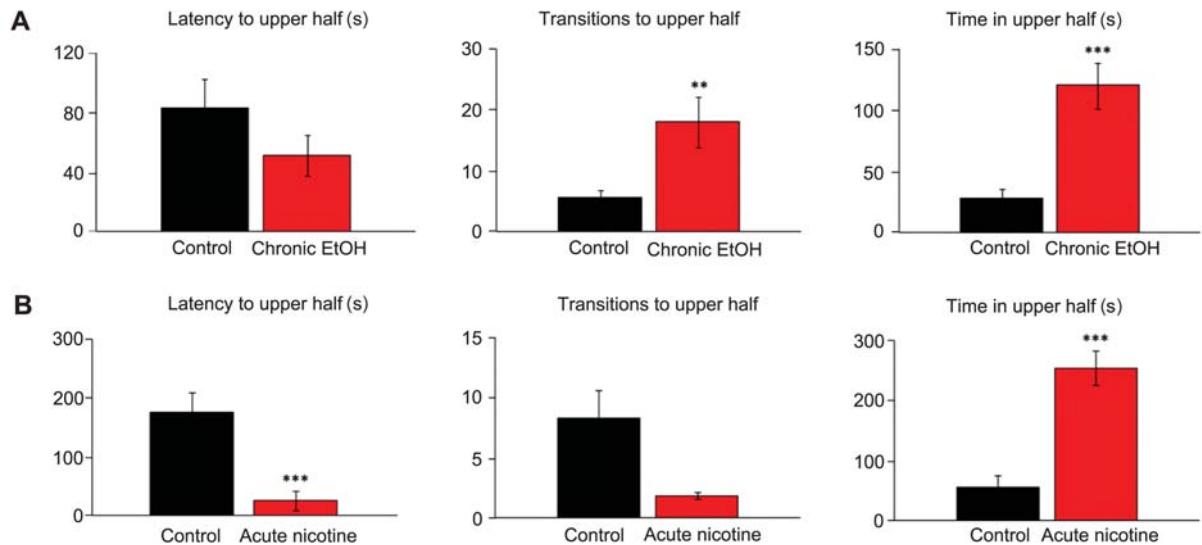


Figure 2 Examples of anxiolytic effects of chronic ethanol and acute nicotine in a 6-min novel tank test. (A) Chronic ethanol (0.3% for 1 week; $n=24$ for each group). (B) Acute nicotine administration (10 mg/l for 5 min; $n=15$ per each group). $**p<0.01$, $***p<0.001$, U -test.

cortisol levels, consistent with its well-known endocrine effects in humans (Bliss et al., 1956) and animals (Sackler et al., 1963; Weltman and Sackler, 1966). Acute administration of another hallucinogen, 3,4-methylenedioxyamphetamine (MDMA), produced less clear-cut behaviors (Figure 3B), although showing a similar reduction in freezing (data not shown). Although this might be relevant to known clinical effects of MDMA and LSD, including the rewarding/euphoric behaviors (Melichar et al., 2001; Passie et al., 2008) and elevated corticoids (Bliss et al., 1956; Sackler et al., 1963; Weltman and Sackler, 1966; de la Torre et al.,

2000; Parrott, 2009), further research is needed to examine zebrafish sensitivity to various hallucinogenic drugs.

Zebrafish can also be used to study plant compounds with potential psychoactive properties. For example, kratom (*Mitragyna speciosa*) is a medicinal leaf, often used as a tea for its calming and energizing effects. Mitragynine, the major alkaloid identified from kratom, is a partial opioid agonist producing similar effects to morphine in mammals (Babu et al., 2008). When administered acutely, kratom extract has a mild sedative effect in the zebrafish novel tank (Figure 4) but did not have psychoactive/anxiolytic action over a wide range

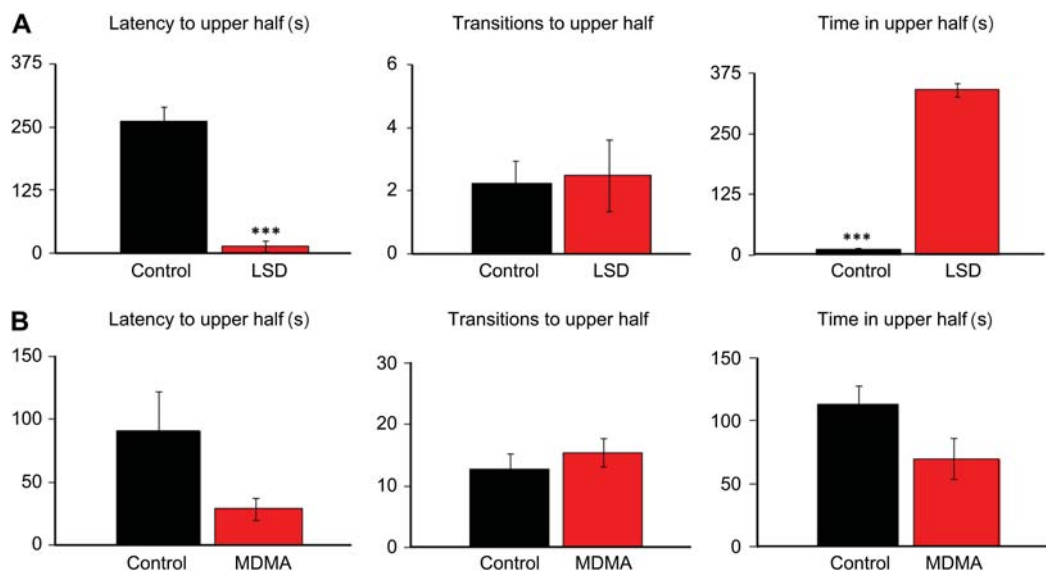


Figure 3 Examples of behavioral effects of hallucinogenic drugs in a 6-min novel tank test. (A) Acute lysergic acid diethylamide (LSD) administration (250 $\mu\text{g/l}$ for 20 min; $n=10$ per each group). (B) Acute 3,4-methylenedioxyamphetamine (MDMA) administration (5 mg/l for 20 min; $n=10$ per each group). $***p<0.001$, U -test.

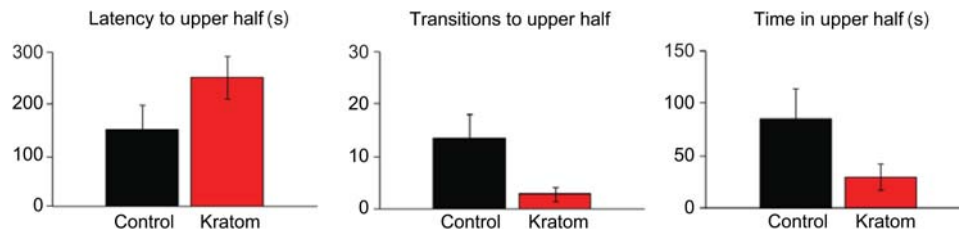


Figure 4 Examples of behavioral effects of kratom extract in a 6-min novel tank test. Fish were acutely exposed to 6 g/l of kratom extract for 20 min (n=10 per each group).

of concentrations (1–12 g/l) tested in our laboratory. This suggests that zebrafish can display differential sensitivity to the complex behavioral profiles of the drugs.

Tolerance and withdrawal

Commonly observed for drugs of abuse in both clinical (Joseph et al., 2010; Roberts and Dollard, 2010) and animal (Aley and Levine, 1997; Gerlai et al., 2006) studies, ‘tolerance’ is the progressive reduction of drug sensitivity, which requires higher doses to obtain the same effects. Tolerance represents an important drug abuse-related phenotype (Figure 1), mediated by the brain’s adaptive mechanisms (Wang et al., 2007; Nagy, 2008; Popik et al., 2010). Recent studies have confirmed tolerance in adult zebrafish, reporting that after chronic exposure to ethanol, zebrafish have a reduced response to the acute effects of the drug (Gerlai et al., 2006). Another study reported tolerance following chronic ethanol exposure, which was also influenced by zebrafish genotype (strain) (Dlugos and Rabin, 2003). Tolerance is also seen in zebrafish following chronic exposure to nicotine (Kily et al., 2008), collectively paralleling known rodent and clinical findings.

‘Withdrawal’ is another key phenotype associated with drug abuse (Cachat et al., 2010) (Figure 1), extensively studied in various rodents following cessation of ethanol (Morris et al., 2010), cocaine (Santucci and Rosario, 2010), benzodiazepines (De Ross et al., 2008), and opiates (Becker et al., 2010). These symptoms are also sensitive to various pharmacological (Bhutada et al., 2010; Rawls et al., 2010), genetic (Morice et al., 2010), and behavioral (Smith and Yancey, 2003; Saadipour et al., 2009) factors. Withdrawal is believed to be mediated by the tendency of the body to maintain homeostasis, in which counter-regulatory mechanisms produce unopposed effects when a drug is abruptly removed (Tyler and Seivewright, 1984; Bayard et al., 2004; Khong et al., 2004; Cruz et al., 2008; Nagy, 2008; Ista et al., 2010).

Researchers have recently turned their attention to studying the withdrawal phenomena in zebrafish (Table 1). Acute discontinuation of drug treatment – acute (single) withdrawal – is a common form of withdrawal, evoking strong behavioral effects in humans and rodents (Ashton, 1984; Kokkinidis et al., 1986; Koob et al., 1989; Wiese et al., 2000; Jonkman et al., 2008). In all studies, the most common behavioral manifestations of withdrawal include anxiety, seizures, sedation,

and pain (Harris and Gewirtz, 2004; Gowing et al., 2009; Strong et al., 2009; Joseph et al., 2010; Minozzi et al., 2010). In zebrafish, acute ethanol discontinuation decreases zebrafish shoaling behavior (Gerlai et al., 2009), whereas cocaine withdrawal evokes marked hyperlocomotion marked by erratic movements and increased exploratory behavior (Lopez-Patino et al., 2008a,b). Acute discontinuation of ethanol, diazepam, and morphine produces robust anxiogenic-like behavioral responses in zebrafish such as hypolocomotion, decreased top transitions, and increased freezing bouts in the novel tank (Cachat et al., 2010).

This novelty-based test (Levin et al., 2006; Egan et al., 2009; Stewart et al., 2010) is an effective paradigm for observing withdrawal behavior in zebrafish owing to these clear-cut anxiety-like responses (see other papers in this issue for details on zebrafish models of anxiety). Our experiments with CDP, a sedative benzodiazepine known to produce anxiolytic effects in both rodents (Mathiasen et al., 2008) and zebrafish (Sackerman et al., 2010), have tested its withdrawal potential in zebrafish. Two groups of fish (n=15) were administered chronic CDP (10 mg/l) for 4 months, with another group of similar size used as a drug-free control. One of the CDP-treated groups was withdrawn from CDP for 7 days before testing in the novel tank test (Figure 5). The withdrawal group displayed an increased latency to the top half of the tank, less transitions to the top, and a shorter duration in the top, compared to controls. The withdrawal group also exhibited an increased freezing duration, compared to both the control and the chronic CDP groups (Figure 5). Similar to the response observed in both humans (Bearn et al., 2001; Fox et al., 2009; Shi et al., 2009) and rodents (Borlikova et al., 2006; Rabbani et al., 2009), withdrawal elevates zebrafish cortisol, correlating with the expected higher levels of stress and anxiety (Cachat et al., 2010) (also see Figure 5 for CDP data).

In humans, chronic drug abuse represents a cyclical process of repeated reward and withdrawal. Therefore, to more accurately model clinical withdrawal phenomena, ‘repeated withdrawal’ models are needed in addition to ‘acute withdrawal’ studies. Repeated drug withdrawal paradigms have been recently developed for rodents, showing that both the rat and human share common triggers of relapse (such as the drug of abuse, stress, stimuli, or the environment conditioned to the drug of abuse), and that withdrawal selectively potentiates responses to anxiogenic stimuli (Miczek and Vivian, 1993; Fendt and Mucha, 2001; Vorel et al., 2001; Harris and Aston-Jones, 2003; Jonkman et al., 2008). A recent study

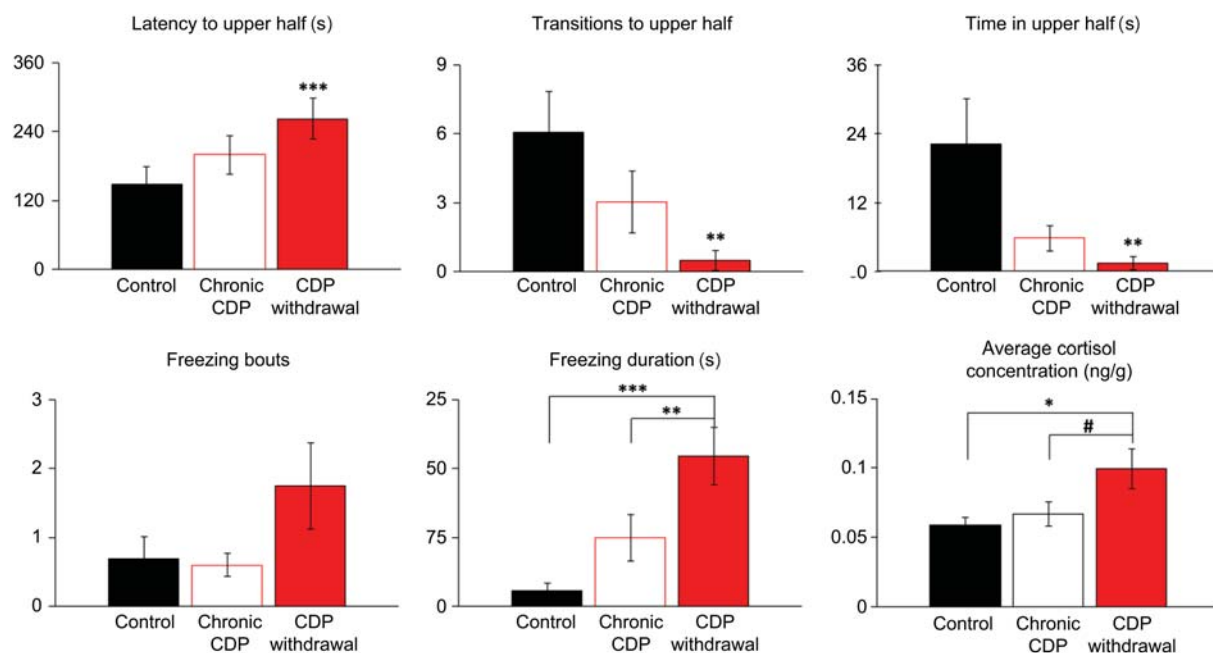


Figure 5 Behavioral effects of 100 mg/l chronic chlordiazepoxide (CDP) in a 6-min novel tank test. CDP was administered for 4 months, after which one group was withdrawn for 7 days ($n=15$ per each group). There was significant group effect for latency to upper half ($F_{(2,44)}=8.7, p<0.001$), transitions to top ($F_{(2,44)}=5.2, p<0.01$), time in top ($F_{(2,44)}=6.1, p<0.01$), and freezing duration ($F_{(2,44)}=14.7, p<0.001$). * $p<0.05$; ** $p<0.01$; *** $p<0.001$; # $p=0.05-0.1$ (trend) between the groups; post-hoc Tukey test for significant one-way analysis of variance data.

successfully applied repeated withdrawal protocol to adult zebrafish, reporting that morphine and ethanol withdrawal leads to robust anxiety-like behaviors (Cachat et al., 2010). Interestingly, not all drugs of abuse evoke withdrawal. For example, LSD [known for its low ability to induce withdrawal in humans (Parsons et al., 2009) and animals (Banerjee, 1971)] does not evoke withdrawal in both acute and repeated zebrafish models (data not shown).

The complexity of withdrawal phenotypes (Martinotti et al., 2008; Cooper and Haney, 2009; Cruickshank and Dyer, 2009; Henningfield et al., 2009; Prat et al., 2009; Shoptaw et al., 2009; Teixeira, 2009; Wu et al., 2009), as well as the difficulty in modeling withdrawal syndrome in animals (Keane and Leonard, 1989; Becker, 2000; Braw et al., 2008), represent another challenge. Potentially interesting directions of research could focus on neurochemical alterations, neural circuits, and the long-term consequences (Nava et al., 2006; Li et al., 2008; Zhang et al., 2008; Shi et al., 2009) of drug withdrawal in zebrafish. The genomic profiling of zebrafish withdrawal also provides further insights, including altered gene expression in the zebrafish brain following chronic drug treatment and withdrawal (Gerlai et al., 2006; Kily et al., 2008; Gerlai et al., 2009). Sex differences reported for zebrafish withdrawal-related behaviors (Lopez Patino et al., 2008b) parallel sex differences in human (Fox et al., 2006) and rodent (Alves et al., 2008; Butler et al., 2009; Strong et al., 2009; Taylor et al., 2009) withdrawal responses, therefore increasing populational and construct validity of these models.

Concluding remarks

Because drug abuse is a serious biomedical and societal problem, a better understanding of the mechanisms behind drug reward and abuse is needed to develop innovative and more efficient treatments. Considerable efforts have recently been made to develop reliable and high-throughput assays for zebrafish behavior (Ninkovic and Bally-Cuif, 2006). Mounting evidence indicates that zebrafish cognition is complex and often parallel the abilities of mammals (Stewart et al., 2010). Because cognitive deficits commonly accompany drug abuse (Figure 1) (Barker et al., 2004; Kelley et al., 2005; Rogers et al., 2005; Rapeli et al., 2006), the ability to quantify both affective and cognitive phenomena in zebrafish becomes important to study in relation to drug abuse phenotypes. Therefore, novel behavioral models focusing on reward, cognitive, and affective phenotypes might be needed to more comprehensively model drug abuse in zebrafish.

We have previously emphasized the importance of promoting both larval and adult zebrafish research (Stewart et al., 2010). The rich behavioral repertoire of the latter complements the sensitivity and high-throughput nature of the former (Rihel et al., 2010). Although the genetic aspects of addiction have long been studied in mammalian organisms, zebrafish are also an ideal model for the use in forward genetics because they can be rapidly cloned, have a relatively short generation time, and have large progeny sizes that facilitate large-scale screens (Ninkovic and Bally-Cuif, 2006). Further analysis of the molecular and biochemical pathways underlying pre-

existing genetic differences in zebrafish, such as strain-dependent variances, will advance our understanding of how drug abuse affects the brain (Loucks and Carvan, 2004).

The ease of genetic manipulations and potential for high-throughput screening (He et al., 2006; Hogan et al., 2008; Kily et al., 2008) represent the strengths of zebrafish-based models. However, zebrafish models of drug abuse have other important strengths, including behavioral complexity, three-dimensionality, robustness of drug-evoked phenotypes, and the ability to target a wide spectrum of neurobehavioral phenomena (Figure 1 and Table 1). Therefore, a more comprehensive picture is needed in the field of zebrafish drug abuse research, with a fair balance between their behavioral and genetic strengths. The growing availability of new zebrafish strains through the Zebrafish International Resource Center (Sprague et al., 2008) and current progress of the Sanger Institute's zebrafish genome project complements mounting support to zebrafish behavioral research from the National Science Foundation and recent cross-National Institutes of Health programs to develop new zebrafish genetic models and tools for their phenotyping. Together with multiple lesser initiatives, such as the international Zebrafish Neuroscience Research Consortium and Zebrafish Neurophenome Project (<http://kaluefflab.com/znr.html>), this represents a positive and promising development in the field.

Finally, we acknowledge the importance of applying cross-species and cross-domain modeling (Stewart et al., 2010) to drug abuse-related phenotypes (Figure 1). For example, clinical drug abuse is highly comorbid with anxiety (Schneier et al., 2010), depression (Perkins et al., 2010), bipolar disorder (Swann, 2010), and psychoses (Gouzoulis-Mayfrank, 2008). Therefore, animal models that simultaneously target these comorbidities could lead to valid clinically relevant experimental paradigms of drug abuse. Furthermore, because genetic variability influences addiction sensitivity (Loucks and Carvan, 2004), the overlay between behavioral phenotypes and the propensity for, and the expression of, addictive behavior must be considered. This approach could also improve the ability of animal models to mimic the entire pathway of drug addiction, rather than a single point along the continuum (Warnick et al., 2010). From this point of view, understanding the pathology of drug abuse and addiction will benefit from implementation of innovative approaches and novel model species, such as zebrafish.

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