The utility of the zebrafish model in conditioned place preference to assess the rewarding effects of drugs

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Substance abuse is a significant public health concern both domestically and worldwide. The persistent use of substances regardless of aversive consequences forces the user to give higher priority to the drug than to normal activities and obligations. The harmful and hazardous use of psychoactive substances can lead to a dependence syndrome. In this regard, the genetic and neurobiological underpinnings of reward-seeking behavior need to be fully understood in order to develop effective pharmacotherapies and other methods of treatment. Animal models are often implemented in preclinical screening for testing the efficacy of novel treatments. Several paradigms exist that model various facets of addiction including sensitization, tolerance, withdrawal, drug seeking, extinction, and relapse. Self-administration and, most notably, conditioned place preference (CPP) are relatively simple tests that serve as indicators of the aforementioned aspects of addiction by means of behavioral quantification. CPP is a commonly used technique to evaluate the motivational effects of compounds and experiences that have been associated with a positive or negative reward, which capitalizes on the basic principles of Pavlovian conditioning. During training, the unconditioned stimulus is consistently paired with a neutral set of environmental stimuli, which obtain, during conditioning, secondary motivational properties that elicit approach behavior in the absence of the unconditioned stimulus. For over 50 years, rodents have been the primary test subjects. However, the zebrafish (*Danio rerio*) is gaining favor as a valuable model organism in the fields of biology, genetics, and behavioral neuroscience. This paper presents a discussion on the merits, advantages, and limitations of the zebrafish model and its utility in relation to CPP. *Behavioural Pharmacology* 24:375–383 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Behavioural Pharmacology 2013, **24**:375–383

Keywords: addiction, conditioned place preference, drug seeking, reward, zebrafish

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Received 30 March 2013 **Accepted as revised** 20 May 2013

Introduction

Substance abuse has become a significant public health concern with widespread detrimental consequences both domestically and worldwide. In 2011, an estimated 21.6 million Americans (8.4% of the population) who were 12 years and older needed treatment for problems associated with alcohol and/or drugs, with only 2.3 million receiving it (National Institute on Drug Abuse). The abuse of tobacco, alcohol, and illicit drugs not only causes a host of health issues for the drug user but also accrues ~$600 billion of health, crime, and productivity-related expenditures annually in the USA alone (National Institute on Drug Abuse). In an effort to assess the abuse potential of addictive substances, it is imperative that genetic and neurobiological mechanisms underlying reward-seeking behavior be fully understood. Adoption, family, and twin studies have found that substance abuse and dependence are heritable traits (Agrawal and Lynskey, 2008). Thus, the identification of candidate genes, in addition to gaining a solid understanding of the neurobiological basis of reward seeking, would facilitate development of new pharmacotherapies. Animal models have been used in this endeavor.

Laboratory animal behavioral paradigms have been used to model various facets of addiction, including sensitization, tolerance, withdrawal, extinction, and relapse (Mohn *et al.*, 2004). Animal behavior that can be easily quantified has allowed self-administration and conditioned place preference assays to serve as indicators of the aforementioned facets of addiction. Traditionally, rats and mice have been the go-to laboratory animals when modeling human disease states, principally because of the anatomical, biological, and genomic homology between rodents and humans (Lieschke and Currie, 2007). However, the rodent model is hindered by a number of features, including high cost, difficult husbandry, lengthy developmental periods, and inefficiency in high-throughput techniques.

The zebrafish (*Danio rerio*), compensating for these disadvantages, has been accepted as a valuable model organism in the fields of developmental biology and genetics and is steadily gaining popularity in behavioral neuroscience. Although there is a degree of physiological and phylogenetic disparity between fish and humans, the central nervous system (CNS) of zebrafish develops and is organized in a similar manner to that of their fellow vertebrates, and analogous circuitry that mediates reward has been identified in the zebrafish brain (Rink and Wullimann, 2002). Further, the zebrafish genome has been fully sequenced (Postlethwait *et al.*, 1998; Woods

0955-8810 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/FBP.0b013e328363d14a

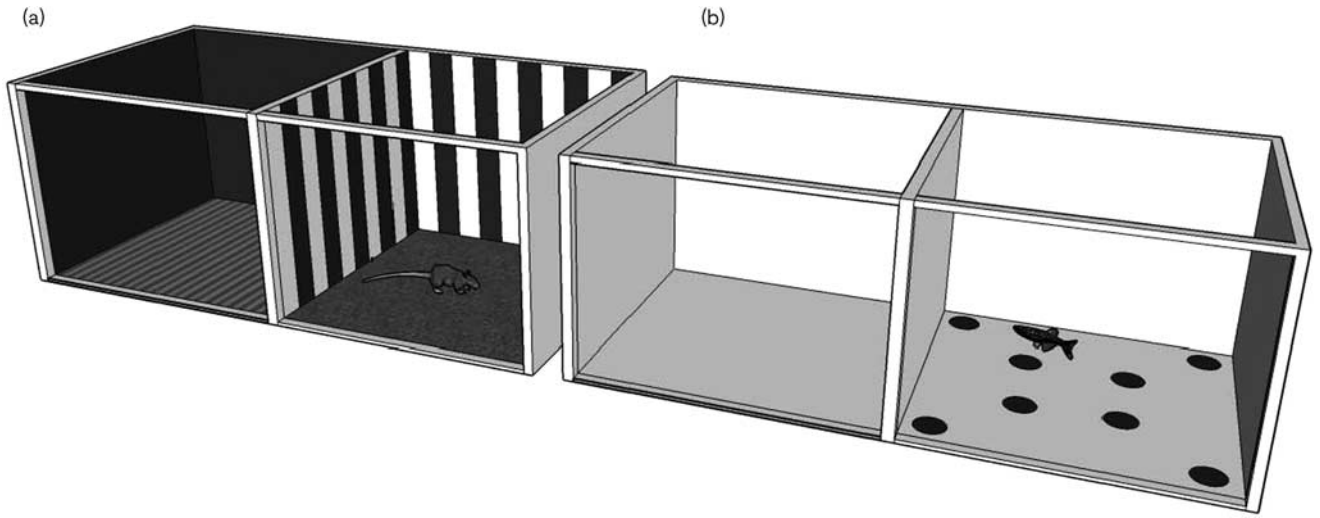
et al., 2000), and a number of genetic tools/mutants are available that allow for investigation of drug-altered gene expression and, more generally, cellular functioning in the vertebrate CNS (Dooley and Zon, 2000; Rinkwitz *et al.*, 2011). The zebrafish model allows for in-vivo, high-throughput screening of the behavioral effects of novel drugs at relatively little cost and personnel time. These attributes help us to establish the zebrafish as an economical and efficient tool for screening new drugs, developing new pharmacotherapies, and identifying genetic mechanisms involved in reward behavior. In this context, researchers are beginning to capitalize on the advantageous characteristics of this species, facilitated by a well-established experimental procedure for assessing the abuse potential and rewarding properties of drugs and natural rewards, conditioned place preference (CPP).

Conditioned place preference

Following the seminal work of Beach (1957) and of Katz and Gormezano (1979), the CPP paradigm has been meticulously utilized, primarily with rodents, to assess the motivational effects of various substances and natural rewards. In CPP procedures, the principal motivational property of the drug or natural reward under investigation serves as the unconditioned stimulus (UCS). The UCS is consistently paired with a neutral set of environmental stimuli, which, during conditioning, gain secondary motivational properties that elicit approach behavior in the absence of the UCS. Subsequently, after repeated pairings, these previously neutral contextual cues serve as the conditioned stimuli (CS). The UCS can be virtually any treatment that possesses rewarding properties, and an array of tactile, olfactory, spatial, and visual contextual cues can be used as the CS. CPP is a form of Pavlovian conditioning, essentially sharing the same basic elements. As such, the CPP task allows for the observation of the phenomena associated with Pavlovian conditioning, including acquisition, extinction, extinction and reinstatement, recovery from extinction, latent inhibition, external inhibition, stimulus generalization and discrimination, and blocking.

A number of comprehensive reviews have been published that catalog the CPP literature in detail and discuss a broad range of relevant issues that are far beyond the scope of this article (Tzschentke, 1998, 2007; Schechter and Calcagnetti, 1998). In short, the CPP apparatus can vary in design; however, it is traditionally a conditioning box (or tank) comprised of two or three distinct compartments. Three-dimensional representations of examples of rodent and zebrafish CPP apparatus are shown in Fig. 1. The latter design uses a third, neutrally designed central chamber as a start box and for passage between the two conditioning compartments. Compartments can be designed in such a way that animals will reliably show a significant preference for one environment over the other before conditioning. In this 'biased' design, treatment is repeatedly paired with the subjects' nonpreferred side. If CPP is observed, rewarding effects of the treatment are inferred as responsible for the animal conquering its innate aversion (Calcagnetti and Schechter, 1993; Ninkovic and Bally-Cuif, 2006). Biased designs have been criticized because of the increased risk of committing a type 1 error if the treatment has anxiolytic properties, which may be responsible for the animal overcoming an intrinsic aversion to the environment. As a result, the 'unbiased' design has been predominantly used and is held in higher regard than the biased design (Sanchis-Segura and Spanagel, 2006). No significant preference for either side is shown during baseline

Fig. 1



A 3D representation of a rodent condition place preference (CPP) apparatus (a) and a zebrafish CPP apparatus (b).

testing in an unbiased apparatus, allowing subjects to be randomly assigned to conditioning chambers. For an in depth discussion on the advantages and disadvantages of each design, refer to Tzschentke (2007).

The CPP protocol typically consists of three phases that take place on consecutive days. During phase 1, the animal is allowed access to all compartments of the apparatus, and baseline preference for each side is measured. During conditioning trials (phase 2), animals are either restricted to the compartment in which they are exposed to the UCS (or control treatment) for a period of time or they are restricted to the remaining compartment without UCS exposure. In the final phase, the animal can access the entire apparatus, and change in preference is calculated by subtracting baseline preference from the final value. This difference is used to quantify place preference behavior, and if the change is significant, CPP is confirmed.

Conditioning chambers acquire motivational properties that elicit approach behavior in the absence of the UCS, demonstrating that CPP adheres to mechanisms of Pavlovian conditioning. This is further validated by extinction of CPP, which occurs when the animal is repeatedly exposed to the CS without the presence of the UCS. CPP can then be reinstated after a period of extinction by re-exposing the animal to the UCS in the formerly associated context. Extinction and reinstatement procedures after establishment of CPP can be used as effective models of drug-primed reinstatement of drug seeking. A 50% basal preference change in zebrafish after 4 weeks of daily conditioning with 1% ethanol has been reported (Parmer *et al.*, 2011). This preference was extinguished (returned to baseline) by conditioning subjects without ethanol for roughly 2 weeks. Animals were then exposed to ethanol in a separate tank and placed in the conditioning apparatus, consequently reinstating place preference. Undoubtedly, it can be a challenge to model 'human' addiction using animal models, but behaviors that manifest in human addicts, including relapse (extinction–reinstatement) and, most notably, drug seeking (CPP), have reliably been modeled in rodents and zebrafish (Fuchs *et al.*, 2002; Sanchez *et al.*, 2003; Aguilar *et al.*, 2009; Cachat *et al.*, 2010).

Nearly identical in concept to CPP is the conditioned place aversion (CPA) paradigm. The key difference between CPP and CPA is in the quality of the presented stimulus. In CPA the aversive stimulus can be (as often is the case) illness or the withdrawal from morphine dependence. The stimulus is then paired with placement of the organism into a distinct environment containing contextual cues. During subsequent testing, time spent avoiding the environment associated with the aversive stimulus is the primary measure of CPA learning (Acquas and Chiara, 1994; Parker and Mcdonald, 2000; Gracy *et al.*, 2001). Drugs found to induce CPP at low concentrations may induce significant aversion at higher doses. It has been reported that zebrafish treated with Salvinorin A (0.2 and 0.5 μg/kg) show CPP behavior, whereas at a higher dose (80 μg/kg), CPA behavior is produced (Braida *et al.*, 2007).

It is extremely important to note that CPP does not unambiguously measure a drug's abuse potential. CPP is an associative learning model to assess reward-seeking behavior. A drug that blocks CPP may do so by disrupting the ability of the organism to form a conditioned association. Conversely, if a drug produces CPP, one cannot simply extrapolate its addiction potential. The motivational effects of a given drug would be best understood with the implementation of additional testing (e.g. self-administration procedures). The ideal laboratory setting would seek to discover the convergence of information over several distinct paradigms (Tzschentke, 1998).

Rodent conditioned place preference behavior

The use of rodents as models to understand human CNS function and dysfunction has been a key factor in the growth of the field of behavioral neuroscience. A quick review of the literature reveals that rodent CPP research has a long and illustrious history. CPP has been induced in rats by addictive substances frequently abused by humans, including D-amphetamine (Yates *et al.*, 2012), cocaine (Bahi *et al.*, 2008; Russo *et al.*, 2008; Zakharova *et al.*, 2009), diazepam (Papp *et al.*, 2002), ethanol (Kotlinska *et al.*, 2011), heroin (Ashby *et al.*, 2003), ketamine (Li *et al.*, 2008), methamphetamine (Goeders and Goeders, 2004; Zakharova *et al.*, 2009), 3,4-methylenedioxy-*N*-methylamphetamine (Starosciak *et al.*, 2012), morphine (Liang *et al.*, 2006), and nicotine (Brielmaier *et al.*, 2008, 2012). For a more comprehensive overview of the effects of drugs of abuse on rodent CPP tests, refer to Tzschentke (2007). The literature reveals that CPP has not been established with drugs that are not normally abused by humans, such as antidepressants, neuroleptics, and antihistamines (Papp *et al.*, 2002). Further, drugs that do elicit self-administration by human addicts are also found to be self-administered by animals (O'Brien and Gardner, 2005). In contrast to self-administration behaviors, CPP has been found to differentially assess drug reward and engage distinct neuropharmacological circuitry (Tzschentke, 1998).

In one study, CPP behavior in male Wistar rats was established after six separate treatments with amphetamine (1 mg/kg), morphine (1 mg/kg), nomifensine (5 mg/kg), cocaine (20 mg/kg), nicotine (0.8 mg/kg), and diazepam (2.5 mg/kg), and the effect of the NMDA antagonist 1-aminocyclopropanecarboxylic acid (ACPC) on CPP was assessed in two subgroups. One group was administered ACPC 30 min before conditioning trials, which was found to inhibit CPP behavior for all six drugs; the second group

received ACPC 30 min before the final place preference test, which blocked morphine, diazepam, and nicotine CPP, but did not block CPP for nomifensine, cocaine, and amphetamine (Papp *et al.*, 2002). These latter drugs are dopamine reuptake inhibitors, which substantially increase the availability of dopamine within the reward pathway, in particular in the nucleus accumbens (Carboni *et al.*, 1989; Kaddis *et al.*, 1995). When the firing rate of dopamine neurons projecting to the nucleus accumbens from the ventral tegmental area increases, reward may translate into motivation (Hernandez *et al.*, 2008; Norton and Bally-Cuif, 2010). It is thus no surprise that a single administration of ACPC on the final day of testing did not block CPP produced by psychostimulants, which aggressively boost dopamine levels. Various natural rewards, including food, sucrose, social interaction, and novelty, were also found to induce CPP, but ACPC had no effect on natural reward place preference (Papp *et al.*, 2002). Thus, natural rewards may be mechanistically different from certain drug rewards. Certainly, there is a likelihood of differences in the intrinsic value. It was accordingly proposed that ACPC administration may be an effective treatment for nicotine, benzodiazepine, and opiate users. In summation, the authors utilized the CPP paradigm to assess the motivational properties of a wide range of stimuli and subsequently discovered a potential pharmacotherapy.

Comparable studies are abundant in the rodent literature, providing the burgeoning zebrafish field with immense data regarding brain circuitry and neurochemicals associated with reward behavior. Although rodents have been the 'gold standard' of vertebrate animal models used to assess CPP behavior since the inception of the procedure, this model is uneconomical, is difficult to handle and maintain, develops slowly, and is not viable for large-scale genetic and embryonic manipulation (Spence *et al.*, 2008). The zebrafish, compensating for these disadvantages, is a more efficient vertebrate model for investigation of the rewarding properties of psychoactive drugs.

The zebrafish model of conditioned place preference behavior

The potential of the zebrafish as a vertebrate model is being realized throughout various fields of study (e.g. developmental biology, genetics, behavioral neuroscience). The zebrafish compensates for many inherent disadvantages of its mammalian counterpart, allowing researchers to replicate and expand upon rodent protocols using a model that is more amenable to high-throughput designs, embryonic manipulation, and large-scale genetic screening. A substantial number of genes are likely involved in brain disorders and behavioral functioning, and 'thus one has to screen thousands of mutants to tackle this complexity and identify appropriate mutations' (Gerlai, 2010). Zebrafish may be ideally suited for this enterprise because they are prolific breeders that externally fertilize a high number of rapidly developing progeny and they can economically be housed with little maintenance. This species is also a viable model of vertebrate development because of their transparent embryos and larvae that allow for direct visualization and quantification of the developing vertebrate organ systems.

The use of technological advancements and new techniques will be necessary to maximize the potential of the zebrafish as a vertebrate model. For example, a transparent mutant line named 'casper' has recently been developed, providing cancer and stem-cell researchers with a model that is amenable to high-resolution, in-vivo single-cell observation during adulthood (White *et al.*, 2008). Another technique is 'Brainbow' imaging, which uses fluorescent proteins of differential hues to visualize and distinguish live cells (Weissman *et al.*, 2011). Although this was originally developed for mice, a Brainbow protocol has been tailored for the zebrafish (Pan *et al.*, 2011). Connectomic approaches such as these may provide researchers with a directly observable blueprint of neural circuitry in zebrafish, which will provide insight into human neurobiological connectivity and functioning. Moreover, to reduce personnel time, eliminate observer bias, and collect accurate data, it is imperative that researchers who utilize protocols of a high-throughput nature adopt automated video tracking software, such as Ethovision XT (Wageningen, Netherlands, Noldus Information Technology, the Netherlands; *http://www.noldus.com*). It is also possible to generate two-dimensional and three-dimensional reconstructions of zebrafish swim paths that represent changes in velocity.

Albeit the zebrafish nervous system is less sophisticated than that of the rodent, adults are capable of performing complex behaviors, related to learning and memory (Darland and Dowling, 2001; Colwil *et al.*, 2005; Sison and Gerlai, 2010, 2011), aggression (Gerlai *et al.*, 2000; Echevarria *et al.*, 2010), anxiety (Blaser *et al.*, 2009), social behavior (Dlugos and Rabin, 2003; Echevarria *et al.*, 2008), and addiction (Lopez-Patino *et al.*, 2008; Mathur and Guo, 2010). In comparison with the rodent literature, zebrafish behavioral assays are currently few in number. Thus, the replication and maturation of behavioral paradigms is paramount to establish good face validity and a systematic methodology across multiple fields, specifically addiction research.

A query in PubMed with the following search terms, 'zebrafish AND conditioned place preference' yielded 11 articles dating back to 2001. It has been argued the most prominent area in which the adult zebrafish has contributed to behavioral genetics is reward (Norton and Bally-Cuif, 2010). With regard to CPP, zebrafish contain several neurochemicals that are homologous to those in humans, such as dopamine, acetylcholine, and serotonin. There is also evidence for homologous brain areas: the medial zone of the dorsal telencephalic region

and the dorsal nucleus of the ventral telencephalic area are believed to be the teleost anatomical homologs of the mammalian amygdala and striatum, respectively (Lau *et al.*, 2011). In addition, several pharmacological agents that have been tested on zebrafish, including monoamine oxidase inhibitors (MAOIs) and lysergic acid diethylamide (LSD), produce behavioral effects analogous to those seen in the rodent model (Stewart *et al.*, 2012). It should be emphasized, however, that fish have morphologically different analogous exteroceptive senses compared with those in rodents and other mammals, which allows for innate and learned responses to UCS and CS (Tierney, 2011).

CPP by adult zebrafish has been reported using D-amphetamine (Ninkovic *et al.*, 2006; Ninkovic and Bally-Cuif, 2006), cocaine (Darland and Dowling, 2001; Braida *et al.*, 2007; Darland *et al.*, 2012), ethanol (Kily *et al.*, 2008; Mathur *et al.*, 2011; Parmer *et al.*, 2011), food (Lau *et al.*, 2006), morphine (Lau *et al.*, 2006; Mathur *et al.*, 2011), nicotine (Kily *et al.*, 2008), and salvinorin A (Braida *et al.*, 2007). Table 1 provides a more comprehensive and detailed account of zebrafish CPP behavior. Two-week-old larval zebrafish have also been reported to show a preference for morphine in a choice chamber paradigm (Bretaud *et al.*, 2007). However, larvae in this study were given the option to self-immerse in a side containing morphine solution or a side with tank water, which more closely resembles self-administration than it does CPP. CPP behavior has not been reported in larval zebrafish, probably because learning-related behaviors do not appear until stages nearing adulthood.

A streamlined protocol that can elicit CPP in zebrafish after only one conditioning session has been published recently (Mathur *et al.*, 2011). Including set-up, clean-up, and video analysis, $\sim$7 h of work is required over a 2 day period for this procedure, which is considerably less time than required for nearly all other reported CPP protocols. Moreover, this protocol incorporates four experimental tanks and video cameras, allowing eight zebrafish to be simultaneously conditioned and video tracked. Adhering to this protocol, CPP was successfully established after a single 20-min exposure to both morphine sulfate (3.0, 7.5 μmol/l) and ethanol (1.5%). This study exemplifies the advantageous characteristics of the zebrafish model and is a prime example of aquatic translation of rodent paradigms into a high-throughput experimental design that requires relatively little time and fewer resources.

Discussion

The objective of this article was two-fold: to emphasize the value of the zebrafish as a behavioral model, primarily to assess the motivational effects of compounds and experiences, and to expound upon the utility of the CPP paradigm. Zebrafish have recently become more prevalent in research laboratories for a number of reasons. On a basic level, they are an advantageous research animal because of prolific breeding, which yields a high number of offspring that reach maturity in a relatively short period of time. A clear chorion allows for zebrafish to be studied during early development, and behavior can be studied in both larvae and adults, albeit larval behavior is limited. A large number of zebrafish can be housed and maintained in a fairly small area, particularly when compared with the amount of space that would be necessary for the equivalent number of rodents. The husbandry of this species is rather simple, which decreases personnel, time, and cost required to maintain the population. Many models of neurological diseases have been established in the zebrafish, providing researchers with a high throughput, in-vivo model amenable to screening candidate compounds.

These characteristics establish the zebrafish as an ideal candidate for use in high-throughput behavioral screens and as a valuable adjunct to other animal models. This is especially true when considering that the mechanisms of learning and memory are suspected to involve a large number of genes, brain regions, and neurotransmitters that would otherwise be tedious to study without high-throughput techniques (Al-Imari and Gerlai, 2008). For zebrafish to be an effective model of human behaviors (e.g. addition), it is crucial to assess the comparative neuroanatomy between the species, particularly with respect to the brain areas commonly implicated in learning and memory processes(which include Pavlovian conditioning). Although the telencephalon and mesodiencephalon develop differently in the teleost brain, its overall systemic architecture resembles that of the mammalian brain (Panula *et al.*, 2010). Certainly, the zebrafish brain is less complex than that of mammalian models: for instance, it lacks the hippocampus and cortex. Nevertheless, behavioral studies have shown this species to be capable of performing well in learning tasks. This suggests that there are structures in the zebrafish brain that are homologous to human brain areas necessary for completion of these tasks, and because zebrafish are operating with a simpler system, minimal neural structures necessary for learning can be discovered, thus appointing this aquatic model a useful 'reductionist tool' (Sison and Gerlai, 2010). Generally, the zebrafish brain is organized in a manner similar to that of their fellow vertebrates (Tropepe and Sive, 2003). Although the mesolimbic system is not conserved among zebrafish and mammals, both the lateral and medial pallium in the zebrafish are analogous to the hippocampus and related mesolimbic circuitry in humans (Gould, 2011). In addition, zebrafish possess a structure called the optic tectum that is likened to the neocortex in mammals because of its size and position (Eddins *et al.*, 2009). The anatomical organization of the nervous system is fairly conserved among vertebrates, and the existing data

Table 1 Zebrafish conditioned place preference behavior

Treatment	Dose(s)	Number of conditioning trials	Strain(s)/age	Apparatus	Effect	References
D-Amphetamine	40 μg/g i.p. injection	3	AB, ache^{sb55}/+ mutants 3–6 months	Two chambers. ½ brown ½ white w/ black spots Conditioning chambers separated by a transparent slider Biased	Decreased CPP in ache^{sb55}/+ mutants compared to AB.	Ninkovic *et al.* (2006)
D-Amphetamine	40 μg/g i.p. injection	3	AB, ABO, tuebingen 3–6 months	Two chambers. ½ brown ½ white w/ black spots Conditioning chambers separated by a transparent slider Biased	40 μg/g CPP in all strains tested.	Ninkovic and Bally-Cuif (2006)
Cocaine	1, 5, 10, 15 and 20 mg/l added to tank water	1	Strain not specified 8–12 months	Two chambers. ½ white ½ white w/ black dots Conditioning chambers separated by a perforated wall Unbiased	5, 10 and 20 mg/l CPP	Darland and Dowling (2001)
Cocaine	20 mg/kg added to tank water	1	Strain not specified Adult fish	Two chambers. ½ white ½ white w/ black dots Conditioning chambers separated by a perforated wall Unbiased.	20 mg/kg CPP	Braida *et al.* (2007)
Cocaine + SCH23390 + sulpiride	10 mg/l + 10 μmol/l + 10 μmol/l added to tank water	2	AB 6–8 months	Three chambers Duct tape wrapped around one end of tank/other end remained clear Central habituation chamber Unbiased	10 mg/l CPP + SCH23390 did not block cocaine CPP + sulpiride blocked cocaine CPP	Darland *et al.* (2012)
Ethanol	0.5,1 and 1.5% (20 min in tank water)	1	Tuebingen wild type 4 months	Two chambers. ½ black spots ½ black and white stripes Biased	1% CPP Water treated fish also displayed CPP	Kily *et al.* (2008)
Ethanol	0.5, 1.0, 1.5% (20 min in tank water)	1	AB wild type 6–9 months	Three chambers. ½ white ½ blue dotted Brown central chamber Unbiased	1.5% CPP	Mathur *et al.* (2011)
Ethanol	1% (20 min in tank water)	3	Not specified	Two chambers ½ vertical black stripes ½ black spots Unbiased	1% CPP	Parmer *et al.*, (2011)
Food + naloxone	Live brine shrimp + 2.7 μmol/l pre-exposure in tank	1	AB/EK hybrid Age not specified	Three chambers White/white with black dots Conditioning compartments separated by gray central alley Unbiased	Food CPP blocked by naloxone	Lau *et al.* (2006)
Morphine	1.5, 3, 7.5 and 15 μmol/l added to tank water	1	AB/EK hybrid Adult fish	Three chambers White/white with black dots Conditioning compartments separated by gray central alley Unbiased	3 and 7.5 μmol/l induced CPP	Lau *et al.* (2006)

Morphine + naloxone + SCH23390 + sulpiride	3 μmol/l + 2.7 μmol/l + 9.3 μmol/l + 0.15 mmol/l Added to tank water	1	AB/EK hybrid Adult fish	Three chambers White/white with black dots Conditioning compartments separated by gray central alley Unbiased	Naloxone blocked morphine CPP + SCH23390 blocked morphine CPP + sulpiride blocked morphine CPP	Lau *et al.* (2006)
Morphine sulfate	1.5, 3, 7.5 and 15 μmol/l added to tank water	1	AB wild type 6–9 months	Three chambers. ½ white ½ blue dotted Brown central chamber Unbiased	3 and 7.5 μmol/l CPP	Mathur *et al.* (2011)
Nicotine	3, 30, 60, 150 and 300 μmol· added to tank water	1	Tuebingen wild type/4 month	Two chambers. ½ black dots ½ black and white striped Unbiased	3, 30 60 and 300 CPP Water treated fish also displayed CPP	Kily *et al.* (2008)
Salvinorin A + norbinaltorphimine + rimonabant	0.2, 0.5, 1 and 80 μg/kg + 10 mg/kg + 1 mg/kg i.m. injection pretreatment	1	Unspecified strain Adult fish	Two chambers. ½ white ½ white w/ black dots Conditioning chambers separated by a perforated wall Unbiased	0.2 and 0.5 μg/kg CPP. 80 μg/kg CPA + salvinorin A CPP blocked by norbinaltorphimine + salvinorin A CPP blocked by rimonabant	Braida *et al.* (2007)
Spiradoline	1 mg/kg i.m. injection	1	Unspecified strain Adult fish	Two chambers. ½ white ½ white w/ black dots Conditioning chambers separated by a perforated wall Unbiased	1 mg/kg CPA	Braida *et al.* (2007)

CPA, conditioned place aversion; CPP, conditioned place preference; EK, ekkwill; i.p., intraperitoneal; i.m., intramuscular.

present sufficient similarities to allow for confident extrapolation of results from zebrafish to mammals.

Despite the neuroanatomical differences between zebrafish and mammals, they should both be capable of functioning in a similar manner during learning and remembering owing to their neurochemical similarities. Behavioral responses of zebrafish to nicotine have been found to mirror the effects seen in rodents, suggesting that they share some of the neurotransmitter and receptor systems necessary for cognitive processing (Eddins *et al.*, 2009). In addition to sharing all of the major areas with the mammalian CNS, the zebrafish system uses the same neurotransmitters, including γ-aminobutyric acid, glutamate, dopamine, noradrenaline, serotonin, histamine, and acetylcholine (Panula *et al.*, 2010).

Although the zebrafish provides many benefits and affords a host of opportunities for use in a variety of scientific subdisciplines, there are some limitations to the use of this species as a model. Although there is growing evidence relating certain zebrafish brain structures to those in their mammalian counterparts, the neuroanatomy is not identical, which will inevitably limit the conclusions that can be drawn with regard to the regional causes of observed behaviors.

Another limitation of the zebrafish model pertains specifically to pharmacological studies. The most common method of drug administration is through a bath solution, in which the fish swim in ambient water containing a given dosage of the drug. Zebrafish are known to absorb most water-soluble drugs through this procedure, but the exact uptake can vary (Best and Alderton, 2008). This issue can be circumvented if preliminary studies conduct measurements to confirm that the absorption into the zebrafish adequately reflects the drug concentration in the water. This method may also not be ideal when screening novel compounds that are likely to be poorly water-soluble. In addition, obtaining the appropriate drug concentration in water may require large amounts of compound, which may not be cost-effective. Alternatively, the drugs may be administered at precise concentrations through intraperitoneal injections, according to a recent protocol developed for this particular species (Kinkel *et al.*, 2010). This method has been found to be more precise and accurate than dissolving drugs in water.

Information garnered from model species can indicate the appropriate avenues of research to investigate further in more complex models, or directly in humans. While acknowledging the limitations of this particular model, the accumulated information shows that the zebrafish is an excellent candidate for modeling a variety of conditions, as it represents an ideal balance between complexity and simplicity, both in anatomy and behavior. In many ways, the trajectory of the zebrafish as a model species

has followed that previously traversed by the rat and mouse models. The maturation of the field of genetics allowed for the advent of transgenic mice and, with them, necessitated a tool to assess the effects of these specific induced mutations. Behavioral tests were developed to satisfy this need. Upon their frequent use by molecular neurobiologists, it was acknowledged that these behavioral paradigms required more exhaustive review and modification to ensure that false positive or negative findings were not being reported (Gerlai, 2001). The relative paucity of behavioral studies on learning and addiction in zebrafish can be decreased through increased use of the CPP paradigm. Effectively, use of this single apparatus allows for the convergence of information from zebrafish studies, as data can be compiled to provide a fuller understanding of reward-seeking behavior. Coupled with the fact that the CPP is appropriate for testing multiple species (i.e. humans, mice, rats, gerbils, adult zebrafish), it also provides opportunities to utilize the benefits of each model and assemble the fullest possible picture of the neurobiology behind these behaviors or, more specifically, the motivational effects of chemical substances and their affiliated experiences as they relate to addiction.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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