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Pharmacological Analyses of Learning and Memory in Zebrafish (*Danio rerio*)

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Abstract

Over the last decade, zebrafish (*Danio rerio*) have become valuable as a complementary model in behavioral pharmacology, opening a new avenue for understanding the relationships between drug action and behavior. This species offers a useful intermediate approach bridging the gap between in vitro studies and traditional mammalian models. Zebrafish offer great advantages of economy compared to their rodent counterparts, their complex brains and behavioral repertoire offer great translational potential relative to *in vitro* models. The development and validation of a variety of tests to measure behavior, including cognition, in zebrafish has set the stage for the use of this animal for behavioral pharmacology studies. This has led to research into the basic mechanisms of cognitive function as well as screening for potential cognition-improving drug therapies, among other lines of research. As with all models, zebrafish have limitations, which spans pharmacokinetic challenges to difficulties quantifying behavior. The use, efficacy and limitations associated with a zebrafish model of cognitive function are discussed in this review, within the context of behavioral pharmacology.

Keywords

Zebrafish; pharmacology; learning; memory

I. Introduction

The small teleost zebrafish (*Danio rerio*) has expanded the landscape of behavioral pharmacology by allowing for more economic and higher-throughput assays of behavioral function, including cognition. Zebrafish provide a key intermediate model between high throughput *in vitro* screens and classic mammalian models as they have the accessibility of *in vitro* models and the complex functional capabilities of mammalian models. The clear

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chorion and embryo during development of the zebrafish allows for continuous visualization of the anatomical changes associated with development, which, along with short maturation times and the capability for complex behavior beginning at just 3 days post fertilization (dpf), makes this model particularly useful for measuring changes to the developing nervous system. Moreover, the rich array of developmental, behavioral, and molecular benefits offered by the zebrafish have contributed to an increasing demand for the use of zebrafish in behavioral pharmacology. Essential for this endeavor has been the development of tests to evaluate a spectrum of behavior and drug-related effects in zebrafish.

Learning and memory function has been quantified in this species, either via the use of procedures with clear rodent analogs or the development of novel, zebrafish-specific assays. As such, zebrafish have been used to 1) characterize the behavioral consequences following exposure to a variety of psychoactive drugs (e.g. therapeutic drugs, drugs of abuse, etc.), 2) to understand mechanistic pathways, and 3) have emerged as an important tool in drug discovery. Zebrafish have been shown to be useful in behavioral toxicology (see Bailey et al., 2013) and behavioral genetics (Norton & Bally-Cuif, 2010) as well as being an excellent model for neurobehavioral disorders (Kalueff et al., 2014). In this review, we present and discuss the use of zebrafish in behavioral pharmacology studies investigating cognition (including effects of drugs of abuse as well as therapeutic drugs). We cover the experimental procedures used to characterize cognitive function in zebrafish and the drawbacks posed by a zebrafish model.

Borrowing largely from a robust mammalian literature, procedures that assess Pavlovian and operant conditioning have been developed for the zebrafish. Any meaningful understanding of the functional effects of a drug includes an understanding of the drug's capacity to affect one's ability to make associations among stimuli or be sensitive to the consequences of one's actions. Every species tested has been shown to be capable of at least simple associative learning, including *Drosophila* and *Aplysia*. Zebrafish, despite their relatively small size and non-mammalian status, are capable of far more than simple CS:US associations. These animals display a wide-range of cognitive function, which can be indexed via tests of habituation learning, conditioned place preference (CPP), passive and active avoidance learning, spatial and visual discrimination, timing (of an interval), and self-control/impulsivity. The procedures described here are limited to those that are thought to measure learning and memory processes; other aspects of cognition (e.g. fear, anxiety, social behavior, models of brain disorders, etc.) are also accessible via a zebrafish model (see Iturriaga-Vasquez et al., 2012) but fall outside the scope of the present review. Associative learning in the zebrafish in general has already been reviewed elsewhere (see Gerlai, 2011) as have other cognitive endpoints (see Jesuthasan, 2012), and the present review is meant to complement these works.

Here, we selected the tools used to measure cognition that are common in the literature or which show particular value in characterizing drug effects. It is important to note that many of the experimental procedures described in this review share common features. These common features may ultimately measure similar behavioral processes that have historically been studied under the rubric of stimulus control in Pavlovian and operant learning preparations. Associating different stimuli with one another and responding differently in

the presence of certain stimuli, or “stimulus control” (Catania, 1992), are measured by all the experimental procedures discussed here. So, the degree to which the tasks described here are meant to describe or tap discrete aspects of cognition should not be over-stated. Rather, a continued appreciation that learning and memory are apical processes, involving multiple systems and levels of information integration, is meant to be emphasized here.

II. Tools used to assess executive function in zebrafish

Habituation learning

Habituation refers to the situation in which a response (often a reflexive response) becomes less likely or less vigorous following repeated stimulation (Thompson & Spencer, 1966; Rankin et al., 2009) and can be understood within the context that animals tend to acclimate to a stimulus that is not particularly harmful, conserving resources for potentially dangerous stimuli. Although superficially quite simple, this process of habituation is indeed a form of learning, a reasonable distinction considering that memory of preceding stimuli mediates responsiveness to later stimuli. A range of behavioral tests for habituation learning have appeared in the zebrafish literature, many modeled after those used with rodents. A tap-elicited startle reflex has been used in adolescent and adult zebrafish to characterize drug effects (e.g. Eddins et al., 2010), and touch- or auditory-elicited reflexes in larvae or young fish are also common (Liu et al., 2012; Mann et al., 2010). In the tap-elicited startle habituation, the stimulus is delivered at a standard interval (e.g. 1 min) for 10 or more stimulus presentations, this way within-session learning curves are generated for each subject (See Figure 1 for an example of the testing apparatus).

Conditioned Place Preference

A procedure sometimes conflated with selfadministration, although a distinctly different tool for assessing the appetitive characteristics of a drug, is conditioned place preference. CPP describes the selection by the zebrafish of an environment where it previously experienced the effects of a drug (Bardo & Bevins, 2000; Winger et al., 2005). This is an arrangement anchored theoretically in associative mechanisms, and ultimately measures an organism's capacity to learn. Although, because presumably *what* is learned in CPP is an association between environmental cues and the rewarding/hedonic effects of a drug, CPP is often described simply as a tool to assess the rewarding value of a drug.

A typical CPP arrangement in zebrafish, as in rodents, involves the differential pairing of two distinct environments (termed the CS) with the drug stimulus of interest (termed the US). The environment that has been paired, often simultaneously, with the drug acquires secondary rewarding effects. Stimuli associated with rewarding effects often elicit approach behavior in animals and it is this response that is captured with CPP; typically duration spent in the drug-paired environment or latency to enter the environment is the primary dependent measure. CPP has been used in zebrafish to characterize the appetitive characteristics of cocaine (Darland & Dowling, 2001), nicotine (Kedikian et al., 2013), opiates (Lau et al., 2006), ethanol (Parker et al., 2014), MK801 (Swain et al., 2004), salvia (Braida et al., 2007) and others (e.g. Collier & Echevarria, 2013), described below. It should be emphasized here that CPP performance is not analogous to “drug abuse”.

Avoidance Learning

Both active avoidance (i.e. the animal learns that a discrete response will result in the avoidance of an aversive stimulus being delivered) and passive avoidance (i.e. the animal learns to avoid an environment previously paired with an aversive stimulus) learning have been established in zebrafish (Kim et al., 2010; Ng et al., 2012; Richetti et al., 2011; Seibt et al., 2011; Truong et al., 2014). In these tests, avoidance learning is typically inferred by the amount of time spent outside the compartment previously associated with an aversive stimulus. Truong et al. (2014) have described an active avoidance procedure where zebrafish must shuttle from one side to the other side of a tank to avoid the delivery of a shock. To establish passive avoidance, methods are employed similar to those used in CPP although the US is aversive rather than appetitive. Here again, the dependent measure is time spent outside the USpaired environment. This sort of passive avoidance learning is frequently used to characterize associative learning and short- and long-term memory in zebrafish and has been used to characterize the effects on learning of antipsychotics (Seibt et al., 2011), scopolamine (Kim et al., 2010; Richetti et al., 2011), MK801 (Ng et al., 2012; Seibt et al., 2011) and others, described below.

Memory

Several tasks used in the zebrafish literature explicitly target memorial processes, though many (if not *all*) other tasks described in this review tap memory to some degree. Maze learning, for example, which is described below in the context of spatial discrimination learning certainly relies on memory. The many and important distinctions between acquisition (i.e. learning) and performance (i.e. memory) endpoints are not belabored here. Rather, two prominent tasks that explicitly tap memory are discussed, while other tasks more often used to characterize other aspects of cognition are described elsewhere.

Adapted from the rodent and primate literatures, the novel object recognition tasks can be used to quantify memorial function in zebrafish. Typically this test is arranged such that memory is tested in a single trial, with no prerequisite training phase. First, a zebrafish is allowed to explore one or two (identical) objects, such as small plastic balls or boxes. After this exploration condition, the zebrafish is presented with the previously encountered object and a novel object. Time spent exploring the novel object, over the familiar one, is characterized as an index of memory (Antunes & Biala, 2012). Often probe trials are introduced following varying delays (usually on the order of hours to days) (Lucon-Xiccato & Dadda, 2014). This arrangement has been used in zebrafish to characterize drug-induced memory impairment as well as models of pathological conditions like Alzheimer's disease. Recently, Braida et al. (2014a) have reported a modified version of novel object recognition in which virtual objects are displayed on monitors located along a tank wall, perhaps offering a higher throughput option for the implementation of this procedure among aquatic species.

The latent learning paradigm has also been used to characterize memory function in zebrafish, and is based largely on the existing rodent literature. Latent learning describes the situation in which an organism is allowed to passively explore a test environment (e.g. a maze), without any programmed consequences. Later, on a probe trial, this organism

navigates the environment more efficiently than a one without prior history in the environment (Tolman & Honzik, 1930). Gomez-Laplaza and Gerlai (2010) demonstrated latent learning when zebrafish, who had previously experienced open or blocked maze arms during exploration, exhibited appropriate side bias during a consequated trial.

Spatial and Visual Discrimination

Spatial (e.g. T-maze, hole-board maze, 3-chamber test or vertical plus maze) and visual (e.g. color/pattern) discrimination procedures have been used to characterize operant learning in zebrafish, that is, sensitivity to the processes of reinforcement and punishment. T- and Y-mazes are simple methods for arranging an operant contingency, and require the acquisition of a spatial discrimination. As a brief example, if the animal makes a “correct” response (e.g. swimming down a central alley, turning left and entering a small compartment), the delivery of food or the image of conspecifics (or some other appetitive stimulus) is initiated. Then, accuracy and latency to engage in the correct response can be measured, and conclusions about learning drawn. Such T- and Y-maze arrangements likely tap similar spatial discrimination as a hole-board or plus maze.

The hole-board arrangement consists of an open arena tank with a centrally located board or box containing several holes, one of which is baited. The location of the baited hole can change across trials, requiring repeated acquisition of the baited arm or remain the same across trials, which more explicitly taps a memory component (for review see van der Staay et al., 2012). Similarly, the vertical column plus maze requires the placement of a single zebrafish into a tank with four compartments stacked 2×2 in the water column – creating bottom left and right and top left and right compartments (Pittman & Lott, 2014). As with other arrangements, one compartment is baited with an appetitive stimulus, and acquisition of that spatial discrimination is measured.

Arthur and Levin (2001) developed a three-chambered spatial discrimination test (Figure 2) in which zebrafish must acquire a spatial discrimination, a left or right turn out of a starting compartment, within a single session. An incorrect choice results in the application of an aversive stimulus (restricted swim space) while a correct choice results in an increased tank space (see Arthur & Levin, 2001; Eddins et al., 2009; Levin et al., 2011; Powers et al., 2011). Choice accuracy and latency to respond are used to characterize acute or long-term drug effects.

Similarly, visual discrimination tasks require the zebrafish to engage in a discrete response (e.g. swim into red or blue compartment) that initiates the delivery of an appetitive stimulus (e.g. food or image of conspecifics) (Colwill et al., 2005). For example, upon approach or entry to a visually distinct zone food is delivered (termed the S+) while approach to an alternate zone does not initiate food delivery (termed the S-). Acquisition of the discrimination, and subsequent memory probes, can be used to characterize both learning and memorial processes, but do so along a qualitatively different dimension than the spatial discrimination arrangement. The three-chambered apparatus described above can also be used to measure qualitative properties of stimuli, e.g. color discrimination (see Arthur & Levin, 2001).

Self-control/Impulsivity

Particularly relevant to questions about substance abuse are paradigms that tap self-control and impulsivity (Stevens et al., 2014; Winstanley et al., 2010). Such tasks are beginning to emerge in the zebrafish literature, following extensive use in mammals (human, non-human primate and rodent). Parker et al. (2012) provides one nice example of this, via the use of a three-choice serial reaction time task in which premature responding was probed using an extended inter-trial interval. Briefly, zebrafish were presented with three stimuli randomly following a fixed-time inter-trial interval. Correct responding (entry into an illuminated response aperture) within a limited hold period following stimulus presentation resulted in the delivery of food. Entry before stimulus presentation resulted in a timeout period. Here, premature responding is characterized as “impulsive” (Parker et al., 2012). Importantly, however, this arrangement and ones like it, have the added benefit of more directly assessing attentional processes. For a review on assessing attention in zebrafish see Echevarria et al. (2011).

Timing

At least nominally, one of the least characterized forms of learning in zebrafish is interval timing, or learning to accurately track the passage of time – interval timing has proven useful for characterizing drug or toxicant effects in other animals including humans (Paule et al., 1999). Cerutti et al. (2013), has, however, reported accurate interval timing in zebrafish using a delayed conditioning arrangement common in the associative learning literature. Here, zebrafish were found to have response latencies proportional to food delays, across several trials. Similarly, other reports have demonstrated that fish can acquire interval timing – although that behavior is often overshadowed by another behavioral endpoint (of primary interest), like shoaling. For example, in an investigation into the social behavior of zebrafish, Jia et al. (2014) found that subjects could accurately predict the location of shoal image following training in which the shoal was presented at different locations after reliable intervals of time. Other studies mentioned in the above sections also capture a component of interval timing. In fact, the capacity to time an interval has proven to be a sensitive marker of cognitive change in humans and rodent models, and is an important “cognitive characteristic” of some diseases (e.g. Parkinson's disease, see Merchant et al., 2008).

III. Cognitive effects of psychoactive drugs in zebrafish

In the following sections we discuss the various uses of zebrafish in studies of behavioral pharmacology (see Table 1). These include using zebrafish to capture some of the apical effects that psychoactive drugs have on behavior and studies that use drugs to probe questions of behavior. Many other investigations, including those which focus on the exploration of molecular and cellular level pathways mediating drug effects and those which target aspects of behavioral effects outside of learning and memory per se (e.g. fear and anxiety, social or aggressive behavior, and sensorimotor ability), are numerous but fall outside the scope of the present review.

Stimulants

The cognitive effects of several stimulant drugs have been evaluated using the zebrafish model, most notably this list includes drugs of abuse (e.g. nicotine), although therapeutic medications also appear in this literature. Methylphenidate, a common stimulant medication, is used clinically for the treatment of attention deficit disorder and attention deficit hyperactivity disorder in children and adolescents with increasing use in adults. In zebrafish, developmental methylphenidate exposure (days 1-5 postfertilization) has been shown to cause reduced accuracy on the three-chamber spatial discrimination task when the fish were tested in young adulthood (Levin et al., 2011). The authors suggest that the developmental nature of this exposure may have altered behavioral plasticity in zebrafish, manifesting as impairments in adapting to environmental contingencies. Stimulant exposure *after* critical periods of development (as described below) is not generally associated with cognitive impairment of this kind – and later life methylphenidate exposure per se has not yet been evaluated. Nicotine, however, is the stimulant drug most widely studied in zebrafish.

Nicotine, a stimulant found in cigarette smoke, is widely classified as a cognitive enhancer and improves indices of learning in zebrafish across a range of tasks, which corresponds to effects seen in other species, including humans. Specifically, nicotine improves performance on a T-maze (Braida et al., 2014b) and increases accuracy during the three-chamber spatial discrimination procedure (Eddins et al., 2009; Levin & Chen, 2004; Levin et al., 2006).

Braida et al. (2014b) demonstrated that cytisine and cytisine-derived partial agonists offered similar improvements as nicotine on T-maze performance, and nAChR antagonists were shown to block the enhancement by nicotine. Similarly, Eddins et al. (2009) showed that nicotine-induced learning enhancement in zebrafish followed increased brain levels of dihydroxyphenylacetic acid (DOPAC) (see Figure 3) and Levin et al. (2006) described the time-course of nicotine's effects on the acquisition of a spatial discrimination (see Figure 4a-b). Nicotine has also been shown to increase a discrimination score on the object recognition test, indicating enhanced visual attention (Braida et al., 2014a) (while scopolamine and mecamylamine decreased the discrimination score). These data are largely consistent with reports from the rodent and human literatures regarding the cognitive enhancement offered by nicotine and stimulant drugs more generally (for relevant reviews see Warburton, 1992; Levin, 2013; Levin & Rezvani, 2002).

As expected from the increased accuracy on the aforementioned operant arrangements, nicotine has also been shown to enhance a conditioned place preference response, an index of associative learning and measure of the rewarding properties of the drug (Kedikian et al., 2013). Cocaine (Darland & Dowling 2001; Darland et al., 2012) and amphetamine (Webb et al., 2009; Ninkovic & Bally-Cuif, 2006) also induce place preference learning in zebrafish. While most often the focus of these investigations involves understanding the genetic contributions to cocaine- and amphetamine-induced CPP (Darland & Dowling, 2001; Webb et al., 2009; Ninkovic & Bally-Cuif, 2006), the CPP data generated here are nearly identical to those described in rodent CPP arrangements and consistent with rodent self-administration assays. These consistencies help increase confidence in the use of zebrafish

in behavioral pharmacology and also point to highly conserved behavioral mechanisms of drug action.

Depressants

A relatively robust literature on the teratogenic effects of developmental ethanol exposure as well as models of fetal alcohol syndrome has emerged using zebrafish in the last decade (e.g. Fernandes et al., 2014; Bailey et al., 2015; and for a review see Cole et al., 2012 or Ali et al., 2011). However, the literature describing the acute effects of ethanol exposure during adolescence or adulthood on learning and memory in zebrafish is more modest. As expected based on the rodent literature, acute and chronic exposure to moderate or high doses of ethanol during adulthood induces CPP in zebrafish (Chacon & Luchiari, 2014; Mathur et al., 2011) and exposure to ethanol during early development results in an exaggerated preference in ethanol-induced CPP during adulthood (Parker et al., 2014). These effects have largely been interpreted as corresponding primarily to the rewarding effects of ethanol, as memory impairment can be produced on simple associative learning tasks (like acquiring the association between a cue and food delivery) at comparable doses (Chacon & Luchiari, 2014) and impaired performance on a vertical column plusmaze has been reported with higher ethanol doses (Pittman & Lott, 2014). Moreover, these effects are consistent with those reported in other model species. Like ethanol, the opiate drug morphine induces a robust CPP response, and opioid receptor antagonists (i.e. naloxone) block this effect in zebrafish (Lau et al., 2006).

Experimental drugs

Several drugs, used as probes to understand mechanisms of action either at the level of behavior or at cellular and molecular levels fall outside of the categories of “therapeutic” or “drugs of abuse” and are therefore included here. Within the zebrafish cognition literature this category is so far primarily limited to the drugs scopolamine and MK-801. Scopolamine is a muscarinic acetylcholine receptor antagonist, and although it has been used therapeutically for motion sickness, it is frequently used experimentally to assess cholinergic-mediated behavioral processes, particularly learning and memory. As in other species, scopolamine has amnestic properties in the zebrafish and has been shown to impair memory as measured by performance on passive avoidance paradigms (Kim et al., 2010; Richetti et al., 2011). Scopolamine also alters Y-maze exploration in zebrafish, reducing exploration of the novel arm, (Cognato et al., 2012) similarly to rodents.

The NMDA glutamate receptor antagonist dizocilpine (MK-801) is used almost exclusively as an experimental drug to probe glutametergic-mediated behavioral processes. Associative learning procedures have been used to characterize the effects of MK-801 on learning and memory processes in a wide range of species, in which learning deficits or delays are routinely reported (for a review see Castellano et al., 2001). Comparable learning deficits have been demonstrated in MK-801 treated zebrafish where a visual cue was paired with the image of conspecifics (an appetitive unconditioned stimulus) (Sison & Gerlai, 2011). Similarly, deficits have been demonstrated with food-induced CPP (Swain et al., 2004) and on indices of memory during Y-maze performance (Cognato et al., 2012). Avoidance learning paradigms have been widely employed across many species to measure cognitive

deficits associated with MK-801 administration, and a variety of avoidance arrangements have captured MK-801-induced learning or memory impairment in zebrafish (Blank et al., 2009; Ng et al., 2012; Seibt et al., 2011).

Other Psychoactive Drugs

A few other psychiatric and therapeutic drugs have been evaluated for cognitive effects using zebrafish, and are listed briefly here along with their effect on cognition. The histaminergic precursor L-histidine facilitated learning in a delay conditioning (associative learning) task (Cofiel & Mattiolo, 2009). Fluoxetine, the selective serotonin reuptake inhibitor (SSRI) used as an antidepressant, has been shown to impair zebrafish performance on a modified plus maze without otherwise affecting locomotion or activity levels (Pittman & Lott, 2014). The atypical antipsychotics sulpiride and olanzapine were shown to partially correct amnesia in an inhibitory avoidance test induced by the drug MK-801 (Seibt et al., 2011). Finally, piracetam, a nootropic investigated as treatment for mild cognitive impairment and whose mechanism is not fully understood, was found to improve zebrafish performance in a plus-maze test after chronic administration (Grossman et al., 2011).

Hallucinogens and Other Psychedelics

A number of psychedelic drugs of various classes have been administered to zebrafish, although the investigations into the effects of these drugs on zebrafish cognition are still quite sparse. THC, the primary psychoactive ingredient in marijuana and agonist at the endocannabinoid receptor CB1, significantly impaired the performance of a spatial memory task without affecting a color discrimination test or general locomotor activity levels (Ruhl et al., 2014). Lysergic acid diethylamide (LSD), an agonist at a number of dopaminergic, adrenergic, and serotinergic receptors, appears to improve zebrafish performance in the T-maze, reducing arm entry and freezing behaviors (Grossman et al., 2010). Salvinorin A, the hallucinogenic compound found in the *Salvia* plant, was found to be reinforcing for zebrafish, increasing the time the fish spent in the drug-associated compartment in a conditioned place preference test. Antagonists for kappa-opioid receptors and CB1 individually blocked the reinforcing properties of salvinorin A for zebrafish (Braida et al., 2007).

IV. Limitations of zebrafish in the pharmacology of cognition

Route of Administration

While zebrafish have undoubtedly become useful models of the cognitive effects of many drugs (as detailed above), they are not without their limitations. The ease of pharmaceutical administration to zebrafish via immersion is countered by the criticisms sometimes levied against the route, which span water solubility constraints and pharmacokinetic issues to concerns of translational validity. While oral administration (e.g. Kulkarni et al., 2014) and injection (e.g. Parker et al., 2012) have both been successfully used in zebrafish, the vast majority of neuropharmacological studies thus far have utilized aqueous immersion. Regarding translational validity, immersion may not directly correlate to common routes of administration for neuroactive compounds in humans, although the method can be bolstered by measuring drug levels directly in target tissues such as the zebrafish brain, allaying some

pharmacokinetic concerns. Moreover, some drugs may have very different kinetic profiles in zebrafish compared to humans or rodents, or absorption may be impacted by the presence or absence of a chorion and egg washing protocols if exposure is developmental (e.g. ethanol exposure, see Fernandes & Gerlai, 2009; Zhang et al., 2013; Zhang et al., 2014). These points require careful consideration as does the selection of dose ranges. Moreover, it is important to recognize that, while zebrafish are seemingly reliable models for the behavioral effects of neuroactive drugs, due to differences in drug administration and metabolism in zebrafish, full pharmacokinetic testing and validation of prospective compounds may yet still require additional, complimentary model systems.

Biology

As described above, zebrafish are relatively good models of mammalian neurobiology (Del Bene & Wyart, 2012; Feierstein et al., 2014). There are a few differences between zebrafish and mammalian brains, though, which are important to take into consideration. Although zebrafish have no cortex or hippocampus (Panula et al., 2010), analogous structures exist in the zebrafish brain that appear to be sufficiently biochemically and functionally similar to the mammalian brain to permit the use of neuropharmacological manipulations, which are expected to produce behaviors that mirror those seen in mammals. Additionally, zebrafish lack midbrain dopaminergic populations such as the substantia nigra and ventral tegmental area (Panula et al., 2010), although again zebrafish are perfectly capable of displaying behaviors classically attributed to these areas and these behaviors are sensitive to dopamine-targeted pharmacological stimulation (Panula et al., 2006). Other neurotransmitter systems, such as those for histamine and nitric oxide, are not yet fully characterized in zebrafish, and thus the validity of using zebrafish as models for these systems is still unclear (Rico et al., 2011).

The assessment of learning

Finally, an important but under-discussed limitation to measuring learning or memory in zebrafish is the role of motor function, which is intrinsically intertwined, to varying degrees, in all of the aforementioned tasks. Not surprisingly, functional impairments or enhancements in motor ability are a significant hurdle when attempting to draw conclusions about learning or memory from tasks that require motoric responses from the organism. In the rodent model this issue has been dealt with via a number of interventions. For instance, the use of interval rather than ratio schedules of reinforcement can diminish the impact of motor function (e.g. Nevin et al., 2001). So too can employing specific experimental controls for motor function (e.g. Bailey et al., 2010) and by plotting both rate and accuracy-type measures, so that motor changes could be detected. Similar interventions should be emphasized within the context of zebrafish cognition as well, so that motoric effects do not masquerade as changes in cognition.

V. Future Directions

Considering that a zebrafish model of learning and memory is in its relative infancy compared to its rodent counterpart, a number of elegant and sophisticated tasks have been developed or adapted for this small aquatic species. Unsurprisingly, however, a number of

gaps exist in this literature, which fall into two broad categories: un-investigated drug classes and sub-optimal behavioral paradigms. Of course, the zebrafish has not been utilized for the investigation of any number of particular drugs (or drug/dose combinations), but perhaps more surprisingly, entire drug classes still remained largely foreign to the zebrafish model of behavioral pharmacology. The investigations of many antipsychotic medications (e.g. haloperidol), other therapeutics (e.g. opiate drugs, anesthetics) and emerging drugs of abuse (e.g. “bath salts”) have not yet widely utilized the zebrafish model. Moreover, there is a relative dearth in paradigms that tap operant mechanisms of learning in zebrafish. As mentioned above, operant conditioning is thought to characterize a fundamental process via which organisms learn about and adapt to their environment. Procedures that quantify operant learning have proven indispensible in the rodent literature and would likely improve the characterization of drug effects in zebrafish similarly.

VI. Conclusions

Behavior is the primary means by which an organism interacts with its environment (Weiss & Cory-Slechta, 1994). To survive, organisms must be sensitive to events occurring in their environments and respond appropriately. To this end, it is important to emphasize drug effects at the level of behavior. In fact, the final criterion in any study of CNS insult or modification (e.g. drug use) are characteristics associated with the whole animal; characteristics like the development and longevity of the animal are important, but so too is the functional integrity and quality of performance that animal is capable of engaging in, as is the emotional reactivity of the animal (Weiss, 1978). And because the whole animal is our interest, it is necessary that behavior is our subject matter as only it reflects the summed and integrated capacity of an organism to handle the environment within which it exists (Weiss, 1978). The tasks described here, and the data generated from them, build upon many decades of work from Pavlovian conditioning and operant psychology where task development was driven by the desire to understand and predict behavior. Building on these literatures, contemporary scientists are tasked with adapting and developing procedures for the use in new animal models that will produce informative and reliable measures of behavior change so that the field of behavioral pharmacology may continue to make advancements in our understanding of drug effects, and endogenous systems.

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Highlights

- Zebrafish can be used to assess neurobehavioral processes of learning and memory
- Zebrafish provide a complementary model for screening the cognitive effects of drugs
- Zebrafish can be used to help characterize neural systems underlying cognitive function

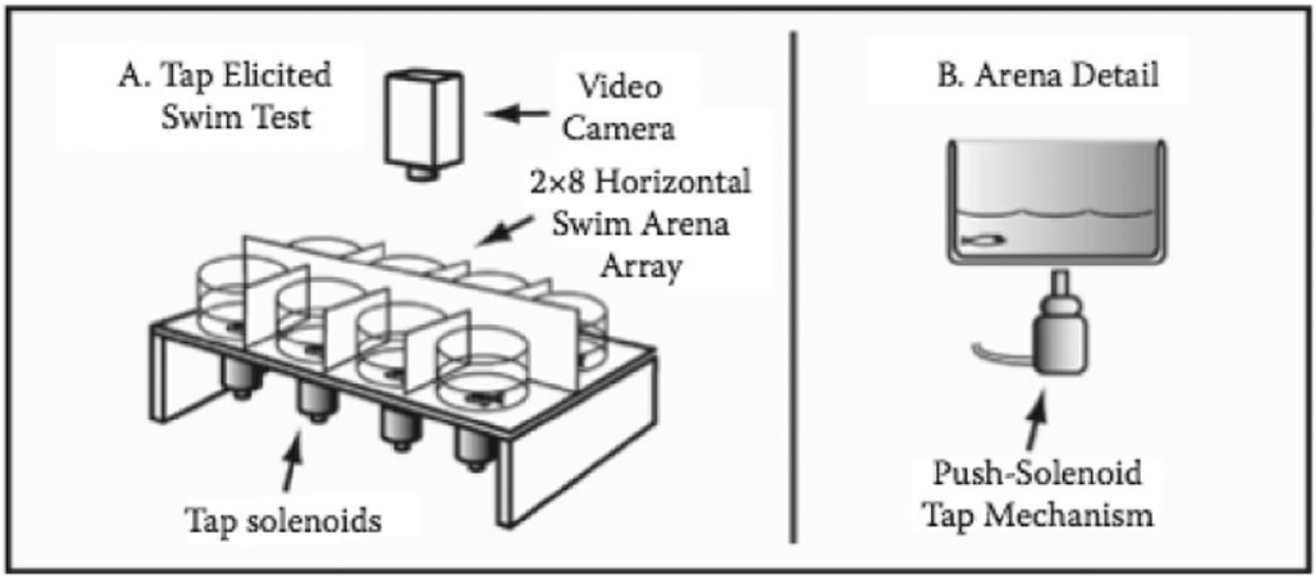


Figure 1.

Diagram of apparatus used to measure startle response. Fish were tested in 8 cylindrical chambers separated by opaque dividers. A pushsolenoid delivered a tap as a startle stimulus at 1 min intervals (10 total trials). A camera mounted overhead acquired video, which was imported into EthoVision tracking software to measure swimming distance.

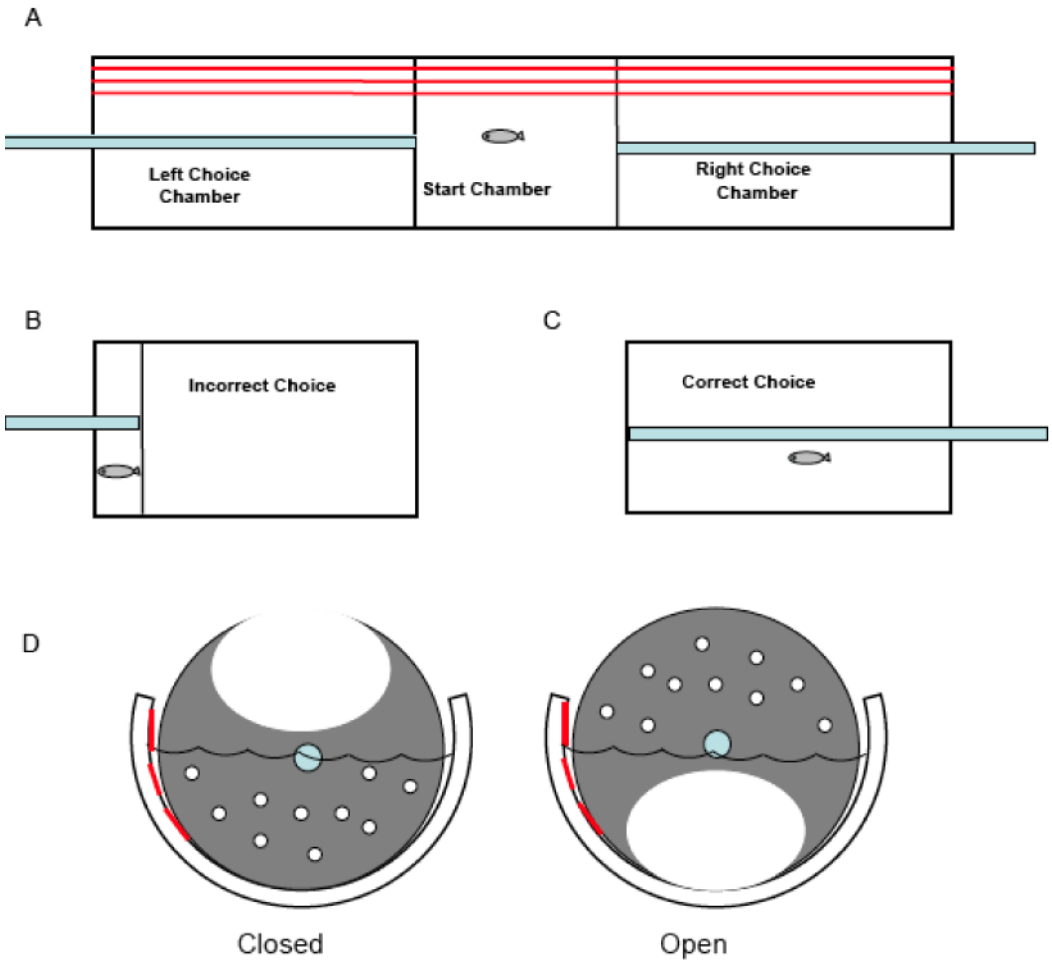


Figure 2. Diagram of the zebrafish 3-chamber task for assessing learning and memory. (Eddins et al., 2009)

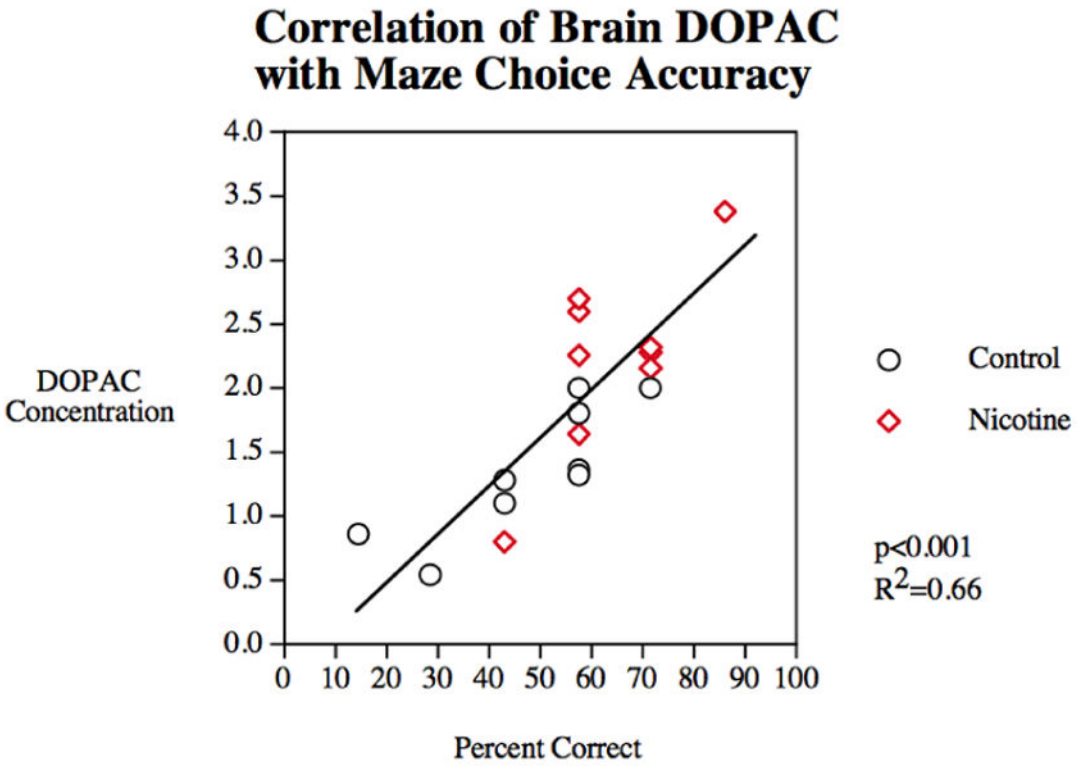
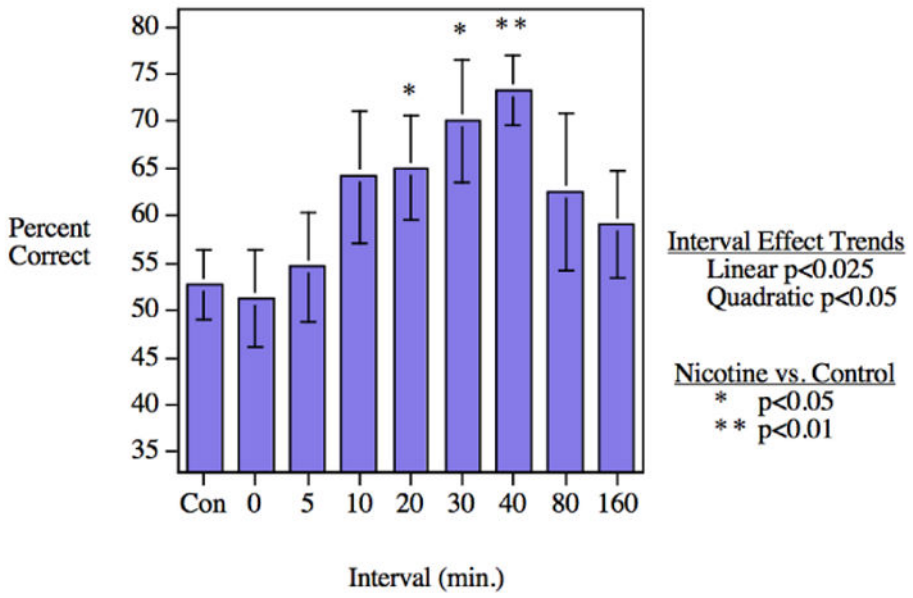


Figure 3. Correlation of percent correct in the 3-chamber task with brain DOPAC concentration. (Eddins et al., 2009)

A.



B.

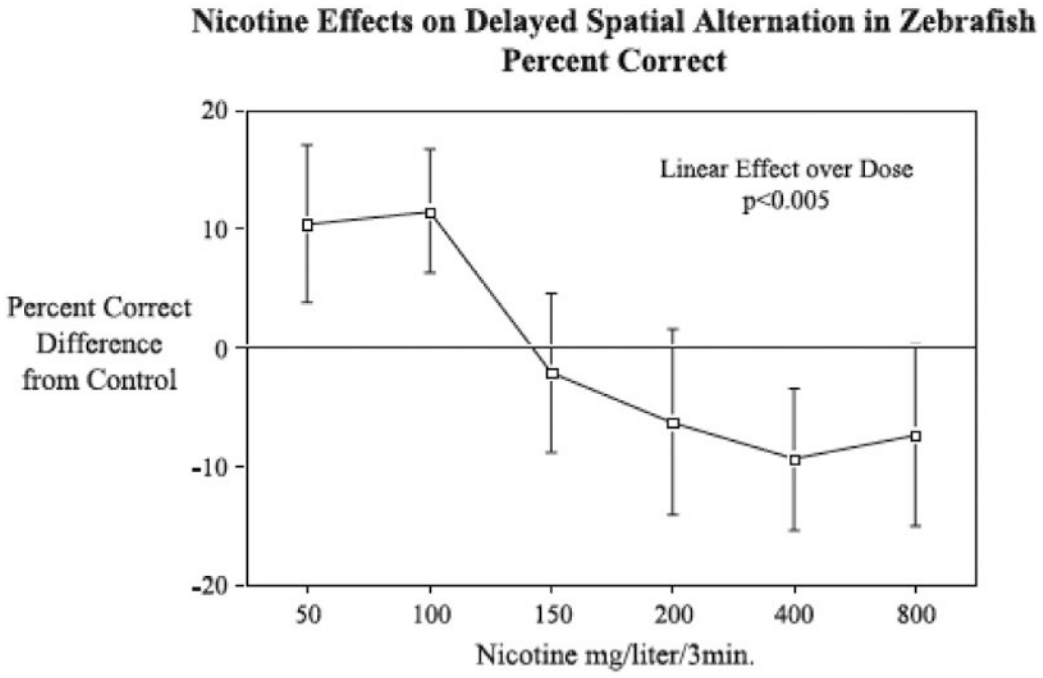


Figure 4. Nicotine effect improving A) spatial discrimination and B) spatial alternation in the 3-chamber task (mean±SEM). (Levin and Chen 2004, Levin et al., 2006)

Table 1
Cognitive Effects of Psychoactive Drugs in Zebrafish

Authors	Compound	Effect	Assay
Braida et al., 2014b	Nicotine	Improved performance	T-maze
Braida et al., 2014b	Nicotine	Increased discrimination score	Object recognition test
Eddins et al., 2009; Levin & Chen, 2004; Levin et al., 2006	Nicotine	Increased accuracy	Spatial discrimination test
Kedikian et al., 2013	Nicotine	Enhanced place preference	Conditioned place preference (for drug)
Braida et al., 2014b	Cytisine (and cytisine-derived partial agonists)	Improved performance	T-maze
Darland & Dowling 2001; Darland et al., 2012	Cocaine	Enhanced place preference	Conditioned place preference (for drug)
Webb et al., 2009; Ninkovic & Bally-Cuif, 2006	Amphetamine	Enhanced place preference	Conditioned place preference (for drug)
Chacon & Luchiari, 2014; Mathur et al., 2011	Ethanol	Enhanced place preference	Conditioned place preference (for drug)
Chacon & Luchiari, 2014	Ethanol	Memory impairment	Associative learning
Pittman & Lott, 2014	Ethanol	Impaired performance	Vertical column plus-maze
Lau et al., 2006	Morphine	Enhanced place preference	Conditioned Place Preference (for drug)
Kim et al., 2010; Richetti et al., 2011	Scopolamine	Memory impairment	Passive avoidance
Cognato et al., 2012	Scopolamine	Reduced exploration (novel arm)	Y-maze
Sison & Gerlai, 2011	MK-801	Learning impairment	Associative learning
Swain et al., 2004	MK-801	Decreased place preference	Conditioned Place Preference (for food)
Cognato et al., 2012	MK-801	Memory impairment	Y-maze
Blank et al., 2009; Ng et al., 2012; Seibt et al., 2011	MK-801	Learning/memory impairment	Avoidance learning (various)
Cofiel & Mattiolo, 2009	L-histidine	Facilitated learning	Associative learning
Pittman & Lott, 2014	Fluoxetine	Impaired performance	Plus maze
Grossman et al., 2011	Piracetam	Improved performance	Plus maze
Ruhl et al., 2014	THC	Memory impairment	Spatial memory test
Grossman et al., 2010	LSD	Improved performance	T-maze
Braida et al., 2007	Salvinorin A	Enhanced place preference	Conditioned place preference (for drug)