

# Zebrafish: An in vivo model for the study of neurological diseases

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**Abstract:** As the population ages, there is a growing need for effective therapies for the treatment of neurological diseases. A limited number of therapeutics are currently available to improve cognitive function and research is limited by the need for in vivo models. Zebrafish have recently become a focus of neurobehavioral studies since larvae display neuropathological and behavioral phenotypes that are quantifiable and relate to those seen in man. Due to the small size of Zebrafish larvae, assays can be undertaken in 96 well plates and as the larvae can live in as little as 200  $\mu$ l of fluid, only a few milligrams of compound are needed for screening. Thus in vivo analysis of the effects of compounds can be undertaken at much earlier stages in the drug discovery process. This review will look at the utility of the zebrafish in the study of neurological diseases and its role in improving the throughput of candidate compounds in in vivo screens.

**Keywords:** Zebrafish, aging, neurobehavior, neurological disease

## Introduction

Cognitive impairment manifests itself in a number of neurological diseases such as schizophrenia, Huntington's, Parkinson's, and Alzheimer's diseases. As the general population ages, there will be an increased incidence of these disease and disorders and thus a growing need for effective therapies for the treatment of the associated cognitive deficits. A limited number of therapeutics are currently available to improve cognitive function and research is limited by the need for predictive in vivo models.

## Introduction to zebrafish

Zebrafish have recently become a focus of neurobehavioral studies since larvae display learning, sleep, drug addiction, and other neurobehavioral phenotypes that are quantifiable and relate to those seen in man (Zhdanova et al 2001; Cahill 2002; Guo 2004; Orger et al 2004; Ninkovic et al 2006). Furthermore, the organization of the zebrafish genome and the genetic pathways controlling signal transduction and development are highly conserved between zebrafish and man (Postlethwait et al 2000). At seven days post fertilization (d.p.f.) the larvae are approximately 4 mm long. Due to this small size of the larvae, assays can be undertaken in 96 well plates and as the larvae can live in as little as 200  $\mu$ l of fluid; only a few milligrams of compound are needed for screening. Thus in vivo analysis of the effects of compounds can be undertaken at much earlier stages in the drug discovery process than has previously been possible, which is facilitated by the fact that zebrafish are dimethyl sulfoxide (DMSO) tolerant and readily absorb compounds from the water. The relative ease of maintaining large stocks of fish and its high fecundity can provide the investigator with large numbers of larvae to analyze. The above properties have established the zebrafish as an excellent model system that is relevant to studies of human diseases (Grunwald and Eisen 2002). Conventional drug discovery has recently employed systematic, target-based high throughput screening in purified proteins or cells as primary screens with in vivo models as tertiary screens in the cascade after more mechanistic cell assays.

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While the *in vitro* screens have been successful at identifying small molecules affecting known mechanisms, there is still the need to identify modulators of complex *in vivo* phenotypes in the whole organism for less well understood pathways or those that only occur in a physiological/pathophysiological context. The advantages of using larval zebrafish described above allow higher throughput *in vivo* screening for phenotypic endpoints, and the utility of zebrafish in small molecule screening has been the subject of several reviews (MacRae and Peterson 2003; Zon and Peterson 2005; Murphey and Zon 2006; Berger and Currie 2007). However, there are disadvantages to this approach. Namely, that uptake of compound into the zebrafish can be variable and should be measured for accurate interpretation of results (Berghmans et al 2008) and particularly to avoid false negatives, and the larval stage of the zebrafish may not be appropriate in all disease areas.

In a comparison of the zebrafish brain structure with man, there are some differences between teleosts and mammals. Notably, fish have smaller cerebral hemispheres and there are differences in the layout of the forebrain (extensively reviewed by Wullimann and Mueller 2004) and the structure and function of the optic tectum (Luque et al 2005). However, the overall organisation of the zebrafish brain is similar to other vertebrates, having similarly defined areas such the hypothalamus and olfactory bulb, encompassing structures of the lateral pallidum, which appear to be homologous to the mammalian hippocampus (Tropepe and Sive 2003). In addition, the main neurotransmitter systems such as the cholinergic, dopaminergic, and noradrenergic pathways are present and have been mapped throughout the brain (Rink and Wullimann 2004; Wullimann and Mueller 2004). Zebrafish have a developmentally regulated blood-brain barrier. Functional analysis using fluorescent dyes and anatomical analysis by transmission electron microscopy provides evidence that the zebrafish blood brain barrier is functional at 10 d.p.f. (Goldsmith and Fleming 2007). Additionally, it was also shown that zebrafish paralogues of P-glycoprotein (Pgp) are first detected in the vasculature endothelium of the central nervous system (CNS) at 8 d.p.f., which coincides with the efflux of the Pgp substrate, rhodamine 123 from the zebrafish brain. These data suggest that the zebrafish is a relevant model for the study of neurological diseases.

## Zebrafish neurodegenerative disease models

### Huntington's disease

Huntington's disease (HD) is a monogenic polyglutamine (polyQ) neurodegenerative disorder which results in cognitive deficits in attentional and executive functions along with

defects in visual working memory (Montoya et al 2006). Neurodegeneration occurs primarily in the striatal medium-sized spiny neurons which project to the substantia nigra and globus pallidus. HD has a prevalence of approximately 5 in 100,000 worldwide, the median age of onset being 39 (Cowan and Raymond 2006). In zebrafish, the huntingtin (Htt) gene has been cloned and sequenced with a 3121 predicted amino acid protein, which has 70% identity with the human peptide sequence (Karlovič et al 1998). Knockdown of Htt using morpholino technology disrupted a number of features of zebrafish development resulting in small head and eyes, delayed or paler pigmentation and colorless hypochromic blood (Lumsden et al 2007). In a separate study, a 'Huntington's like' zebrafish was created by inserting mRNA of the N-terminal fragment of Htt with different length polyQ repeats linked to a GFP-fusion protein (Schiffer et al 2007). The increasing polyQ length was associated with an increase in abnormalities and apoptosis in the embryos as early as 24 hours p.f. The embryos containing the Q102-GFP developed inclusions in the cytoplasm, which increased in size by incorporation of the soluble Q102 peptide leading to insoluble deposits. These findings confirmed a previous study where expression of poly Q56 or greater exhibited toxicity and abnormalities in the zebrafish embryos with inclusion bodies formed in more than 70% of embryos (Miller et al 2005). These studies also investigated the effect of aggregation inhibitors which suggested that the prevention of aggregation did not reduce the toxic effect on the fish, implying that the formation of smaller intermediate aggregates were the main cause of toxicity. Thus, these models could be used to screen for novel compounds for the treatment of HD by evaluating either the prevention of aggregate formation, enhanced clearance of aggregates or the reduction in embryo death.

### Alzheimer's disease

Alzheimer's disease (AD) is the most common cause of dementia, with nearly 50% of dementia cases worldwide being attributed to AD. The prevalence increases from 1.53% of the population between 65–69 years to as high as 30% between 80–85 years (Vandenberghe and Tournoy 2005; Mount and Downton 2006). AD is characterized histopathologically by amyloid-beta (A $\beta$ ) containing plaques and intracellular neurofibrillary tangles consisting of abnormally phosphorylated tau protein in the brain (Selkoe 2000; Mudher and Lovestone 2002). Cognitive impairments manifest themselves as progressive episodic memory loss and effects on executive functions, which are usually accompanied by mini-mental state examination scores below 24 (Vandenberghe and Tournoy 2005). Zebrafish possess two homologues of amyloid precursor

protein (APP) with good (about 70%) homology to human APP (Musa et al 2001). Studies have demonstrated the presence of the functional  $\gamma$ -secretase machinery to produce A $\beta$  in zebrafish (for a full review see Newman et al 2007). Other studies have investigated the tau protein in zebrafish (Tomasiewicz et al 2002). Microinjection of four repeat human tau GFP constructs into 1–2 cell stage embryos showed disruption to the cytoskeletal structure and tau trafficking by 48 hours post injection. This eventually led to hyperphosphorylated fibrillar tau staining similar to that seen with neurofibrillary tangles in pathology of AD patients. These models offer the ability to screen for novel therapeutics that decrease A $\beta$  load and decrease the hyperphosphorylation seen in tauopathies.

### Parkinson's disease

Although predominantly a movement disorder, there are a number of cognitive impairments associated with Parkinson's disease (PD) (Galvin 2006). These include executive dysfunction and impaired memory retrieval and the prevalence increases from 2.7% per year at 55–64 years to 13.7% at 70–79 years. Parkinson's disease is characterized neuropathologically by degeneration of dopaminergic neurons and the appearance of intracytoplasmic inclusions called Lewy bodies. Genetically, six genes linked to Parkinsonism have been identified: Parkin, DJ-1, PINK1,  $\alpha$ -Synuclein, UCHL-1, and LRRK2 (Abeliovich and Beal 2006). Of these genes two have been studied in zebrafish with preliminary work beginning on the others (Paquet et al 2006; Shankaran et al 2006).

Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) was originally identified in siblings with a strong family history of PD (Leroy et al 1998). In zebrafish, UCH-L1 mRNA was expressed in neuronal cells at 1 d.p.f. (Son et al 2003). UCH-L1 was detected in the diencephalon and ventral region of the mid and hindbrain, which are associated with motoneuron development (although which mammalian motoneurons these were correlated with was not investigated). Interestingly, in the ventral diencephalon, an area functionally homologous to the substantia nigra in humans, the UCH-L1 was co-expressed with tyrosine hydroxylase substantiating the association of UCH-L1 with dopaminergic neurons.

Patients with autosomal recessively inherited DJ-1 mutations typically present with early onset PD. In embryonic and adult zebrafish DJ-1 is expressed throughout the body with higher abundance in the brain, eyes, heart and muscle of the adult (Bretaud et al 2007). The zebrafish DJ-1 protein has high homology with human (83%) and mouse DJ-1 (80%). Knockdown of DJ-1 in the zebrafish did not affect the number of dopaminergic neurons in a similar manner to the

mouse DJ-1 null mutant (Chen et al 2005). In keeping with the suggested role of DJ-1, the knockdown zebrafish embryos were more susceptible to oxidative stress and had significantly elevated SOD1 levels. Furthermore, simultaneous knockdown of DJ-1 and p53 caused dopaminergic neuronal loss demonstrating a strong interaction between these genes.

The main pharmacological approach to studying PD in animal models has been using 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) which reproduces some of the effects of idiopathic Parkinson's disease (Smeyne and Jackson-Lewis 2005). Zebrafish embryos treated with MPTP demonstrated a loss of TH and DAT-expressing neurons which could be rescued using the monoamine oxidase-B inhibitor deprenyl (Bretaud et al 2004; Lam et al 2005; McKinley et al 2005). A reduction was also seen in the locomotor activity mimicking motor effects seen in PD patients; however this did not always occur simultaneously with the reduction in dopaminergic cells (Salzmann et al 2006) and in the adult a reduction is seen in the locomotor activity without an effect being seen in dopaminergic cells (Anichtchik et al 2004). The utility of these various models will aid screening for novel compounds for both the hereditary and the idiopathic forms of PD.

### Schizophrenia

Schizophrenia affects approximately 1% of the world's population and is characterized by neuronal dysfunction resulting in deficits in a number of different cognitive areas such as visual and verbal memory and learning and attention (Nuechterlein et al 2004; Tamminga 2006). Patients with schizophrenia and other conditions including Huntington's disease have been shown to exhibit impaired prepulse inhibition (PPI) (Swerdlow et al 1995; Braff et al 2001). PPI is a neurological phenomenon in which a weak prestimulus or prepulse suppresses the response to a subsequent startling stimulus and is highly conserved among vertebrates. A recent study of sensorimotor gating in zebrafish described an assay for PPI in 6 d.p.f. larvae (Burgess and Granato 2007). In this study, the effective interstimulus interval for inhibition along with the dopaminergic and glutamergic modulation of PPI was found to be comparable to that in mammals. Again, using this paradigm will allow the screening of novel therapeutics for schizophrenia at an earlier stage of the drug discovery process.

### Assays for assessing learning and memory in zebrafish

Mild cognitive impairment, a risk factor for AD, consists of a number of etiologies and is characterized by a slight impairment (usually memory) in cognitive function of everyday activities

(Levey et al 2006). Its prevalence in epidemiological studies ranges from 3%–19% in adults over 65 years old, with half of adults diagnosed with MCI progressing to dementia (Gauthier et al 2006). There have been a number of models developed to facilitate the study of learning and memory in zebrafish. Nonassociative learning has been studied in larval zebrafish by monitoring the reduction in a startle response to a series of acoustic stimuli (Best et al 2007). This habituation to the stimuli was modulated by current therapies for improving cognitive ability in Alzheimer's disease patients, namely donepezil and memantine, as well as by the phosphodiesterase (PDE) 4 inhibitor rolipram, a compound shown to increase learning and memory in rodent studies. Associative learning has been demonstrated by visual discrimination in a T maze with food reward linked to certain colours (Colwill et al 2005). The adult zebrafish learned to associate one of a pair of colours with food and reversal and extinction were also shown in this system. A T maze has also been used to determine the roles of glutamergic transmission (Swain et al 2004) and histamine in learning and memory in adult zebrafish (Peitsaro et al 2003). Furthermore, adult zebrafish have been shown to display quick and reliable learning by swimming to certain locations within a tank to avoid adverse stimulus, such as a moving net (Arthur and Levin 2001) or electrical shocks (Xu et al 2007) and show cognitive flexibility in that they show reversal when the contingencies are changed over. Active avoidance to avoid mild electrical shocks was used in a similar experimental paradigm to demonstrate the possible involvement of cell adhesion molecule L1.1 in memory consolidation (Pradel et al 2000). A three compartment maze with a central chamber and a choice of moving to the left or right chambers has been used to demonstrate spatial and non spatial visual discrimination learning (Arthur and Levin 2001) and to demonstrate the improvement in learning in response to nicotine treatment (Levin et al 2006a) in adult zebrafish. A spatial alternation paradigm has been used to show that adult zebrafish learn to alternate between ends of the test tank for food reward, rapidly achieving high correct response levels (Williams et al 2002). This behavior is recalled if the zebrafish are tested again after 10 days. This type of learning can be impaired in zebrafish treated with ethanol (10 mM) or lead (10  $\mu$ M) for the first 24 hours post fertilization of development as they demonstrated significant learning and memory deficits when treated in the three compartment maze assay (Carvan et al 2004). The progress in the area of learning and memory experimental paradigms in zebrafish suggest the possible utility of this model organism in behavioral pharmacology studies.

## Pharmacology mediating cognition

One of the key challenges with CNS drug discovery is the need to isolate specific areas of the brain mediating a particular disorder or disease. Therapies have become available for cognitive impairment, although these have been dominated by two classes of drug, acetylcholinesterase inhibitors and N-methyl-D-aspartate (NMDA) antagonists exemplified by donepezil and memantine, respectively. Since a number of publications and reviews have covered these therapies, the pathways they mediate and their effects in both preclinical models (Levin and Simon 1998; Levin et al 2006b; Cosman et al 2007) and clinical trials (Burns et al 1999; Birks 2006; Geerts and Grossberg 2006; Ringman and Cummings 2006; Robinson and Keating 2006), as well as the effects of these pathways on zebrafish behavior (Levin and Chen 2004; Nam et al 2004; Levin et al 2006a; McDearmid and Drapeau 2006), the latter part of this review will concentrate on four other interesting targets; these being phosphodiesterases, histamine H3 receptors, 5HT6 receptors, and AMPAkinases, which are currently being investigated preclinically and the utility of zebrafish to aid in the characterization of these targets.

## Phosphodiesterase inhibitors

The role of phosphodiesterases in cognition have been well documented (for reviews see Rose et al 2005; Hebb and Robertson 2007). Currently, in mammals there are 11 distinct families encoding 21 genes. Of these, PDE 1, 2, 4, and 10 are primarily located in the mammalian brain. PDE1B and PDE10 are located in the striatum and expression levels of both were found to decline in the HD mouse models R6/1 and R6/2, suggesting a role for these PDEs in the deficits associated with HD (Hebb et al 2004). The PDE 4 A, B, and C isoforms are distributed throughout regions of the brain, with PDE4A and 4D observed in the hippocampus along with 4D, and all three isoforms were seen to different extents in the cortical regions (Cherry and Davis 1999). Furthermore, although the levels of PDE4A were low in the substantia nigra there was no change in levels between the control and HD mice (Hebb et al 2004). Since the levels of PDE4s may not change in Huntington's disease it may provide a viable target for the associated cognitive deficits.

Treatment of rodents with rolipram, a nonselective PDE4 inhibitor, demonstrated either enhancement of memory and long term potentiation or a reversal of pharmacologically induced memory deficit (Imanishi et al 1997; Barad et al 1998; Zhang et al 2000; Zhang and O'Donnell 2000). Other studies with the PDE5 inhibitor sildenafil, also demonstrated cognitive improvement in the object recognition

and attenuated the scopolamine induced learning deficit in mice suggesting this family as a possible therapeutic target (Prickaerts et al 2002; Devan et al 2004). Furthermore, more recent studies have investigated PDE10A as a therapeutic target for treating schizophrenia and HD (Hebb et al 2004; Siuciak et al 2006). However, despite the substantial data to support the role of PDEs in learning and memory, so far only the only reported studies for the clinical use of phosphodiesterase inhibitors, has been in the treatment of depression using rolipram (reviewed by Zhu et al 2001; Renau 2004).

In zebrafish, 2',3'-cyclic-nucleotide 3'-phosphodiesterase was first reported as being induced in an optic nerve regeneration study (Ballesterio et al 1999). Further searches of the National Center for Biotechnology Information (NCBI) protein sequence finder revealed zebrafish possess proteins with similarity to the phosphodiesterases 1, 3–7, 9–11. Two enzymes of primary interest are the PDE4 (<http://www.ncbi.nlm.nih.gov/BLAST>, accession number: CAK10806), with an identity to the human PDE4C1 of 63%, rat 4D 67% and mouse 4C 61%, and PDE10A (<http://www.ncbi.nlm.nih.gov/BLAST>, accession number: NP 957396), which has an identity with the human, rat, and mouse PDE10A of 83% (for summary see Table 1). Specific orthologues of the other splice forms may also exist but have yet to be reported. As yet, little has been reported in zebrafish as to the effects of PDE inhibitors on learning and memory; though this lab has reported that rolipram enhances the acoustic startle response in 7 d.p.f. larval zebrafish and delays habituation to consecutive tones (Best et al 2007). These effects in zebrafish are similar to those seen in previous rat studies (Kehne et al 1991), suggesting the presence of functional phosphodiesterase 4 enzymes or at the very least high-affinity rolipram binding sites. Clearly more investigations are needed to elucidate the distribution of PDEs in fish and their role in fish behavior and cognition.

**Table 1** Homology of the rat and zebrafish with the human receptors which have been associated with learning and memory

Human receptor	Rat	Zebrafish
PDE 4	71%	63%
PDE 10	95%	83%
H3	91%	50%
5HT6	89%	54%
GluR1	97%	71%
GluR2	98%	87%
GluR3	98%	88%
GluR4	99%	88%

## Histamine 3 receptor antagonists

The histamine 3 (H3) receptor is a G protein coupled receptor identified in the 1980s and pursued as a drug target for a number of indications (for reviews see Witkin and Nelson 2004; Esbenshade et al 2006; Bonaventure et al 2007). H3 receptors are centrally located with mRNA distributed in regions connected to memory and learning, such as the hippocampus and cortex (Lovenberg et al 1999). In recombinant receptor systems H3 receptors have been shown to have constitutive activity (reviewed by Arrang et al 2007) and research has centred on investigating inverse agonists/antagonists for cognitive disorders. Thioperamide has been used to examine the effect of H3 receptor antagonists on learning and memory and clobenpropit has been shown to reverse scopolamine-induced learning deficits as well as increasing social memory, attention and inhibitory avoidance. However, thioperamide and clobenpropit impair fear conditioning suggesting H3 antagonists may be specific in their role in mediating cognition (Passani et al 2004; Witkin and Nelson 2004; Esbenshade et al 2006; Bonaventure et al 2007). A number of pharmaceutical companies have programs based around H3 antagonists with clinical candidates reported in Phase I (see Esbenshade et al 2006).

Three histamine receptors H1, H2, and H3 have been cloned and expressed in zebrafish (Peitsaro et al 2007). When compared with the distribution in mammalian brain, the histaminergic neurons are more tightly located around the posterior recess and are more ventral to the mammillary body than in rodents. However, the rostrocaudal distribution is very similar to that seen in the rat and overall the projections patterns are highly conserved (Kaslin and Panula 2001). Comparison of the H3 receptor peptide sequence showed 50% identity with the human (see Table 1) (Peitsaro et al 2007), and binding studies demonstrated the H3 receptor was expressed throughout the zebrafish brain with the greatest intensity in the optic tectum and hypothalamus (Peitsaro et al 2000). Zebrafish at 5 d.p.f. treated with thioperamide (100  $\mu$ M) demonstrated a decrease in locomotor activity although no toxicity assessment was given to determine whether this decrease was an effect was specific antagonism or a reduction due to generalised toxicity. Other studies in goldfish have demonstrated involvement of H1 and H2 in inhibitory avoidance (Cofiel and Mattioli 2006).

## 5HT6 receptor antagonists

5-Hydroxytryptamine (5-HT) receptors so far are composed of seven different subtypes which have been implicated in mediating cognition (Meneses 1999, 2007). They are all

found in areas of the brain connected with learning and memory, but attention has focussed on 5-HT<sub>6</sub>, which appears to have a greater role in long-term memory (for review see Mitchell and Neumaier 2005). A number of antagonists (eg, RO4368554, SB-271046) have been shown to reverse scopolamine-induced deficits in passive avoidance assays in rats as well as enhancing object recognition (Foley et al 2004; Mitchell and Neumaier 2005; Hirst et al 2006; Schreiber et al 2007), although the consistency of the effect at this target has been disputed, which raises questions as to brain penetrance and selectivity of the various compounds used (Russell and Dias 2002). Again, despite the literature supporting their effect in learning and memory, there are currently no reports of clinical trials for 5HT<sub>6</sub> antagonists.

In zebrafish, 5-HT neurons are expressed in the spinal cord as early as 1 d.p.f. with populations in the telencephalon, hindbrain and throughout the brain by 5 d.p.f. (Drapeau et al 2002; McLean and Fetcho 2004). In the adult these neurons cluster in the area of the hypothalamus and habenula (Kaslin and Panula 2001). A search of the NCBI protein sequence finder database and blast search shows that zebrafish have at least three recognised 5-HT receptors namely 1, 2 and 7 (<http://www.ncbi.nlm.nih.gov/BLAST>). This has been confirmed with studies using methysergide a 5-HT<sub>1,2,7</sub> antagonist which modulated locomotor activity in larval zebrafish (Drapeau et al 2002; Brustein and Drapeau 2005). Studies in goldfish have demonstrated the presence of 5-HT<sub>1A</sub> in the retina (Schmeer et al 2001). Interestingly, a blast search using the human 5-HT<sub>6</sub> peptide sequence revealed 54% identity with a hypothetical protein (accession number: XP 696681) which also has 52% and 50% identity with the rat and mouse 5-HT<sub>6</sub> sequences respectively (see Table 1).

## AMPA receptor potentiators

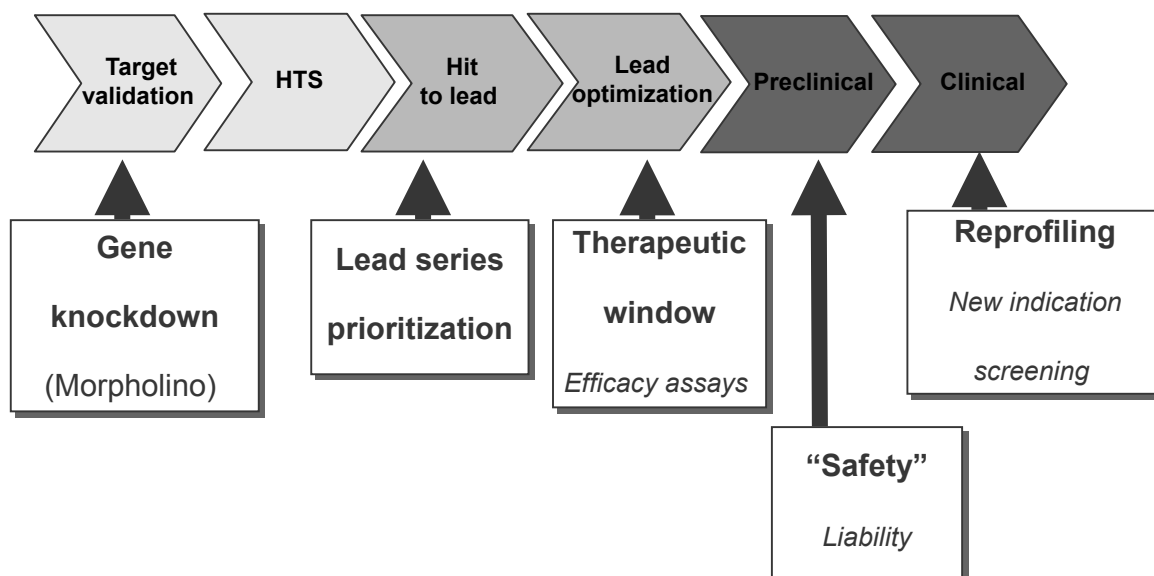
Alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors are glutamate receptors comprising four different subunits GluRA1-A4, are found throughout the CNS mediating fast excitatory transmission (for a comprehensive review see O'Neill et al 2004). Early studies have shown the presence of multiple AMPA receptors in the CA1/CA2 region of the hippocampus suggesting this as a target for cognition (Wenthold et al 1996). Ampakines are a diverse class of compounds which positively modulate glutamateric AMPA receptors with pyrrolidones (eg, piracetam) and piperidines (CX516) being examples (see O'Neill et al 2004; O'Neill and Dix 2007). These compounds have been studied in a number of different rodent memory tasks such as spatial memory, delayed-nonmatch-to sample and extinction

learning and have been shown to improve performance (Staubli et al 1994; Hampson et al 1998; Zushida et al 2007). However, despite some early encouraging data (Lynch and Gall 2006), recent clinical results have been mixed with the ampakine farampator increasing short-term memory trial of the symbol digit recall test in elderly patients (Wezenberg et al 2007) whereas CX516 used as an add-on therapy did not alter the composite cognitive score compared to the placebo group in schizophrenic patients (Goff et al 2007) or improve cognitive outcomes in patients with fragile X syndrome (Berry-Kravis et al 2006) suggesting these compounds may not have been dosed adequately or that they need to be used more selectively for cognitive impairments.

Zebrafish possess the AMPA receptors subunits GluR1-4 with each one consisting of a subtype A and B (NCBI protein database; <http://www.ncbi.nlm.nih.gov/sites/entrez>). A blast search between the subtypes shows a similarity between the A and B subtypes ranges from 82% for GluR1, 88% GluR3 to 92% for GluR2 and 4. Compared with human, rat, and mouse, the zebrafish receptors have very good identity, the 1A subtype has 71% identity with the human, rat, and mouse GluR1 sequences. The GluR2-4A receptors have higher identities with human, rat and mouse of between 85%–90% with the equivalent protein sequence (see Table 1). AMPA receptors have been found and studied in the retina, hindbrain, spinal cord, and Mauthner neurons of zebrafish (Ali et al 2000; Yazulla and Studholme 2001; Patten and Ali 2007). They are also associated with the neuromuscular junction in zebrafish (Todd et al 2004). Not much has been reported in the zebrafish literature as to the affect of AMPAkinones on their learning and memory; however studies on nonassociative learning in this lab investigating piracetam have found that it increased the ASR and prolonged the habituation (unpublished data) similar to the other cognition enhancers tested.

## Conclusion

It is now recognised that zebrafish possess a great deal of similarity to mammals and are an extremely useful model for screening compounds at several stages of the drug discovery process (see Figure 1). As more is reported on the efficacy models in zebrafish its utility as an early stage screening tool for the discovery of CNS therapeutics should enable increased throughput of *in vivo* analysis of novel compounds for neurological disorders. In this review we have described the application of the zebrafish models to neurodegenerative disorders, schizophrenia and learning and memory. Several of these diseases affect an aging population and are adult onset raising questions as



**Figure 1** Utility of zebrafish in a screening and drug discovery strategy. Flow diagram showing where zebrafish can aid the drug development and discovery process but either increasing throughput or assessing candidate compounds more economically.

to the appropriateness of using a rapidly developing larval system. However, early phenotypes are observable and in comparison with rodents the zebrafish larvae are not foetal but are closer to juveniles in that the nervous system is mature, organs are functioning and tissue architecture is fully developed by the time at which many of the assays are performed. In this review we have also described four validated pharmacological targets which are being investigated preclinically for impaired learning and memory; phosphodiesterases, histamine 3, 5HT-6 and AMPA, and have illustrated how zebrafish may be used in the assessment of these targets. As more information is reported on neurological assays in zebrafish, the utility of this model organism as an early stage screening tool for CNS disorders should help increase in vivo throughput and ameliorate the cost associated with drug screening in mammals.

## References

- Abeliovich A, Beal MF. 2006. Parkinsonism genes: culprits and clues. *J Neurochem*, 99:1062–72.
- Ali DW, Buss RR, Drapeau P. 2000. Properties of miniature glutamatergic EPSCs in neurons of the locomotor regions of the developing zebrafish. *J Neurophysiol*, 83:181–91.
- Anichtchik OV, Kaslin J, Peitsaro N, et al. 2004. Neurochemical and behavioural changes in zebrafish *Danio rerio* after systemic administration of 6-hydroxydopamine and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *J Neurochem*, 88:443–53.
- Arrang JM, Morisset S, Gbahou F. 2007. Constitutive activity of the histamine H(3) receptor. *Trends Pharmacol Sci*, 1249:139–51.
- Arthur D, Levin ED. 2001. Spatial and non-spatial visual discrimination learning in zebrafish (*Danio rerio*). *Animal Cognition*, 4:125–31.
- Ballesteros RP, Dybowski JA, Levy G, et al. 1999. Cloning and characterization of zRICH, a 2',3'-cyclic-nucleotide 3'-phosphodiesterase induced during zebrafish optic nerve regeneration. *J Neurochem*, 72:1362–71.
- Barad M, Bourtoouladze R, Winder DG, et al. 1998. Rolipram, a type IV-specific phosphodiesterase inhibitor, facilitates the establishment of long-lasting long-term potentiation and improves memory. *Proc Natl Acad Sci USA*, 95:15020–5.
- Berger J, Currie P. 2007. The role of zebrafish in chemical genetics. *Curr Med Chem*, 14:2413–20.
- Berghmans S, Butler B, Goldsmith P, et al. 2008. Early drug safety assessment for the cardiac, visual and gut functions using the zebrafish. *J Pharmacol Toxicol Methods*, in press.
- Berry-Kravis E, Krause SE, Block SS, et al. 2006. Effect of CX516, an AMPA-modulating compound, on cognition and behavior in fragile X syndrome: a controlled trial. *J Child Adolesc Psychopharmacol*, 16:525–40.
- Best JD, Berghmans S, Hunt JJ, et al. 2007. Non-associative learning in larval zebrafish. *Neuropsychopharmacology*, Jun 20; [Epub ahead of print].
- Birks J. 2006. Cholinesterase inhibitors for Alzheimer's disease. *Cochrane Database Syst Rev*, 1:CD005593.
- Bonaventure P, Letavic M, Dugovic C, et al. 2007. Histamine H3 receptor antagonists: from target identification to drug leads. *Biochem Pharmacol*, 73:1084–96.
- Braff DL, Geyer MA, Swerdlow NR. 2001. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berl)*, 156:234–58.
- Bretau S, Allen C, Ingham PW, et al. 2007. p53-dependent neuronal cell death in a DJ-1-deficient zebrafish model of Parkinson's disease. *J Neurochem*, 100:1626–35.
- Bretau S, Lee S, Guo S. 2004. Sensitivity of zebrafish to environmental toxins implicated in Parkinson's disease. *Neurotoxicol Teratol*, 26:857–64.
- Brustein E, Drapeau P. 2005. Serotonergic modulation of chloride homeostasis during maturation of the locomotor network in zebrafish. *J Neurosci*, 25:10607–16.
- Burgess HA, Granato M. 2007. Sensorimotor gating in larval zebrafish. *J Neurosci*, 27:4984–94.

- Burns A, Rossor M, Hecker J, et al. 1999. The effects of donepezil in Alzheimer's disease – results from a multinational trial. *Dement Geriatr Cogn Disord*, 10:237–44.
- Cahill GM. 2002. Clock mechanisms in zebrafish. *Cell Tissue Res*, 309:27–34.
- Carvan MJ, 3rd, Loucks E, Weber DN, et al. 2004. Ethanol effects on the developing zebrafish: neurobehavior and skeletal morphogenesis. *Neurotoxicol Teratol*, 26:757–68.
- Chen L, Cagniard B, Mathews T, et al. 2005. Age-dependent motor deficits and dopaminergic dysfunction in DJ-1 null mice. *J Biol Chem*, 280:21418–26.
- Cherry JA, Davis RL. 1999. Cyclic AMP phosphodiesterases are localized in regions of the mouse brain associated with reinforcement, movement, and affect. *J Comp Neurol*, 407:287–301.
- Cofiel LP, Mattioli R. 2006. Involvement of histamine receptors in the acquisition of inhibitory avoidance in *Carassius auratus*. *Prog Neuropsychopharmacol Biol Psychiatry*, 30:1246–50.
- Colwill RM, Raymond MP, Ferreira L, et al. 2005. Visual discrimination learning in zebrafish (*Danio rerio*). *Behav Processes*, 70:19–31.
- Cosman KM, Boyle LL, Porsteinsson AP. 2007. Memantine in the treatment of mild-to-moderate Alzheimer's disease. *Expert Opin Pharmacother*, 8:203–14.
- Cowan CM, Raymond LA. 2006. Selective neuronal degeneration in Huntington's disease. *Curr Top Dev Biol*, 75:25–71.
- Devan BD, Sierra-Mercado D, Jr., Jimenez M, et al. 2004. Phosphodiesterase inhibition by sildenafil citrate attenuates the learning impairment induced by blockade of cholinergic muscarinic receptors in rats. *Pharmacol Biochem Behav*, 79:691–9.
- Drapeau P, Saint-Amant L, Buss RR, et al. 2002. Development of the locomotor network in zebrafish. *Prog Neurobiol*, 68:85–111.
- Esbenshade TA, Fox GB, Cowart MD. 2006. Histamine H3 receptor antagonists: preclinical promise for treating obesity and cognitive disorders. *Mol Interv*, 6:77–88, 59.
- Foley AG, Murphy KJ, Hirst WD, et al. 2004. The 5-HT(6) receptor antagonist SB-271046 reverses scopolamine-disrupted consolidation of a passive avoidance task and ameliorates spatial task deficits in aged rats. *Neuropsychopharmacology*, 29:93–100.
- Galvin JE. 2006. Cognitive change in Parkinson disease. *Alzheimer Dis Assoc Disord*, 20:302–10.
- Gauthier S, Reisberg B, Zaudig M, et al. 2006. Mild cognitive impairment. *Lancet*, 367:1262–70.
- Geerts H, Grossberg GT. 2006. Pharmacology of acetylcholinesterase inhibitors and N-methyl-D-aspartate receptors for combination therapy in the treatment of Alzheimer's disease. *J Clin Pharmacol*, 46:8S–16S.
- Goff DC, Lamberti JS, Leon AC, et al. 2007. A Placebo-Controlled Add-On Trial of the Ampakine, CX516, for Cognitive Deficits in Schizophrenia. *Neuropsychopharmacology*.
- Goldsmith P, Fleming A (2007). Screening methods employing zebrafish and the blood brain barrier. IN OFFICE, E. P. (Ed.).
- Grunwald DJ, Eisen JS. 2002. Headwaters of the zebrafish – emergence of a new model vertebrate. *Nat Rev Genet*, 3:717–24.
- Guo S. 2004. Linking genes to brain, behavior and neurological diseases: what can we learn from zebrafish? *Genes Brain Behav*, 3:63–74.
- Hampson RE, Rogers G, Lynch G, et al. 1998. Facilitative effects of the ampakine CX516 on short-term memory in rats: enhancement of delayed-nonmatch-to-sample performance. *J Neurosci*, 18:2740–7.
- Hebb AL, Robertson HA. 2007. Role of phosphodiesterases in neurological and psychiatric disease. *Curr Opin Pharmacol*, 7:86–92.
- Hebb AL, Robertson HA, Denovan-Wright EM. 2004. Striatal phosphodiesterase mRNA and protein levels are reduced in Huntington's disease transgenic mice prior to the onset of motor symptoms. *Neuroscience*, 123:967–81.
- Hirst WD, Stean TO, Rogers DC, et al. 2006. SB-399885 is a potent, selective 5-HT6 receptor antagonist with cognitive enhancing properties in aged rat water maze and novel object recognition models. *Eur J Pharmacol*, 553:109–19.
- Imanishi T, Sawa A, Ichimaru Y, et al. 1997. Ameliorating effects of rolipram on experimentally induced impairments of learning and memory in rodents. *Eur J Pharmacol*, 321:273–8.
- Karlovič CA, John RM, Ramirez L, et al. 1998. Characterization of the Huntington's disease (HD) gene homologue in the zebrafish *Danio rerio*. *Gene*, 217:117–25.
- Kaslin J, Panula P. 2001. Comparative anatomy of the histaminergic and other aminergic systems in zebrafish (*Danio rerio*). *J Comp Neurol*, 440:342–77.
- Kehne JH, Boulis NM, Davis M. 1991. Effects of the phosphodiesterase inhibitor rolipram on the acoustic startle response in rats. *Psychopharmacology (Berl)*, 105:27–36.
- Lam CS, Korzh V, Strahle U. 2005. Zebrafish embryos are susceptible to the dopaminergic neurotoxin MPTP. *Eur J Neurosci*, 21:1758–62.
- Leroy E, Boyer R, Auburger G, et al. 1998. The ubiquitin pathway in Parkinson's disease. *Nature*, 395:451–2.
- Levey A, Lah J, Goldstein F, et al. 2006. Mild cognitive impairment: an opportunity to identify patients at high risk for progression to Alzheimer's disease. *Clin Ther*, 28:991–1001.
- Levin ED, Chen E. 2004. Nicotinic involvement in memory function in zebrafish. *Neurotoxicol Teratol*, 26:731–5.
- Levin ED, Limpuangthip J, Rachakonda T, et al. 2006a. Timing of nicotine effects on learning in zebrafish. *Psychopharmacology (Berl)*, 184:547–52.
- Levin ED, McClellon FJ, Rezvani AH. 2006b. Nicotinic effects on cognitive function: behavioral characterization, pharmacological specification, and anatomic localization. *Psychopharmacology (Berl)*, 184:523–39.
- Levin ED, Simon BB. 1998. Nicotinic acetylcholine involvement in cognitive function in animals. *Psychopharmacology (Berl)*, 138:217–30.
- Lovenberg TW, Roland BL, Wilson SJ, et al. 1999. Cloning and functional expression of the human histamine H3 receptor. *Mol Pharmacol*, 55:1101–7.
- Lumsden AL, Henshall TL, Dayan S, et al. 2007. Huntingtin-deficient zebrafish exhibit defects in iron utilization and development. *Hum Mol Genet*.
- Luque MA, Perez-Perez MP, Herrero L, et al. 2005. Involvement of the optic tectum and mesencephalic reticular formation in the generation of saccadic eye movements in goldfish. *Brain Res Rev*, 49:388–97.
- Lynch G, Gall CM. 2006. Ampakines and the threefold path to cognitive enhancement. *Trends Neurosci*, 29:554–62.
- MacRae CA, Peterson RT. 2003. Zebrafish-based small molecule discovery. *Chem Biol*, 10:901–8.
- McDeamid JR, Drapeau P. 2006. Rhythmic motor activity evoked by NMDA in the spinal zebrafish larva. *J Neurophysiol*, 95:401–17.
- McKinley ET, Baranowski TC, Blavo DO, et al. 2005. Neuroprotection of MPTP-induced toxicity in zebrafish dopaminergic neurons. *Brain Res Mol Brain Res*, 141:128–37.
- McLean DL, Fetcho JR. 2004. Ontogeny and innervation patterns of dopaminergic, noradrenergic, and serotonergic neurons in larval zebrafish. *J Comp Neurol*, 480:38–56.
- Meneses A. 1999. 5-HT system and cognition. *Neurosci Biobehav Rev*, 23:1111–25.
- Meneses A. 2007. Do serotonin(1–7) receptors modulate short and long-term memory? *Neurobiol Learn Mem*, 87:561–72.
- Miller VM, Nelson RF, Gouvion CM, et al. 2005. CHIP suppresses polyglutamine aggregation and toxicity in vitro and in vivo. *J Neurosci*, 25:9152–61.
- Mitchell ES, Neumaier JF. 2005. 5-HT6 receptors: a novel target for cognitive enhancement. *Pharmacol Ther*, 108:320–33.
- Montoya A, Price BH, Menear M, et al. 2006. Brain imaging and cognitive dysfunctions in Huntington's disease. *J Psychiatry Neurosci*, 31:21–9.
- Mount C, Downton C. 2006. Alzheimer disease: progress or profit? *Nat Med*, 12:780–4.
- Mudher A, Lovestone S. 2002. Alzheimer's disease-do tauists and baptists finally shake hands? *Trends Neurosci*, 25:22–6.



- Murphey RD, Zon LI. 2006. Small molecule screening in the zebrafish. *Methods*, 39:255–61.
- Musa A, Lehrach H, Russo VA. 2001. Distinct expression patterns of two zebrafish homologues of the human APP gene during embryonic development. *Dev Genes Evol*, 211:563–7.
- Nam RH, Kim W, Lee CJ. 2004. NMDA receptor-dependent long-term potentiation in the telencephalon of the zebrafish. *Neurosci Lett*, 370:248–51.
- Newman M, Musgrave FI, Lardelli M. 2007. Alzheimer disease: amyloidogenesis, the presenilins and animal models. *Biochim Biophys Acta*, 1772:285–97.
- Ninkovic J, Folchert A, Makhankov YV, et al. 2006. Genetic identification of AChE as a positive modulator of addiction to the psychostimulant D-amphetamine in zebrafish. *J Neurobiol*, 66:463–75.
- Nuechterlein KH, Barch DM, Gold JM, et al. 2004. Identification of separable cognitive factors in schizophrenia. *Schizophr Res*, 72:29–39.
- O'Neill MJ, Bleakman D, Zimmerman DM, et al. 2004. AMPA receptor potentiators for the treatment of CNS disorders. *Curr Drug Targets CNS Neurol Disord*, 3:181–94.
- O'Neill MJ, Dix S. 2007. AMPA receptor potentiators as cognitive enhancers. *IDrugs*, 10:185–92.
- Orger MB, Gahtan E, Muto A, et al. 2004. Behavioral screening assays in zebrafish. *Methods Cell Biol*, 77:53–68.
- Paquet D, Schmid B, Haass C. 2006. Analysis of the function of the Parkinson's disease gene pink1 in zebrafish. *7th International Conference on Zebrafish Development and Genetics.*, University of Wisconsin-Madison, Madison, Wisconsin USA, June 14–18.
- Passani MB, Lin JS, Hancock A, et al. 2004. The histamine H3 receptor as a novel therapeutic target for cognitive and sleep disorders. *Trends Pharmacol Sci*, 25:618–25.
- Patten SA, Ali DW. 2007. AMPA receptors associated with zebrafish Mauthner cells switch subunits during development. *J Physiol*, 581:1043–56.
- Peitsaro N, Anichtchik OV, Panula P. 2000. Identification of a histamine H(3)-like receptor in the zebrafish (*Danio rerio*) brain. *J Neurochem*, 75:718–24.
- Peitsaro N, Kaslin J, Anichtchik OV, et al. 2003. Modulation of the histaminergic system and behaviour by alpha-fluoromethylhistidine in zebrafish. *J Neurochem*, 86:432–41.
- Peitsaro N, Sundvik M, Anichtchik OV, et al. 2007. Identification of zebrafish histamine H1, H2 and H3 receptors and effects of histaminergic ligands on behavior. *Biochem Pharmacol*, 73:1205–14.
- Postlethwait JH, Woods IG, Ngo-Hazelett P, et al. 2000. Zebrafish comparative genomics and the origins of vertebrate chromosomes. *Genome Res*, 10:1890–902.
- Pradel G, Schmidt R, Schachner M. 2000. Involvement of L1.1 in memory consolidation after active avoidance conditioning in zebrafish. *J Neurobiol*, 43:389–403.
- Prickaerts J, van Staveren WC, Sik A, et al. 2002. Effects of two selective phosphodiesterase type 5 inhibitors, sildenafil and vardenafil, on object recognition memory and hippocampal cyclic GMP levels in the rat. *Neuroscience*, 113:351–61.
- Renau TE. 2004. The potential of phosphodiesterase 4 inhibitors for the treatment of depression: opportunities and challenges. *Curr Opin Investig Drugs*, 5:34–9.
- Ringman JM, Cummings JL. 2006. Current and emerging pharmacological treatment options for dementia. *Behav Neurol*, 17:5–16.
- Rink E, Wullimann MF. 2004. Connections of the ventral telencephalon (subpallium) in the zebrafish (*Danio rerio*). *Brain Res*, 1011:206–20.
- Robinson DM, Keating GM. 2006. Memantine: a review of its use in Alzheimer's disease. *Drugs*, 66:1515–34.
- Rose GM, Hopper A, De Vivo M, et al. 2005. Phosphodiesterase inhibitors for cognitive enhancement. *Curr Pharm Des*, 11:3329–34.
- Russell MG, Dias R. 2002. Memories are made of this (perhaps): a review of serotonin 5-HT(6) receptor ligands and their biological functions. *Curr Top Med Chem*, 2:643–54.
- Salzmann J, Anichtchik OV, Best JD, et al. 2006. Effect of MPTP treatment on locomotor activity and dopaminergic cells in the zebrafish larvae. *Workshop – Neurodegeneration; basic mechanisms of motor and cognitive dysfunctions*, Cracow, Poland, September 2–4.
- Schiffner NW, Broadley SA, Hirschberger T, et al. 2007. Identification of anti-prion compounds as efficient inhibitors of polyglutamine protein aggregation in a zebrafish model. *J Biol Chem*, 282:9195–203.
- Schmeier C, Obregon F, Urbina M, et al. 2001. Further characterization of 5-HT1A receptors in the goldfish retina: role of cyclic AMP in the regulation of the in vitro outgrowth of retinal explants. *Neurochem Res*, 26:213–23.
- Schreiber R, Vivian J, Hedley L, et al. 2007. Effects of the novel 5-HT(6) receptor antagonist RO4368554 in rat models for cognition and sensorimotor gating. *Eur Neuropsychopharmacol*, 17:277–88.
- Selkoe DJ. 2000. Toward a comprehensive theory for Alzheimer's disease. Hypothesis: Alzheimer's disease is caused by the cerebral accumulation and cytotoxicity of amyloid beta-protein. *Ann N Y Acad Sci*, 924:17–25.
- Shankaran S, Schmid B, Kahle P, et al. 2006. Functional Analysis of Parkinson's Disease Gene LRRK2 in Zebrafish. *7th International Conference on Zebrafish Development and Genetics.*, University of Wisconsin-Madison, Madison, Wisconsin USA, June 14–18.
- Siuciak JA, Chapin DS, Harms JF, et al. 2006. Inhibition of the striatum-enriched phosphodiesterase PDE10A: a novel approach to the treatment of psychosis. *Neuropharmacology*, 51:386–96.
- Smeyne RJ, Jackson-Lewis V. 2005. The MPTP model of Parkinson's disease. *Brain Res Mol Brain Res*, 134:57–66.
- Son OL, Kim HT, Ji MH, et al. 2003. Cloning and expression analysis of a Parkinson's disease gene, uch-L1, and its promoter in zebrafish. *Biochem Biophys Res Commun*, 312:601–7.
- Staubli U, Rogers G, Lynch G. 1994. Facilitation of glutamate receptors enhances memory. *Proc Natl Acad Sci USA*, 91:777–81.
- Swain HA, Sigstad C, Scalzo FM. 2004. Effects of dizocilpine (MK-801) on circling behavior, swimming activity, and place preference in zebrafish (*Danio rerio*). *Neurotoxicol Teratol*, 26:725–9.
- Swerdlow NR, Paulsen J, Braff DL, et al. 1995. Impaired prepulse inhibition of acoustic and tactile startle response in patients with Huntington's disease. *J Neurol Neurosurg Psychiatry*, 58:192–200.
- Tamminga CA. 2006. The neurobiology of cognition in schizophrenia. *J Clin Psychiatry*, 67:e11.
- Todd KJ, Slatter CA, Ali DW. 2004. Activation of ionotropic glutamate receptors on peripheral axons of primary motoneurons mediates transmitter release at the zebrafish NMJ. *J Neurophysiol*, 91:828–40.
- Tomasiewicz HG, Flaherty DB, Soria JP, et al. 2002. Transgenic zebrafish model of neurodegeneration. *J Neurosci Res*, 70:734–45.
- Tropepe V, Sive HL. 2003. Can zebrafish be used as a model to study the neurodevelopmental causes of autism? *Genes Brain Behav*, 2:268–81.
- Vandenbergh R, Tournoy J. 2005. Cognitive aging and Alzheimer's disease. *Postgrad Med J*, 81:343–52.
- Wenthold RJ, Petralia RS, Blahos J, II, et al. 1996. Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. *J Neurosci*, 16:1982–9.
- Wezenberg E, Verkes RJ, Ruijt GS, et al. 2007. Acute effects of the amphetamine farampator on memory and information processing in healthy elderly volunteers. *Neuropsychopharmacology*, 32:1272–83.
- Williams FE, White D, Messer WS. 2002. A simple spatial alternation task for assessing memory function in zebrafish. *Behav Processes*, 58:125–32.
- Witkin JM, Nelson DL. 2004. Selective histamine H3 receptor antagonists for treatment of cognitive deficiencies and other disorders of the central nervous system. *Pharmacol Ther*, 103:1–20.
- Wullimann MF, Mueller T. 2004. Teleostean and mammalian forebrains contrasted: Evidence from genes to behavior. *J Comp Neurol*, 475:143–62.
- Xu X, Scott-Scheiern T, Kempker L, et al. 2007. Active avoidance conditioning in zebrafish (*Danio rerio*). *Neurobiol Learn Mem*, 87:72–7.

- Yazulla S, Studholme KM. 2001. Neurochemical anatomy of the zebrafish retina as determined by immunocytochemistry. *J Neurocytol*, 30:551–92.
- Zhang HT, Crissman AM, Dorairaj NR, et al. 2000. Inhibition of cyclic AMP phosphodiesterase (PDE4) reverses memory deficits associated with NMDA receptor antagonism. *Neuropsychopharmacology*, 23:198–204.
- Zhang HT, O'Donnell JM. 2000. Effects of rolipram on scopolamine-induced impairment of working and reference memory in the radial-arm maze tests in rats. *Psychopharmacology (Berl)*, 150:311–16.
- Zhdanova IV, Wang SY, Leclair OU, et al. 2001. Melatonin promotes sleep-like state in zebrafish. *Brain Res*, 903:263–8.
- Zhu J, Mix E, Winblad B. 2001. The antidepressant and antiinflammatory effects of rolipram in the central nervous system. *CNS Drug Rev*, 7:387–98.
- Zon LI, Peterson RT. 2005. In vivo drug discovery in the zebrafish. *Nat Rev Drug Discov*, 4:35–44.
- Zushida K, Sakurai M, Wada K, et al. 2007. Facilitation of extinction learning for contextual fear memory by PEPA: a potentiator of AMPA receptors. *J Neurosci*, 27:158–66.