

## **The Developing Utility of Zebrafish in Modeling Neurobehavioral Disorders**

**Adam Stewart, Ferdous Kadri, John DiLeo, Kyung Min Chung,  
Jonathan Cachat, Jason Goodspeed, Christopher Suci, Sudipta Roy, Siddharth Gaikwad, Keith Wong, Marco Elegante, Salem Elkhayat, Nadine Wu, Thomas Gilder, David Tien, Leah Grossman, Julia Tan, Ashley Denmark, Brett Bartels, Kevin Frank, Esther Beeson, Allan V. Kalueff**  
*Tulane University Medical School, U.S.A.*

The zebrafish (*Danio rerio*) is becoming increasingly popular in the field of neurobehavioral research, including experimental, genetic, and pharmacological models of human brain disorders. While zebrafish research is rapidly expanding, its application as a translational neurobehavioral model is still in its relative infancy. Therefore, further investigation of new models is needed for targeting more domains and new, more complex brain disorders. The main aim of this paper is to discuss recent developments in the field of zebrafish neurobehavioral research, and to outline important emerging topics for further studies.

The zebrafish (*Danio rerio*) is a promising model organism in neurobehavioral and biological psychiatry research. The robustness of zebrafish phenotypes makes this species an excellent animal for studying experimental, genetic, and pharmacological models of neurobehavioral disorders. As fish represent perhaps the dawn of the evolution of vertebrates' emotional behavior, the main aim of this paper is to outline recent developments in the field of zebrafish neurobehavioral research, and to summarize the emerging important new topics for further studies in this field. Another aim of this paper is to discuss what can be done to further improve and promote zebrafish neurobehavioral research.

### **Exploratory-Based Models**

Zebrafish behavioral assays are currently used for high-throughput phenotyping and testing of various psychotropic drugs (Blaser & Gerlai, 2006; Levin, Bencan, & Cerutti, 2007; Lopez-Patino, Yu, Cabral, & Zhdanova, 2008) (see Fig. 1a,b). A popular method of behavioral analysis in zebrafish research is the *novel tank test* (Fig. 2a), conceptually similar to the open field test used for rodents, which exhibit anxiety-like behavior by staying close to the walls (thigmotaxis), but increase exploration as they become acclimated to the new environment (Choleris, Thomasb, Kavaliersa, & Prat, 2001). Similarly, exposure to a novel environment evokes a robust anxiety response in zebrafish (Blaser & Gerlai, 2006), as they dive to the bottom (geotaxis) until they feel safe to swim in

The study was supported by Tulane University's Neuroscience Program, the Gordon and the G. Lurcy Fellowships, Provost's Scholarly Enrichment Fund, Newcomb Fellows Grant, Tulane Intramural Research Fund, LA Board of Regents' Pfund award, and NARSAD Young Investigator Award. Corresponding Author: Allan V. Kalueff, PhD, Department of Pharmacology, Tulane University Medical School, 1430 Tulane Ave. New Orleans, LA 70112, U.S.A. (avkalueff@gmail.com).

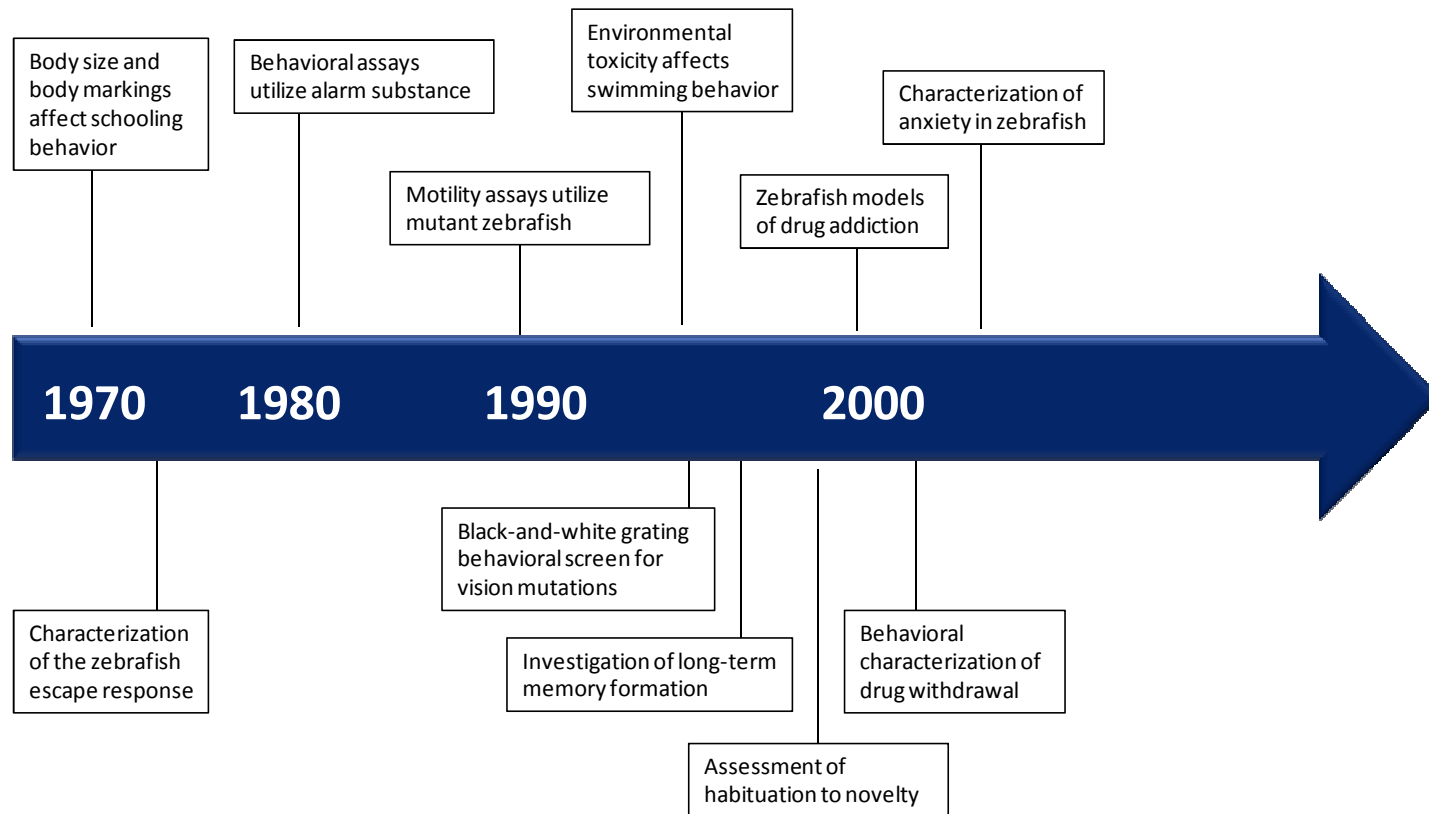
the upper regions of the tank. Typical endpoints in this test include the latency to enter the top, the number of transitions to the top, time spent in top, top:bottom time ratio, the number of fear/escape-like erratic movements, as well as freezing frequency and duration (Cachat et al., 2009; Levin et al., 2007; Wong et al., 2009).

Until recently, quantification of zebrafish behavior was primarily performed manually, making it vulnerable to human error and incorrect data interpretation. However, automated video-tracking technologies are becoming widely used to analyze animal behavior, providing a standardized unbiased observation of behavioral endpoints (Egan et al., 2009a). Another advantage of using the video-tracking approach is the ability to store, replay, and reanalyze videos. Finally, video-tracking tools can calculate additional behavioral endpoints that are not available through manual observation, such as distance traveled in top/bottom, velocity, meandering and angular velocity. Comparisons of data produced by the video-tracking system with that recorded manually show a high correlation between the two (Egan et al., 2009a; Gerlai, 2005), confirming the video-tracking approach as a reliable method of analysis in zebrafish neurobehavioral research.

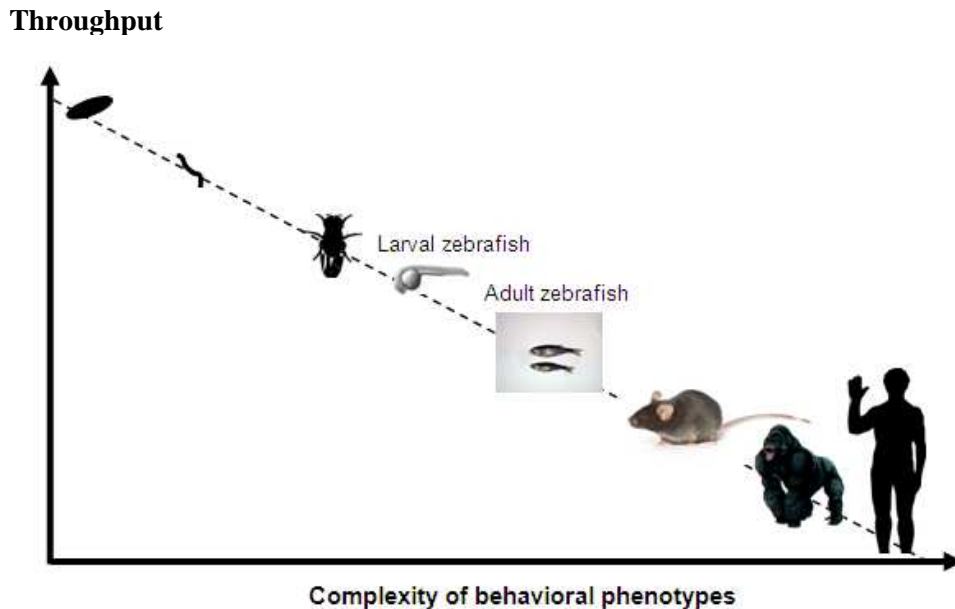
*The light/dark box* is traditionally used in rodent behavioral neuroscience, and is based on the innate aversion to open illuminated areas (scotophilia, scototaxis) (Bourin & Hascoet, 2003). Previous research has shown that while anxiolytic compounds can facilitate exploratory activity (i.e. increased entries and duration in the light part), anxiogenic drugs cause the opposite effect (Bourin & Hascoet, 2003). Importantly, this test is now being applied to zebrafish, in which they exhibit a natural preference for the dark side (Serra, Medalha, & Mattioli, 1999) (Fig. 2b). Several different modifications exist for the fish light-dark box test (e.g., Blaser, Chadwick, & McGinnis, 2010; Serra et al., 1999), consistently demonstrating the utility of light-dark situation to model zebrafish anxiety. Our own observations also support this notion, showing that “more aversive” light behaviors in zebrafish may be modulated by anxiogenic and anxiolytic drugs, strikingly paralleling the mouse light-dark behaviors.

*The open field test*, another apparatus traditionally used in experimental biopsychology in rodents (Carola, D'Olimpio, Brunamonti, Mangia, & Renzi, 2002; Choleris et al., 2001; Koplik, Salieva, & Gorbunova, 1995; Walsh & Cummins, 1976), also offers a promising new area of research in zebrafish. For example, some studies have applied the open field test to larval models (Lockwood, Bjerke, Kobayashi, & Guo, 2004). The utility of the open field test for adult zebrafish research also seems very logical. As in mice, zebrafish exhibit a natural tendency to stay close to walls of the apparatus, especially the corners. As they habituate to the novel arena, zebrafish predictably stray into the open central area, showing increased exploration (Fig. 2c).

Overall, this brief summary of zebrafish exploration-based paradigms leads to several important observations. First, zebrafish exploration appears to be driven by the same, evolutionarily conserved factors as rodent behavior, which is much better studied and understood. These factors include the balance between exploration (novelty-seeking, curiosity) and avoidance of aversive stimuli



**Figure 1.** The developing utility of zebrafish models in behavioral neuroscience research. A) Timeline of key findings in the field.

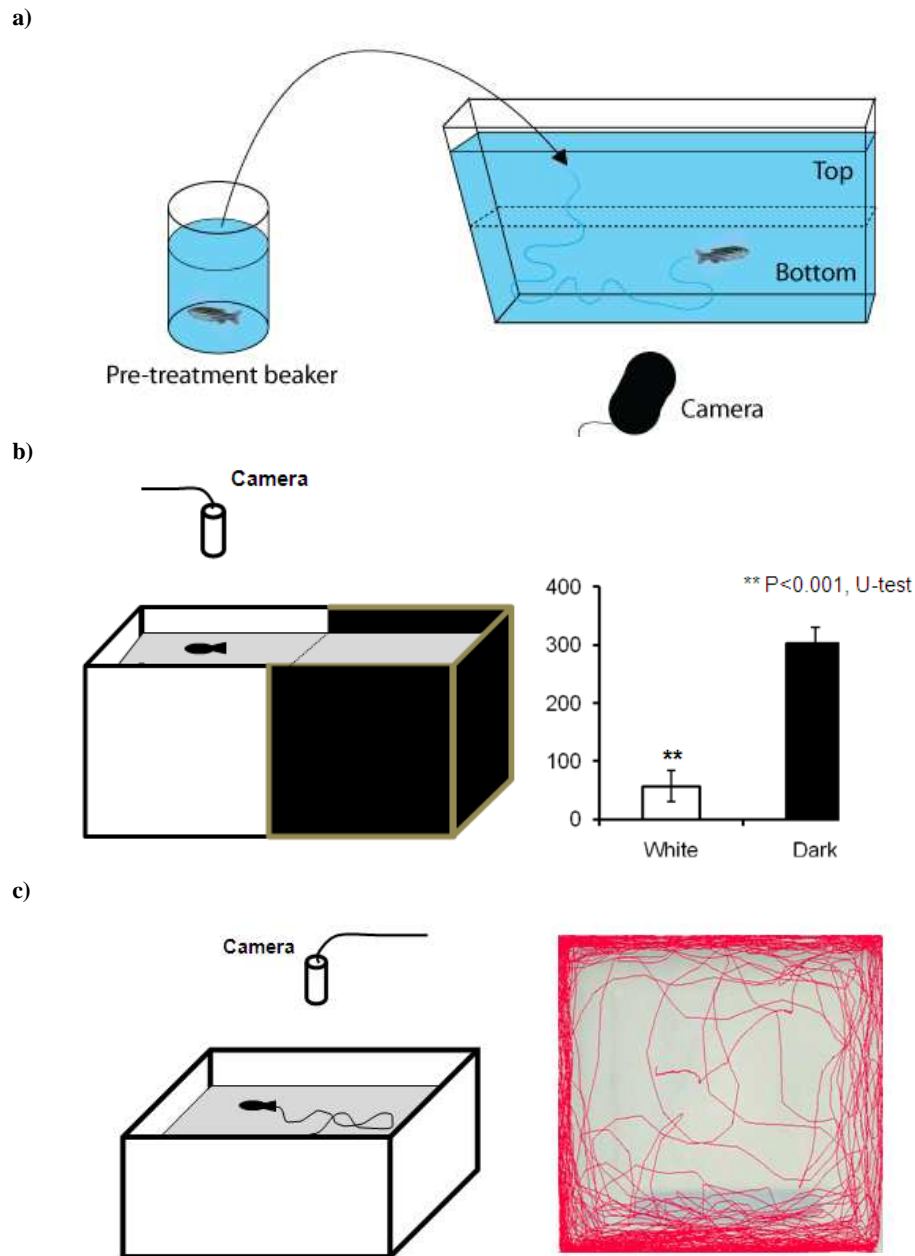


**Figure 1 (continued).** B) Zebrafish as a model species for biomedical research (other popular models include *E. coli*, *C. Elegans*, *Drosophila*, rodents, primates and humans).

(thigmotaxis, scototaxis), thereby reconfirming the use of zebrafish in experimental and comparative biopsychology research. Finally, unlike rodent models, zebrafish behavior is 3-dimensional, and includes an additional *vertical* dimension (geotaxic top-bottom behavior), thereby introducing a novel aspect to their exploration-based phenotypes.

### ***Zebrafish habituation behavior***

Relevant to both exploration and emotionality, habituation is the simplest form of learning, and has long been used to examine animal cognitive phenotypes (Bolivar, 2009; Salomons, van Luijk, Reinders, Kirchhof, Arndt, & Ohl, 2009). Habituation to novelty represents attenuation of innate behaviors, as subjects become accustomed to the environment (Leussis & Bolivar, 2006; Thompson & Spencer, 1966). Intra-session habituation reflects spatial working memory, whereas inter-session habituation is commonly used to assess middle- and long-term spatial memory (Muller et al., 1994). Despite being widely studied in various rodents (Bolivar, 2009; File & Mabbutt, 1990; Leussis & Bolivar, 2006; Ohl, Roedel, Storch, Holsboer, & Landgraf, 2002; Platel & Porsolt, 1982; Thompson & Spencer, 1966), habituation has not been extensively evaluated in zebrafish until recently (Best et al., 2008; Egan et al., 2009b; Gerlai, 2003; Goldsmith, 2004; Leimer et al., 1999; Shin & Fishman, 2002; Zon & Peterson, 2005).



**Figure 2.** Experimental paradigms to study zebrafish behavior. a) The novel tank test examines novelty-evoked anxiety. When a zebrafish is exposed to a novel (potentially dangerous) environment, it initially dives to the bottom, and then gradually explores the top. Inhibited exploration, reduced speed, and increased frequency of escape-like erratic behaviors are usually associated with higher levels of anxiety elicited by different stressors. b) Normal light-dark preference in adult zebrafish ( $n = 15$ ) tested in a 6-min light-dark box test (data obtained from manual registration of video-recorded behaviors). c) Open field thigmotaxis behavior in representative adult zebrafish tested for 30 min (top view; behavioral traces are generated using Noldus Ethovision XT7 video-tracking software).

Until recently, fish behavior was generally assumed to be instinctively-driven, with little cognitive ability (rev. in Burt de Perera, 2004). However, it is currently known that fish are capable of forming spatial memories and cognitive maps (Burt de Perera, 2004; Riedel, 1998), providing an opportunity to explore their habituation behaviors in depth. Several recent studies suggest that zebrafish can habituate to various stimuli, including conditioned place preference (Kily et al., 2008; Ninkovic & Bally-Cuif, 2006), light/dark locomotion (MacPhail, Brooks, Hunter, Padnos, Irons, & Padilla, 2009) and startle reflex (Eddins, Cerutti, Williams, Linney, & Levin, 2009; Levin et al., 2009) testing.

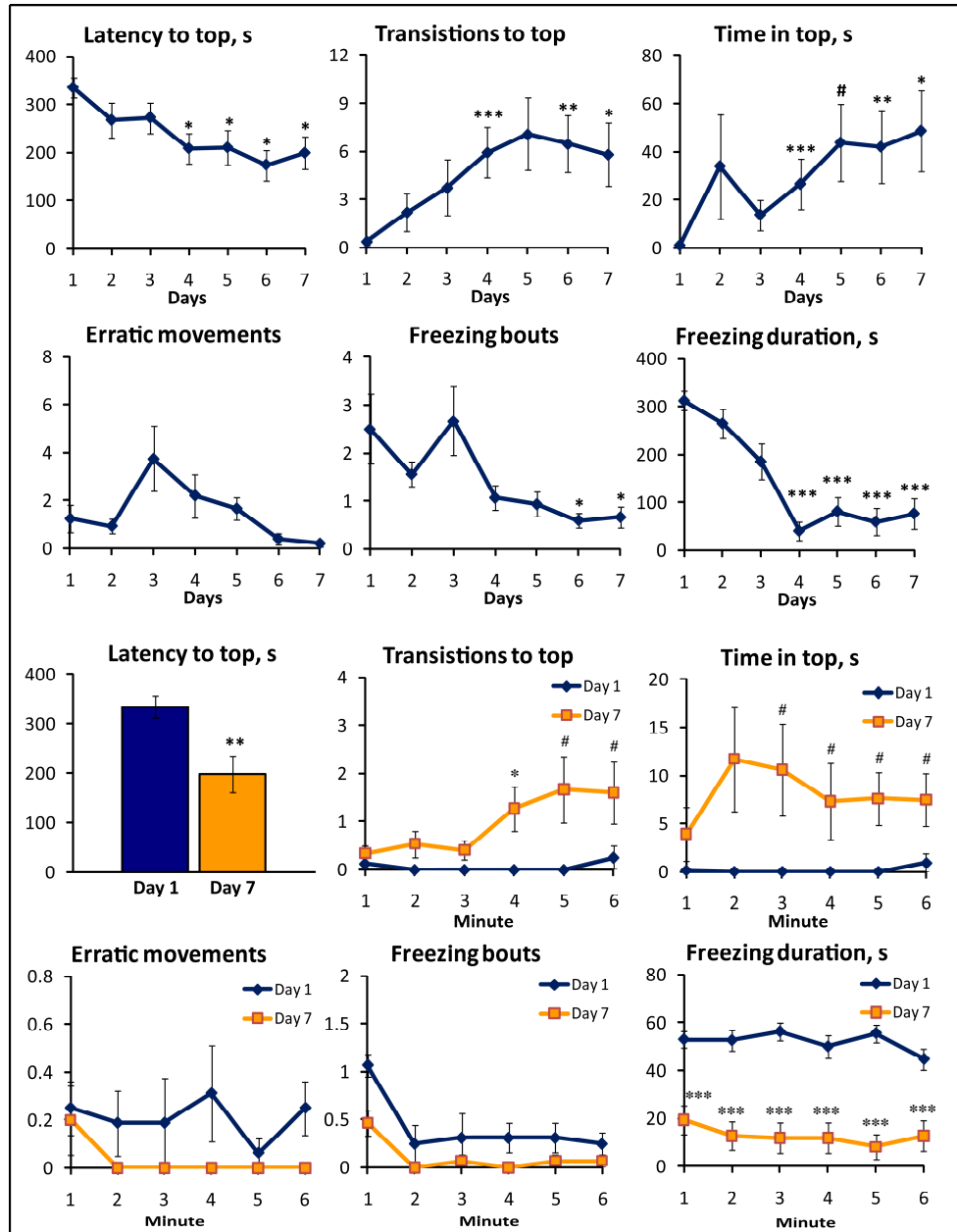
Experiments undertaken in our laboratory have comprehensively characterized zebrafish habituation to novelty (Wong et al., 2009). Using short 6-min novel tank trials, we found significant increases in exploratory behavior and decreases in freezing behavior over time. We have found that during 30-min intra-session habituation trials, the zebrafish exhibited a steady increase over time in transitions to the top of the novel tank, time spent in the top, as well as a marked decrease in freezing scores, but not in erratic movements (Wong et al., 2009). Finally, by analyzing inter-session habituation (Fig. 3), with each successive day, we found significantly increased transitions to the top, a similar trend for time spent there, as well as reduced freezing behaviors. Collectively, these findings confirm robust habituation phenotypes in zebrafish (similar to that observed in various rodent models) and emphasize the utility of zebrafish to study both cognitive and emotional behaviors.

### ***Other behavioral models***

Fostering high-throughput translational paradigms in zebrafish, one area that is rapidly developing is the neurobiology of the acoustic startle reflex (ASR). In humans, ASR assesses hearing sensitivity as well as the intact activity of multiple neuronal circuits (Musiek, 2003). The startle response is evolutionarily advantageous (because it provides a form of protection from dangers in the natural habitat), and its endpoints are thought to reflect sensitization, habituation, and prepulse inhibition (PPI) (Koch, 1999). In humans, lowered PPI has been reported in patients with neurological damage, schizophrenia or Huntington's disease (Musiek, 2003). For example, Burgess and Granato (2007) have shown that PPI does modulate ASR in zebrafish just as in higher vertebrates, such as mice, also reporting that reduced PPI (modulated by dopamine agonists) can be counteracted by antipsychotic drugs (Rigdon & Weatherspoon, 1992).

Olfaction plays a key role in zebrafish behavioral responses. In line with this, recent studies (Braubach, Wood, Gadbois, Fine, & Croll, 2009) have characterized zebrafish olfactory behaviors and their modifications through learning. Exposing zebrafish to L-alanine and L-valine (two amino acids that induce appetitive behavior), the researchers measured zebrafish appetitive behavior by counting >90° turns in a circular flow-through tank. These experiments paired the amino acids (unconditioned stimulus) with the conditioned stimulus

(phenylethyl alcohol), thereby eliciting a conditioned response to a neutral stimulus by pairing it with an olfactory cue (Braubach et al., 2009).



**Figure 3.** Habituation responses in zebrafish tested daily in the 6-min novel tank for 7 days ( $n = 23$ ), adapted from (Wong et al., 2009); \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\*  $p < 0.005$ , # $p = 0.05-0.1$ , trend (U-test with Bonferroni correction, where applicable, vs. Day 1 of the test) for significant ANOVA data).

Finally, social behaviors are commonly seen in zebrafish, raising the possibility of modeling social behavior in these animals. In rodents, social defeat stress has been shown to induce anxiety and depression in the “loser” animals (Becker, Zeau, Rivat, Blugeot, Hamon, & Benoliel, 2008; Koolhaas, De Boer, De Rutter, Meerlo, & Sgoifo, 1997). While most research in social defeat has been focused on higher organisms (Bjorkqvist, 2001; Koolhaas et al., 1997), zebrafish are also capable of establishing dominant-subordinate relationships and exhibiting agonistic behavior (Larson, O’Malley, & Melloni, 2006), opening an interesting new avenue for further study. For example, it may be possible that with repeated aggression (induced through many ongoing social defeat trials), some fish would emerge as persistent “losers”, leading to chronic social defeat similar to that observed in rodents.

### **Current Approaches and Methodological Considerations**

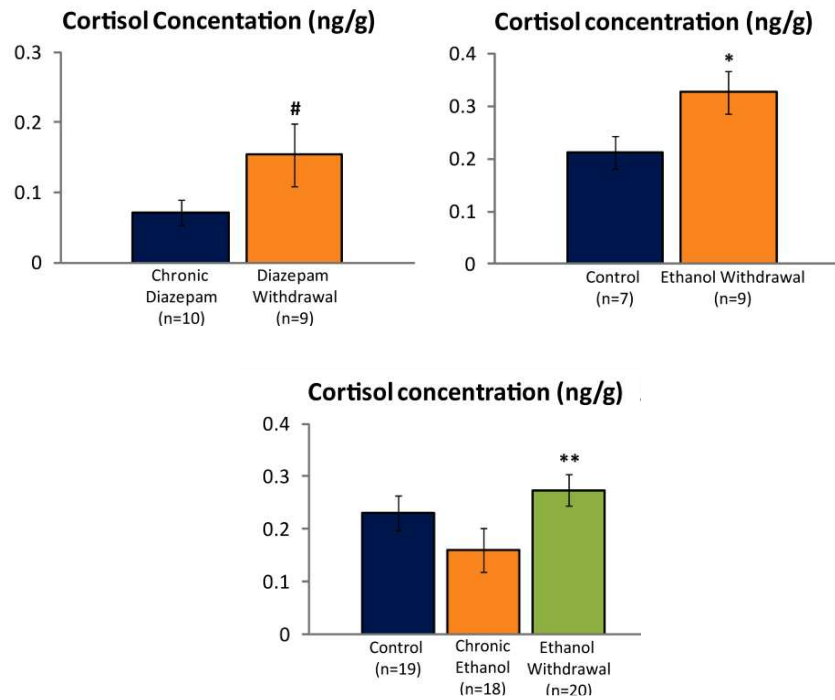
#### ***Larval vs. adult zebrafish research***

Larval zebrafish have emerged as a popular model for a number of brain pathologies. Larvae display learning, sleep, drug addiction, and other quantifiable neurobehavioral phenotypes (Best & Alderton, 2008). Another advantage of using zebrafish larva is the ability to study multiple animals simultaneously within a high-throughput battery (Best & Alderton, 2008; Best et al., 2008; Creton, 2009). However, such models have some limitations, since they do not exhibit the rich behavior of the adult animals (e.g., Creton, 2009, Fig. 1). Also, larval models have somewhat limited developmental applications, for example, lacking fully established mediatory and endocrine systems (Kimmel, Ballard, Kimmel, Ullmann, & Schilling, 1995), as well as some neural circuits and projections (Kastenhuber, Kratochwil, Ryu, Schweitzer, & Driever, 2010). Likewise, behavioral endpoints observed in larval animals may not be fully translated (or have good homology) to adult subjects’ behavior. Thus, larval research is unable to fully replace the adult zebrafish studies. This notion is important, since zebrafish neuroscientists sometimes remind us of Montekki and Capuletti in the larval vs. adult zebrafish dilemma. Although testing multiple adult fish simultaneously may be an interim solution, a better strategy may be to accept both approaches and use them complementarily to advance zebrafish research.

#### ***Thinking outside the brain: endocrine responses to stress in zebrafish***

Thinking outside of the traditional “box” is important in experimental modeling of brain disorders (Kalueff, LaPorte, Murphy, & Sufka, 2008; Kalueff, Wheaton, & Murphy, 2007). Here we argue that focusing on bodily processes, in addition to pure brain mechanisms, may be a fruitful direction of zebrafish biobehavioral research. The hypothalamic-pituitary-adrenal (HPA) axis mediates the endocrine response to stress in humans and mammals (Alsop & Vijayan, 2008). Under stress, the paraventricular nucleus of the hypothalamus produces

corticotropin releasing factor (CRF), which is delivered to the anterior pituitary gland via the hypothalamic-hypophysial portal blood vessel system (Suzuki, Kawasaki, Ohnishi, Nakamura, & Ueta, 2009). CRF stimulates the anterior pituitary gland, causing release of adrenocorticotrophic hormone (ACTH) into the blood stream (Tsigos & Chrousos, 2002). When stimulated by ACTH, the adrenal cortex synthesizes glucocorticoid hormones that modulate the stress reaction (Dedovic, Duchesne, Andrews, Engert, & Pruessner, 2009; Pruessner et al., 2010).



**Figure 4.** Zebrafish endocrine responses (whole-body cortisol, ng/g fish) to withdrawal from diazepam and ethanol. Left to right then down: 72-h withdrawal from chronic diazepam (72  $\mu\text{g}/\text{mL}$ , 2 weeks); 12-h withdrawal from chronic ethanol (0.3%, 1 week); chronic ethanol exposure (0.3%, 1 week) and 12-h withdrawal from chronic ethanol (0.3%, 1 week). Data are presented as mean  $\pm$  SEM (\* $p < 0.05$ , \*\* $p < 0.01$ , # $p = 0.05$ -0.1, trend, U-test).

A similar evolutionarily conserved mechanism has been found in zebrafish (To et al., 2007), whose hypothalamus-pituitary-interrenal (HPI) axis is homologous to the HPA axis. With cortisol as the main mediator of the physiological response to stress (Winberg, Nilsson, Hylland, Soderstrom, & Nilsson, 1997), zebrafish may be an excellent model for endocrine research (Winberg et al., 1997). Figure 4 summarizes recent data generated by our lab from a series of experiments evoking strong anxiety in zebrafish. The consistency of increased whole-body cortisol concentrations following stressful stimuli is in line with behavioral data gathered in these and previous studies (Egan et al., 2009a).

Such ability to parallel physiological responses with behavioral phenotypes provides researchers with an important tool for investigating stress-related phenomena.

### ***Neurodegenerative disorders***

The two key endophenotypes of Alzheimer's disease (AD) include a buildup of amyloid-beta plaques in the nervous system, and a parallel production of uncoordinated meshwork of neurofibrillary tangles caused by damaged Tau protein (Best & Alderton, 2008; Paquet et al., 2009). As suggested, these neuronal damages can lead to memory impairment, and have specifically been noted in 50% of patients with dementia (Vandenberghe & Tournoy, 2005). Therefore, it is possible that learning and memory acquisition in zebrafish can be effectively modeled based on amyloid-beta plaques and tangled neuron formation.

Paradigms which use ASR, raised platform, or T-maze arenas can assess learning and memory capabilities in fish (Best & Alderton, 2008), including tauopathic zebrafish (Barut & Zon, 2000; Paquet et al., 2009) highly relevant to AD. Likewise, Parkinson's disease (PD), the most common movement disorder in humans, is also well-studied in zebrafish (Paquet, Schmid, & Haass, 2006; Shankaran, Schmid, & Kahle, 2006). In addition, various PD-inducing drugs have also been evaluated in both larval and adult zebrafish (e.g., Guo, 2009).

### ***Addiction***

Recent analyses of gene expression changes following acute or chronic exposure to drugs of abuse (Kily et al., 2008), have established the genetic correlates of addiction. For example, chronic treatment of zebrafish with ethanol and nicotine alters the expression of multiple CNS genes, some of which have been identified as components of the addiction pathways in mammals (Kily et al., 2008). Further evidence also suggests the sensitivity of zebrafish to drug withdrawal, which is the cornerstone of addictive behavior (Cachat et al., 2009).

For instance, ethanol discontinuation disrupts zebrafish shoaling behavior (Gerlai, Chatterjee, Pereira, Sawashima, & Krishnannair, 2009), while cocaine withdrawal evokes marked alterations in their locomotion (Lopez-Patino et al., 2008; Lopez Patino, Yu, Yamamoto, & Zhdanova, 2008). Our laboratory has demonstrated that withdrawal also modulates zebrafish cortisol levels (Fig. 4), implicating their cortisol abnormalities as a phenotype (Cachat et al., 2009) consistent with glucocorticoid dysregulations in human and rodent withdrawal syndrome (Borlikova, Le Merrer, & Stephens, 2006; Keedwell, Poon, Papadopoulos, Marshall, & Checkley, 2001; Lovallo, 2006; Rabbani, Hajhashemi, & Mesripour, 2009).

## **New Potential Applications of Zebrafish Models**

### ***Serotonin syndrome***

As the clinical use of selective serotonin reuptake inhibitors (SSRIs) is rapidly increasing, their toxicity also becomes a serious biomedical problem. Serotonin syndrome (SS) is a severe adverse drug interaction characterized by altered mental status, autonomic dysfunction, and neuromuscular abnormalities. SS symptoms can include agitation, delirium, coma, mydriasis, diaphoresis, hyperthermia, tachycardia, fluctuating blood pressure, tremors, rigidity, myoclonus, and seizures. Although mild cases of the condition usually resolve within 24-72 h (Martin, 1996), SS is particularly difficult to diagnose, and may have a rapid development (Boyer & Shannon, 2005). While SS has been previously modeled in rodents (Fox, Jensen, Gallagher, & Murphy, 2007; Gingrich & Hen, 2001; Kalueff, Fox, Gallagher, & Murphy, 2007), this condition has not been assessed in zebrafish, although they also have a well-developed serotonergic system.

Here we suggest that when attempting to model SS in zebrafish, we may need to select behavioral (e.g., anxiety, immobility) endpoints, and focus on the toxic effects of a combination of several serotonergic drugs (since a single drug may not reliably and effectively induce SS). For instance, a monoamine oxidase A inhibitor administered in conjunction with an SSRI can be expected to induce SS-like states in zebrafish, similar to the SS-like states that the two drugs would evoke in mice or humans. Agents that can be expected to achieve this result include fluoxetine, tranylcypromine, olanzapine or clomipramine. In parallel to behavioral abnormalities, endocrine and/or neurochemical (e.g., brain serotonin levels) endophenotypes can be assessed to more fully mimic fish SS-like behaviors.

### ***Depression***

Though attempts to model depression in zebrafish have so far been non-existent, some attempts have been made to model bipolar depression in mice. One such model is based on administration of one drug followed by another drug causing the opposing behavioral effects. For example, a single administration of psychostimulants such as amphetamine or methamphetamine causes hyperactivity, which is then used to test the efficacy of anti-manic treatments such as lithium and valproate. Furthermore, behavioral sensitization by repeated administration of psychostimulants such as amphetamine, methamphetamine, and cocaine has also been used as a model of bipolar disorder in mice (Kato, Kubota, & Kasahara, 2007). Since repeated exposure to cocaine can induce an oscillation or cycling in a variety of neurochemical and physiological systems (Antelman et al., 1998), we suggest that it may also be possible to evoke “bipolar” behavior in zebrafish, for example, by using a combination of cocaine and anti-psychotic agents.

### ***LPS response and sickness behavior***

Cytokine-mediated sickness behavior is an animal syndrome that includes decreased locomotor activity, inhibited exploration of their physical and social environment, reduced food and water intake, and impaired learning and memory (Dantzer, 2001). Notably, zebrafish possess a wide array of cytokines, similar to humans and mice (Lieschke, 2001). Drawing on already established knowledge that bacterial lipopolysaccharide (LPS) is capable of inducing sickness behavior in zebrafish via the induction of pro-inflammatory cytokines (Henry et al., 2008), LPS exposure may possibly serve as a model for sickness behavior in zebrafish.

The inflammatory response is initiated by the uptake of bacteria and their products by the cells of the innate immune system, which, in turn, continues with various mechanisms, including the elevation of cytokines and/or chemokines such as TNF- $\alpha$ , IL-1, IL-6, and IL-8 (Decker, 2004). While affective pathogenesis is attributed to various exogenous stressors (Nemeroff, 2007; Nutt, 2000), recent studies have directly linked affective disorders with various cytokines (Asberg et al., 2009; Hoge, Brandstetter, Moshier, Pollack, Wong, & Simon, 2009; Jonsdottir, Hagg, Glise, & Ekman, 2009; Lu, Jensen, Huang, Kealey, Blair, & Whitehead, 2009). Therefore, the induction of a cytokine response via LPS in zebrafish may produce a promising model of cytokine-mediated behavioral syndromes.

### ***Schizophrenia and autism***

Traditional methods of modeling schizophrenia in animals involve dopamine agonist-mediated hyperactivity, and measuring the responses to antipsychotic dopaminergic antagonists, including the elevated levels of dopamine metabolites, dihydroxyphenylacetic acid, and homovanilic acid (found in the cerebral spinal fluid and urine of human patients). We suggest the utilization of the same antipsychotic drugs administered to rodents (such as haloperidol, clozapine, risperidone, and olanzapine), could produce similar behavioral responses in zebrafish. Clearly, more models of schizophrenia in zebrafish are needed. The only current model to date was developed through Burgess and Granato's successful use of sensory gating, in which it was demonstrated that antipsychotic drugs can suppress disruptions in zebrafish PPI induced by dopamine agonists (Burgess & Granato, 2007).

Many of behavioral abnormalities associated with autism spectrum disorders (ASDs) are inherently difficult to model in animals. However, previous experimentation suggests that it is possible to model defects in social interaction in zebrafish, as well as the developmental and cellular defects that correspond to such symptomology. For instance, zebrafish homologues of the genes implicated in ASDs (such as *neurexins*, *reelin*, *mecp2*, and *met*) have been identified, and assays that measure social interaction have been developed (Colman, Baldwin, Johnson, & Scholz, 2009). Thus, like mice, but empowered by the ease of genetic manipulations, zebrafish may lead to new experimental and genetic models relevant to ASD.

## Conclusions

With an organism as promising as the zebrafish, it is imperative to develop new models and to refine and expand upon current models in order to reflect this species' full potential. In addition to establishing new models and paradigms, adding new behavioral endpoints and using novel observation methods, such as automated video-tracking systems, will bolster the utility of zebrafish in neurobehavioral research. Using biomolecular markers (such as gene expression or endocrine measures) to parallel zebrafish physiology with behavioral data is another important direction of research. Finally, expanding the area of zebrafish research by including cross-domain modeling (e.g., drug withdrawal/anxiety), new disorders (e.g., ASD, schizophrenia), and new pathways (e.g., brain genes, central and peripheral cytokines) may lead to new translational models using both larval and adult zebrafish.

## References

- Alsop, D., & Vijayan, M. M. (2008). Development of the corticosteroid stress axis and receptor expression in zebrafish. *American Journal of Physiology Regulatory Integrative and Comparative Physiology*, *294*, R711-R719.
- Antelman, S. M., Caggiula, A. R., Kucinski, B. J., Fowler, H., Gershon, S., Edwards, D. J., et al. (1998). The effects of lithium on a potential cycling model of bipolar disorder. *Progress in Neuro-psychopharmacology & Biological Psychiatry*, *22*, 495-510.
- Asberg, M., Nygren, A., Leopardi, R., Rylander, G., Peterson, U., Wilczek, L., et al. (2009). Novel biochemical markers of psychosocial stress in women. *PLoS One*, *4*, e3590.
- Barut, B. A., & Zon, L. I. (2000). Realizing the potential of zebrafish as a model for human disease. *Physiological Genomics*, *2*, 49-51.
- Becker, C., Zeau, B., Rivat, C., Blugeot, A., Hamon, M., & Benoliel, J. J. (2008). Repeated social defeat-induced depression-like behavioral and biological alterations in rats: Involvement of cholecystokinin. *Molecular Psychiatry*, *13*, 1079-1092.
- Best, J. D., & Alderton, W. K. (2008). Zebrafish: An in vivo model for the study of neurological diseases. *Neuropsychiatric Disease & Treatment*, *4*, 567-576.
- Best, J. D., Berghmans, S., Hunt, J. J., Clarke, S. C., Fleming, A., Goldsmith, P., et al. (2008). Non-associative learning in larval zebrafish. *Neuropsychopharmacology*, *33*, 1206-1215.
- Bjorkqvist, K. (2001). Social defeat as a stressor in humans. *Physiology & Behavior*, *73*, 435-442.
- Blaser, R., & Gerlai, R. (2006). Behavioral phenotyping in zebrafish: Comparison of three behavioral quantification methods. *Behavior Research Methods*, *38*, 456-469.
- Blaser, R. E., Chadwick, L., & McGinnis, G. C. (2010). Behavioral measures of anxiety in zebrafish (*Danio rerio*). *Behavioural Brain Research*, *208*, 56-62.
- Bolivar, V. J. (2009). Intrasession and intersession habituation in mice: From inbred strain variability to linkage analysis. *Neurobiology of Learning & Memory*, *92*, 206-214.
- Borlikova, G. G., Le Merrer, J., & Stephens, D. N. (2006). Previous experience of ethanol withdrawal increases withdrawal-induced c-fos expression in limbic areas, but not

- withdrawal-induced anxiety and prevents withdrawal-induced elevations in plasma corticosterone. *Psychopharmacology* (Berl), 185, 188-200.
- Bourin, M., & Hascoet, M. (2003). The mouse light/dark box test. *European Journal of Pharmacology*, 463, 55-65.
- Boyer, E. W., & Shannon, M. (2005). Medical biology: On the serotonin syndrome. *New England Journal of Medicine*, 352, 1112.
- Braubach, O. R., Wood, H. D., Gadbois, S., Fine, A., & Croll, R. P. (2009). Olfactory conditioning in the zebrafish (*Danio rerio*). *Behavioural Brain Research*, 198, 190-198.
- Burgess, H. A., & Granato, M. (2007). Sensorimotor gating in larval zebrafish. *The Journal of Neuroscience*, 27, 4984-4994.
- Burt de Perera, T. (2004). Fish can encode order in their spatial map. *Proceedings of the Royal Society of London B*, 272, 4.
- Cachat, J., Canavello, P., Elegante, M., Bartels, B., Hart, P., Bergner, C., et al. (2009). Modeling withdrawal syndrome in zebrafish. *Behavioural Brain Research*. (doi: 10.1016/j.bbr.2009.12.004).
- Carola, V., D'Olimpio, F., Brunamonti, E., Mangia, F., & Renzi, P. (2002). Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. *Behavioural Brain Research*, 134, 49-57.
- Choleris, E., Thomas, A. W., Kavaliers, M., & Prat, F. S. (2001). A detailed ethological analysis of the mouse open field test: Effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. *Neuroscience & Biobehavioral Reviews*, 25, 235-260.
- Colman, J. R., Baldwin, D., Johnson, L. L., & Scholz, N. L. (2009). Effects of the synthetic estrogen, 17alpha-ethinylestradiol, on aggression and courtship behavior in male zebrafish (*Danio rerio*). *Aquatic Toxicology*, 91, 346-354.
- Creton, R. (2009). Automated analysis of behavior in zebrafish larvae. *Behavioural Brain Research*, 203, 127-136.
- Dantzer, R. (2001). Cytokine-induced sickness behavior: Where do we stand? *Brain, Behavior, & Immunity*, 15, 7-24.
- Decker, T. (2004). Sepsis: Avoiding its deadly toll. *The Journal of Clinical Investigation*, 113, 1387-1389.
- Dedovic, K., Duchesne, A., Andrews, J., Engert, V., & Pruessner, J. C. (2009). The brain and the stress axis: The neural correlates of cortisol regulation in response to stress. *Neuroimage*, 47, 864-871.
- Eddins, D., Cerutti, D., Williams, P., Linney, E., & Levin, E. D. (2009). Zebrafish provide a sensitive model of persisting neurobehavioral effects of developmental chlorpyrifos exposure: Comparison with nicotine and pilocarpine effects and relationship to dopamine deficits. *Neurotoxicology & Teratology*, 32, 99-108.
- Egan, R., Bergner, C., Hart, R., Cachat, J., Canavello, P., Elegante, M., et al. (2009a). Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. *Behavioural Brain Research*, 205, 38-44.
- Egan, R. J., Bergner, C. L., Hart, P. C., Cachat, J. M., Canavello, P. R., Elegante, M. F., et al. (2009b). Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. *Behavioural Brain Research*, 205, 38-44.
- File, S. E., & Mabbutt, P. S. (1990). Long-lasting effects on habituation and passive avoidance performance of a period of chronic ethanol administration in the rat. *Behavioural Brain Research*, 36, 171-178.

- Fox, M. A., Jensen, C. L., Gallagher, P. S., & Murphy, D. L. (2007). Receptor mediation of exaggerated responses to serotonin-enhancing drugs in serotonin transporter (SERT)-deficient mice. *Neuropharmacology*, *53*, 643-656.
- Gerlai, R. (2005, August). *Event recording and video-tracking: Towards the development of high throughput zebrafish screens*. Paper presented at Measuring Behavior 2005, 5<sup>th</sup> International Conference on Methods and Techniques in Behavioral Research, Wageningen, The Netherlands.
- Gerlai, R. (2003). Zebra fish: An uncharted behavior genetic model. *Behavior Genetics*, *33*, 461-468.
- Gerlai, R., Chatterjee, D., Pereira, T., Sawashima, T., & Krishnannair, R. (2009). Acute and chronic alcohol dose: Population differences in behavior and neurochemistry of zebrafish. *Genes, Brain, & Behavior*, *8*, 586-599.
- Gingrich, J. A., & Hen, R. (2001). Dissecting the role of the serotonin system in neuropsychiatric disorders using knockout mice. *Psychopharmacology (Berl)*, *155*, 1-10.
- Goldsmith, P. (2004). Zebrafish as a pharmacological tool: The how, why and when. *Current Opinion in Pharmacology*, *4*, 504-512.
- Guo, S. (2009). Using zebrafish to assess the impact of drugs on neural development and function. *Expert Opinion on Drug Discovery*, *4*, 715-726.
- Henry, C. J., Huang, Y., Wynne, A., Hanke, M., Himler, J., Bailey, M. T., et al. (2008). Minocycline attenuates lipopolysaccharide (LPS)-induced neuroinflammation, sickness behavior, and anhedonia. *Journal of Neuroinflammation*, *5*, 15.
- Hoge, E. A., Brandstetter, K., Moshier, S., Pollack, M. H., Wong, K. K., & Simon, N. M. (2009). Broad spectrum of cytokine abnormalities in panic disorder and posttraumatic stress disorder. *Depression & Anxiety*, *26*, 447-455.
- Jonsdottir, I. H., Hagg, D. A., Glise, K., & Ekman, R. (2009). Monocyte chemotactic protein-1 (MCP-1) and growth factors called into question as markers of prolonged psychosocial stress. *PLoS One*, *4*, e7659.
- Kalueff, A. V., Fox, M. A., Gallagher, P. S., & Murphy, D. L. (2007). Hypolocomotion, anxiety and serotonin syndrome-like behavior contribute to the complex phenotype of serotonin transporter knockout mice. *Genes, Brain & Behavior*, *6*, 389-400.
- Kalueff, A. V., LaPorte, J. L., Murphy, D. L., & Sufka, K. (2008). Hybridizing behavioral models: A possible solution to some problems in neurophenotyping research? *Progress in Neuro-psychopharmacology & Biological Psychiatry*, *32*, 1172-1178.
- Kalueff, A. V., Wheaton, M., & Murphy, D. L. (2007). What's wrong with my mouse model? Advances and strategies in animal modeling of anxiety and depression. *Behavioural Brain Research*, *179*, 1-18.
- Kastenhuber, E., Kratochwil, C. F., Ryu, S., Schweitzer, J., & Driever, W. (2010). Genetic dissection of dopaminergic and noradrenergic contributions to catecholaminergic tracts in early larval zebrafish. *The Journal of Comparative Neurology*, *518*, 439-458.
- Kato, T., Kubota, M., & Kasahara, T. (2007). Animal models of bipolar disorder. *Neuroscience & Biobehavioral Reviews*, *31*, 832-842.
- Keedwell, P. A., Poon, L., Papadopoulos, A. S., Marshall, E. J., & Checkley, S. A. (2001). Salivary cortisol measurements during a medically assisted alcohol withdrawal. *Addiction Biology*, *6*, 247-256.
- Kily, L. J., Cowe, Y. C., Hussain, O., Patel, S., McElwaine, S., Cotter, F. E., et al. (2008). Gene expression changes in a zebrafish model of drug dependency suggest

- conservation of neuro-adaptation pathways. *Journal of Experimental Biology*, 211, 1623-1634.
- Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B., & Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Developmental Dynamics*, 203, 253-310.
- Koch, M. (1999). The neurobiology of startle. *Progress in Neurobiology*, 59, 107-128.
- Koolhaas, J. M., De Boer, S. F., De Rutter, A. J., Meerlo, P., & Sgoifo, A. (1997). Social stress in rats and mice. *Acta Physiologica Scandinavica. Supplementum*, 640, 69-72.
- Koplik, E. V., Salieva, R. M., & Gorbunova, A. V. (1995). [The open-field test as a prognostic criterion of resistance to emotional stress in Wistar rats]. *Zhurnal Vyssheĭ Nervnoĭ Deiatelnosti Imeni I P Pavlova*, 45, 775-781.
- Larson, E. T., O'Malley, D. M., & Melloni, R. H. (2006). Aggression and vasotocin are associated with dominant-subordinate relationships in zebrafish. *Behavioural Brain Research*, 167, 94-102.
- Leimer, U., Lun, K., Romig, H., Walter, J., Grunberg, J., Brand, M., et al. (1999). Zebrafish (*Danio rerio*) presenilin promotes aberrant amyloid beta-peptide production and requires a critical aspartate residue for its function in amyloidogenesis. *Biochemistry*, 38, 13602-13609.
- Leussis, M. P., & Bolivar, V. J. (2006). Habituation in rodents: A review of behavior, neurobiology, and genetics. *Neuroscience & Biobehavioral Reviews*, 30, 1045-1064.
- Levin, E. D., Aschner, M., Heberlein, U., Ruden, D., Welsh-Bohmer, K. A., Bartlett, S., et al. (2009). Genetic aspects of behavioral neurotoxicology. *Neurotoxicology*, 30, 741-753.
- Levin, E. D., Bencan, Z., & Cerutti, D. T. (2007). Anxiolytic effects of nicotine in zebrafish. *Physiology & Behavior*, 90, 54-58.
- Lieschke, G. J. (2001). Zebrafish--an emerging genetic model for the study of cytokines and hematopoiesis in the era of functional genomics. *International Journal of Hematology*, 73, 23-31.
- Lockwood, B., Bjerke, S., Kobayashi, K., & Guo, S. (2004). Acute effects of alcohol on larval zebrafish: A genetic system for large-scale screening. *Pharmacology, Biochemistry, & Behavior*, 77, 647-654.
- Lopez-Patino, M. A., Yu, L., Cabral, H., & Zhdanova, I. V. (2008). Anxiogenic effects of cocaine withdrawal in zebrafish. *Physiology & Behavior*, 93, 160-171.
- Lopez Patino, M. A., Yu, L., Yamamoto, B. K., & Zhdanova, I. V. (2008). Gender differences in zebrafish responses to cocaine withdrawal. *Physiology & Behavior*, 95, 36-47.
- Lovallo, W. R. (2006). Cortisol secretion patterns in addiction and addiction risk. *International Journal of Psychophysiology*, 59, 195-202.
- Lu, Z. Y., Jensen, L. E., Huang, Y., Kealey, C., Blair, I. A., & Whitehead, A. S. (2009). The up-regulation of monocyte chemoattractant protein-1 (MCP-1) in Ea.hy 926 endothelial cells under long-term low folate stress is mediated by the p38 MAPK pathway. *Atherosclerosis*, 205, 48-54.
- MacPhail, R. C., Brooks, J., Hunter, D. L., Padnos, B., Irons, T. D., & Padilla, S. (2009). Locomotion in larval zebrafish: Influence of time of day, lighting and ethanol. *Neurotoxicology*, 30, 52-58.
- Martin, T. G. (1996). Serotonin syndrome. *Annals of Emergency Medicine*, 28, 520-526.
- Muller, U., Cristina, N., Li, Z. W., Wolfer, D. P., Lipp, H. P., Rulicke, T., et al. (1994). Behavioral and anatomical deficits in mice homozygous for a modified beta-amyloid precursor protein gene. *Cell*, 79, 755-765.

- Musiek, F. E. (2003). What can the acoustic startle reflex tell us? *The Hearing Journal*, *56*, 55.
- Nemeroff, C. B. (2007). The burden of severe depression: A review of diagnostic challenges and treatment alternatives. *Journal of Psychiatric Research*, *41*, 189-206.
- Ninkovic, J., & Bally-Cuif, L. (2006). The zebrafish as a model system for assessing the reinforcing properties of drugs of abuse. *Methods*, *39*, 262-274.
- Nutt, D. (2000). Treatment of depression and concomitant anxiety. *European neuropsychopharmacology*, *10*, S433-437.
- Ohl, F., Roedel, A., Storch, C., Holsboer, F., & Landgraf, R. (2002). Cognitive performance in rats differing in their inborn anxiety. *Behavioral Neuroscience*, *116*, 464-471.
- Paquet, D., Bhat, R., Sydow, A., Mandelkow, E. M., Berg, S., Hellberg, S., et al. (2009). A zebrafish model of tauopathy allows in vivo imaging of neuronal cell death and drug evaluation. *The Journal of Clinical Investigation*, *119*, 1382-1395.
- Paquet, D., Schmid, B., & Haass, C. (2006, June). *Analysis of the function of the Parkinson's disease gene pink1 in zebrafish*. 7<sup>th</sup> International Conference on Zebrafish Development and Genetics, Madison, Wisconsin, USA.
- Platel, A., & Porsolt, R. D. (1982). Habituation of exploratory activity in mice: A screening test for memory enhancing drugs. *Psychopharmacology (Berl)*, *78*, 346-352.
- Pruessner, J. C., Dedovic, K., Pruessner, M., Lord, C., Buss, C., Collins, L., et al. (2010). Stress regulation in the central nervous system: Evidence from structural and functional neuroimaging studies in human populations - 2008 Curt Richter Award Winner. *Psychoneuroendocrinology*, *35*, 179-191.
- Rabbani, M., Hajhashemi, V., & Mesripour, A. (2009). Increase in brain corticosterone concentration and recognition memory impairment following morphine withdrawal in mice. *Stress*, *12*, 451-456.
- Riedel, G. (1998). Long-term habituation to spatial novelty in blind cave fish (*Astyanax hubbsi*): Role of the telencephalon and its subregions. *Learning & Memory*, *4*, 451-461.
- Rigdon, G. C., & Weatherspoon, J. K. (1992). 5-Hydroxytryptamine 1a receptor agonists block prepulse inhibition of acoustic startle reflex. *The Journal of Pharmacology & Experimental Therapeutics*, *263*, 486-493.
- Salomons, A. R., van Luijk, J. A., Reinders, N. R., Kirchhoff, S., Arndt, S. S., & Ohl, F. (2009). Identifying emotional adaptation: Behavioural habituation to novelty and immediate early gene expression in two inbred mouse strains. *Genes, Brain, & Behavior*, *9*, 1-10.
- Serra, E. L., Medalha, C. C., & Mattioli, R. (1999). Natural preference of zebrafish (*Danio rerio*) for a dark environment. *Brazilian Journal of Medical and Biological Research*, *32*, 1551-1553.
- Shankaran, S., Schmid, B., & Kahle, P. (2006, June). *Functional analysis of Parkinson's disease gene LRRK2 in zebrafish*. 7<sup>th</sup> International Conference on Zebrafish Development and Genetics, Madison, Wisconsin, USA.
- Shin, J. T., & Fishman, M. C. (2002). From zebrafish to human: Modular medical models. *Annual Review of Genomics and Human Genetics*, *3*, 311-340.
- Suzuki, H., Kawasaki, M., Ohnishi, H., Nakamura, T., & Ueta, Y. (2009). Regulatory mechanism of the arginine vasopressin-enhanced green fluorescent protein fusion gene expression in acute and chronic stress. *Peptides*, *30*, 1763-1770.
- Thompson, R. F., & Spencer, W. A. (1966). Habituation: A model phenomenon for the study of neuronal substrates of behavior. *Psychological Review*, *73*, 16-43.

- To, T. T., Hahner, S., Nica, G., Rohr, K. B., Hammerschmidt, M., Winkler, C., et al. (2007). Pituitary-interrenal interaction in zebrafish interrenal organ development. *Molecular Endocrinology*, *21*, 472-485.
- Tsigos, C., & Chrousos, G. P. (2002). Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *Journal of Psychosomatic Research*, *53*, 865-871.
- Vandenberghe, R., & Tournoy, J. (2005). Cognitive aging and Alzheimer's disease. *Postgraduate Medical Journal*, *81*, 343-352.
- Walsh, R. N., & Cummins, R. A. (1976). The open-field test: A critical review. *Psychological Bulletin*, *83*, 482-504.
- Winberg, S., Nilsson, A., Hylland, P., Soderstrom, V., & Nilsson, G. E. (1997). Serotonin as a regulator of hypothalamic-pituitary-interrenal activity in teleost fish. *Neuroscience Letters*, *230*, 113-116.
- Wong, K., Elegante, M., Bartels, B., Elkhayat, S., Tien, D., Roy, S., et al. (2009). Analyzing habituation responses to novelty in zebrafish (*Danio rerio*). *Behavioral Brain Research*. doi:10.1016/j.bbr.2010.01.031
- Zon, L. I., & Peterson, R. T. (2005). In vivo drug discovery in the zebrafish. *Nature Reviews Drug Discovery*, *4*, 35-44.