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Recent advances with a novel model organism: Alcohol tolerance and sensitization in zebrafish (*Danio rerio*)

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Abstract

Alcohol abuse and dependence is a rapidly growing problem with few treatment options available. The zebrafish has become a popular animal model for behavioural neuroscience. This species may be appropriate for investigating the effects of alcohol on the vertebrate brain. In the current review, we examine the literature by discussing how alcohol alters behaviour in zebrafish and how it may affect biological correlates. We focus on two phenomena that are often examined in the context of alcohol-induced neuroplasticity. Alcohol tolerance (a progressive decrease in the effect of alcohol over time) is often observed following continuous (chronic) exposure to low concentrations of alcohol. Alcohol sensitization also called reverse tolerance (a progressive increase in the effect of alcohol over time) is often observed following repeated discrete exposures to higher concentrations of alcohol. These two phenomena may underlie the development and maintenance of alcohol addiction. The phenotypical characterization of these responses in zebrafish may be the first important steps in establishing this species as a tool for the analysis of the molecular and neurobiological mechanisms underlying human alcohol addiction.

Keywords

alcohol tolerance; alcohol sensitisation; alcohol addiction; ethanol; behavioural phenotyping; zebrafish

Introduction

Alcohol (ethanol or ethyl alcohol) continues to be a popular recreational drug, with binge drinking and abuse becoming an ever increasing problem (Naimi et al., 2003; Kanny et al., 2011). Recent reports suggest the lifetime prevalence for alcohol dependence can be as high as 17% in the United States (Brett et al., In press). Previous reports have identified over 20

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million people in the US alone who met the diagnosis for alcohol abuse or dependence over a 12 month period (Grant et al., 2004). The economic cost of alcohol abuse is staggering with billions of dollars in loss of revenue (Goetz et al., 2003; Rice 1995). Due to the complexity of how alcohol interacts with the central nervous system, treatment options for alcohol addiction currently remain limited (Vengeliene et al., 2008; Hyman & Malenka, 2006).

Repeated and long-term consumption of alcohol is associated with the development of addiction in humans. Animal models have been employed to examine the underlying neural mechanisms which may mediate alcohol dependence (Kaun et al., 2011; Spanagel, 2003). Laboratory animal species such as the mouse, rat, and fruit fly have been used to model alcohol-dependent behaviours such as conditioned place preference and alcohol-induced withdrawal symptoms (Spanagel, 2010; Crabbe & Phillips, 2004; Crabbe et al., 2010; Kaun et al., 2011). Two neuroplastic changes that are commonly examined following long-term alcohol use are the development of tolerance and sensitization (reverse tolerance) (Hyman & Malenka, 2001; Spanagel, 2010). The development of alcohol tolerance in humans is commonly observed following chronic and long-term consumption, such that individuals require larger quantities to achieve the same desired effects (Bennet et al., 1993; King et al., 2002), which may lead to excessive drinking. Sensitization is thought to increase the motivational value of obtaining alcohol by reinforcing drug seeking tendencies (see Berridge & Robinson, 1998; Leyton, 2007) and is associated with alcohol addiction in humans (Newlin & Thomson, 1991; 1999). These two phenomena may prove to be essential components regarding the development and maintenance of alcohol dependence. Despite the available research tools and scientific progress over the past several decades, the mechanisms underlying alcohol's actions *in vivo* and alcohol-induced neuroplasticity (i.e. tolerance and sensitization) remain poorly understood (Hyman & Malenka, 2006; Vengeliene et al., 2008). The establishment of novel animal models for alcohol sensitization and tolerance may facilitate the investigation of such underlying mechanism.

Zebrafish as an *in vivo* animal model for alcohol research

Zebrafish have been at the forefront of developmental biology for the past decade (see Grunwald & Eisen, 2003). Researchers have taken advantage of this small vertebrate's developmental transparency to examine structural and anatomical alterations following embryonic alcohol exposure (Biolota et al., 2002; Carven et al., 2004). Adult zebrafish have also been used in the study of addiction, including cocaine (Lopez Patino et al., 2008), amphetamine (Kyzar et al., 2013), nicotine (Miller et al., 2013), and alcohol (Gerlai et al., 2000; Echevarria et al., 2011). In particular, zebrafish have been used to examine the molecular mechanisms underlying alcohol's actions *in vivo* (Gerlai et al., 2000; Cachat et al., 2010; Kily et al., 2008; Mathur & Guo, 2010). Zebrafish have been suggested to be an appropriate animal model to study alcohol addiction for several reasons. First, it is a simple vertebrate with high enough psychopharmacological similarities as compared to rodents and humans (Gerlai et al., 2000; Kily et al., 2008; Rico et al., 2011b). Second, it possesses a number of practical advantages including small size, reasonably short generation time, and very high fecundity, optimal features for high throughput mutagenesis (i.e. forward genetics) as well as small molecule screening (see Sison & Gerlai, 2010). Third, its genome has been

entirely sequenced and high nucleotide sequence homologies have been identified between its genes and human genes (Barbazuk et al., 2000; Klee et al., 2012). Finally, and from our perspective perhaps most importantly, drug delivery in this species is often simple and non-invasive: water soluble drugs including alcohol can be mixed directly into the tank water and the fish can be immersed and maintained in the drug solution (Gerlai et al., 2000). We also emphasize that increasing the knowledge about alcohol's mechanisms in multiple species will allow comparative approaches which could identify evolutionarily conserved mechanisms across fish, rodents, and humans, for example. These conserved mechanisms are likely most important to the core aspects of alcohol-induced functional changes of the brain, including sensitization and tolerance.

Alcohol-induced alterations in zebrafish have been reported following one of two exposure regimens. One approach is to house animals in low concentrations of alcohol for extended periods of time to examine adaptation related changes that ensue (Gerlai et al., 2000; 2009; Tran & Gerlai, 2013). The second approach involves repeated discrete, i.e. intermittent, exposures that modify the baseline response. For example, repeated exposures to higher concentrations of alcohol have been shown to induce behavioural sensitization (Blaser et al., 2010) and conditioned place preference in zebrafish (Kily et al., 2008; Mathur et al., 2011). We will first review the literature on how different concentrations of alcohol alter zebrafish behaviour (acute exposure). Next we will examine how prolonged continuous alcohol immersion (chronic exposure) and repeated intermittent alcohol exposure regimens may alter the response to the substance. We will also discuss potential underlying mechanisms that have recently been reported in the literature.

Acute alcohol exposure in zebrafish

Alcohol is known to act on the mammalian central nervous system in a biphasic manner. It initially acts as a stimulant/anxiolytic as blood and brain alcohol concentrations begin to rise. However, as alcohol levels continue to increase in the brain, the effects become depressant/anxiogenic-like (Koob, 2004; Erblich et al., 2003). In zebrafish, the biphasic effect has also been demonstrated and has been found to be dependent on both concentration and duration of exposure (Tran & Gerlai, 2013; Pannia et al., 2014; Rosemberg et al. 2012). Multiple studies have examined the effects of low to moderate concentrations of alcohol often ranging from 0.1 to 0.5 % v/v on zebrafish behaviour (Tran & Gerlai, 2013; Dlugos & Rabin, 2003; Egan et al., 2009; Wong et al., 2010). These low doses, which have been reported to fall within the range of alcohol consumption in humans (Dlugos & Rabin, 2003; Rosemberg et al., 2012), exert stimulatory effects on zebrafish locomotor activity quantified as increased distance travelled, velocity, or number of zones crossed (Blaser & Penalosa, 2011; Gerlai et al., 2000; Rosemberg et al., 2012; Tran & Gerlai, 2013a). Anxiolytic effects are also observed in response to exposure to low and moderate concentrations of alcohol and they may manifest as reduced frequency or duration of erratic movement (Tran & Gerlai, 2013), freezing (Blaser & Penalosa, 2011), jumping (Luca & Gerlai, 2012), bottom dwelling (Mathur & Guo, 2011; Sackerman et al., 2010; Egan et al., 2009; Tran & Gerlai, 2013; Wong et al., 2010), preference for darkness (Mathur & Guo, 2011), shoaling, (Dlugos & Rabin, 2003; Damodaran et al., 2006; Gerlai et al., 2009), aggression (Echevarria et al., 2010), and distance to a predator (Luca & Gerlai, 2012).

Higher concentrations of alcohol (usually greater than 0.75% v/v) exert depressant-like effects on zebrafish locomotor activity, e.g. observed as a decrease in total distance travelled (Gerlai et al., 2000; Rosemberg et al., 2012). The anxiogenic effects of alcohol also become observable at higher concentrations including increased freezing and bottom dwelling time (Pannia et al., 2014, Rosemberg et al., 2012; Tran & Gerlai et al., 2013a). The anxiogenic effect of acute exposure to higher alcohol concentrations is supported by our recent yet unpublished result showing increased levels of cortisol following a 60 minute exposure to 1.00% v/v alcohol, but not to 0.50% v/v (Tran et al., unpublished results).

It is also important to consider the time-course of alcohol-induced behavioural changes (i.e. the duration of exposure). In response to a higher concentration of alcohol (e.g. 1.00% v/v), in the first 30 minutes as alcohol enters the brain, the stimulant effect becomes prominent with increased activity and decreased anxiety-like behaviours. However, as brain alcohol levels continue to rise, the sedative effects of alcohol begin to appear with decreases in activity levels (Tran & Gerlai, 2013; Rosemberg et al., 2012; Pannia et al., 2014). Further support for the relationship between brain alcohol levels and the motor impairing effects of this substance comes from a recent study by Rosemberg et al. (2012). The authors demonstrated that decreasing the amount of alcohol entering the brain by taurine pretreatment could prevent the motor impairing effects of alcohol. Although it is unclear how taurine pretreatment attenuates alcohol-induced motor impairments, several studies have demonstrated that taurine inhibits the alcohol-induced increase in acetylcholinesterase activity (Rosemberg et al., 2010a) and alters extracellular adenosine monophosphate hydrolysis in the zebrafish brain (Rosemberg et al., 2010b). The behavioural changes reported here are thought to represent functional changes within the brain. However, the possibility of peripheral effects mediating the observed behavioural changes must also be considered. If the behavioural effects of alcohol are mediated by peripheral mechanisms, e.g. because alcohol possibly irritates the eyes, gills and/or the skin of the fish, the behavioural effects should manifest immediately upon exposure to the substance. However, analysis of the time course of the locomotor enhancing effects of a relatively high concentration of alcohol (1% vol/vol) administered acutely showed that the activity enhancing effect was absent for at least the first three 3 minutes of exposure (Tran & Gerlai, 2013). It is also important to note that alcohol takes time to enter the brain and the levels of alcohol were found to increase to about 15% of the external bath concentration within 10 minutes and further rise and plateau at about 20% of the external bath concentration (Tran & Gerlai, unpublished results). This temporal trajectory matches the biphasic (initial rise and subsequent depression of activity) behavioral effect of high dose of acute alcohol exposure (Tran & Gerlai, 2013), which also correlates well with the changes observed in the levels of neurochemicals dopamine and serotonin and their metabolites during acute alcohol exposure (Chatterjee & Gerlai, 2009). Nevertheless, future studies will need to systematically analyze the amount of alcohol entering different parts of the body, including the muscles and the eyes of the fish and will need to attempt to dissociate potential direct effects of this substance on muscle function and perceptual function including vision..

Biological correlates of acute alcohol exposure

Following the behavioural characterization of alcohol-induced changes, researchers have started to examine the neurochemical basis of alcohol's actions (Gerlai et al., 2009; Rosemberg et al., 2010a). Acute alcohol exposure has been shown to alter neurotransmitter systems (Gerlai et al., 2009), hormonal systems (Cachat et al., 2010; Tran et al., unpublished manuscript), cell signalling cascades (Peng et al., 2009), enzymatic activity (Chatterjee et al., 2014) and gene expression in the zebrafish brain (Rosemberg et al., 2010a). One limitation regarding the use of zebrafish in neurobiological research is the organism's small size and thus the difficulties associated with processing and handling its tiny brain. However, recent studies have successfully quantified changes in levels of neurochemicals following acute alcohol exposure using high precision liquid chromatography (HPLC), a sensitive method for the analysis of neurochemicals and metabolites in small volumes of tissue (Lopez Patino et al., 2008; Chatterjee & Gerlai, 2009; Chatterjee et al., 2014; Pan et al., 2012). The levels of dopamine, serotonin, and their metabolites from whole brain tissue have been reported to increase in a dose- and time-dependent manner during a 60 minute acute alcohol exposure period (Chatterjee & Gerlai, 2009; Gerlai et al., 2009; Chatterjee et al., 2014). Increases in dopamine in the nucleus accumbens is thought to underlie the rewarding effects of alcohol (Tupala & Tiitonen, 2004; Berridge & Robinson, 1998). However, the mammalian homolog of the nucleus accumbens has not been identified in the zebrafish brain (Rink & Wullmann, 2002).

One of the limitations with HPLC as employed for zebrafish is the lack of regional specificity, i.e. our inability to sample and quantify neurochemicals from specific brain regions. Furthermore, it is unknown whether the alterations found in the levels of certain neurochemicals are the result of changes in their synthesis/storage and/or release. Nevertheless, using a combination of neurochemical and enzymatic measures Chatterjee et al. (2014) recently determined that the increase in dopamine and its metabolite induced by the acute alcohol treatment employed significantly correlated with increase in tyrosine hydroxylase activity (TH, a rate limiting enzyme in the synthetic pathway of catecholamines, including dopamine) but was not accompanied by changes in monoamine oxidase activity (a family of enzymes responsible for the oxidative deamination of monoamines, e.g. dopamine, e.g. Elsworth & Roth, 1997). A recent study also reported increases in the expression of tyrosine hydroxylase genes (*th1* and *th2*) in larval zebrafish following acute alcohol exposure (Puttonen et al., 2013). These results suggest that acute alcohol treatment affects the synthesis but not the metabolism of dopamine in zebrafish.

Although the dopaminergic system may play a major role in mediating the effects of alcohol, especially in the domain of motivation and reward (Kalivas & Stewart, 1991; Phillips et al., 1997), other neurotransmitter systems are also expected to be affected by this substance. In zebrafish, several amino acid neurotransmitters including glutamate, aspartate, glycine, taurine, and GABA were found to respond to acute alcohol exposure (Tran & Gerlai, unpublished results; Chatterjee et al., 2014). These changes may explain the biphasic effect of alcohol. For example, the levels of excitatory neurotransmitters (glutamate and aspartate) were found to decrease in a dose-dependent manner following acute ethanol exposure, whereas inhibitory neurotransmitters (glycine, GABA, and taurine) increase/

decreased in a strain dependent manner (Chatterjee et al., 2014). Reported strain differences in alcohol's stimulatory and inhibitory effects (Gerlai et al., 2008; Pannia et al., 2014) may be due to strain specific changes in the levels of excitatory and inhibitory neurotransmitters (Chatterjee et al., 2014; see Phillips & Shen, 1996). In addition to monoamine and amino acid neurotransmitters, alcohol may also act on cholinergic neurons. Acute alcohol exposure has been reported to increase the activity of acetylcholinesterase (AChE) in a dose-dependent manner (Rosemberg et al., 2010a). AChE is responsible for the breakdown of acetylcholine following the release of this neurotransmitter to the synaptic cleft. Furthermore, behavioural sensitivity to the stimulant effect of alcohol has also been linked to the gene encoding adenylyl cyclase 5 and the phosphorylation of extracellular signal-regulated kinase (Peng et al., 2009).

Alcohol has also been shown to differentially affect genetically distinct zebrafish lines demonstrating a large underlying genetic component (Gerlai et al., 2008; 2009; Pannia et al., 2014). For example, an inbred strain such as the AB line appears to be more sensitive to higher concentrations of alcohol compared to the genetically heterogeneous short-fin (SF) wild-type (Gerlai et al., 2009; Chatterjee et al., 2014). The blunted alcohol effect in the SF strain may be a classic case of hybrid vigor, a phenomenon often manifesting as elevated buffering against fluctuations in the external environment (high concentrations of alcohol in this case). It is likely due to high proportion of loci being heterozygous, i.e., the reduced probability of deleterious recessive alleles being in a homozygous form in the genome of individuals from this zebrafish population (Chatterjee et al., 2014). Further characterization of the SF line also identified reduced levels of a number of neurochemicals relevant to alcohol addiction, which may represent a "hypo-reactive" state in these fish (Pan et al., 2012). Using qPCR, we have also found elevated expression of the gene encoding the dopamine D1 receptor in SF compared to AB (Gerlai unpublished results). In addition, SF zebrafish were also found to exhibit reduced expression of the gene encoding the GABA-B1 receptor and the solute carrier family 6 member protein (Pan et al., 2012), the latter being responsible for the reuptake of neurotransmitters following neurotransmission (Hahn & Blakely, 2007).

Chronic exposure – Development of Alcohol Tolerance

In zebrafish, behavioural adaptation to alcohol has been demonstrated following a minimum of two weeks of chronic alcohol exposure (Tran & Gerlai, 2013; Gerlai et al., 2009; 2006; Dlugos & Rabin, 2003). The majority of studies have used low to moderate concentrations of alcohol for chronic exposure ranging from 0.25 to 0.50 % v/v (Gerlai et al., 2006; Gerlai et al., 2009; Egan et al., 2009; Cachat et al., 2010; Dlugos & Rabin, 2003; Damodaran et al., 2006; Dlugos et al., 2011; Tran & Gerlai, 2013). A common procedure described in several studies as "dose escalation" has been adopted to reduce mortality associated with abrupt exposure to higher concentrations of alcohol. The procedure entails initially housing animals at a lower concentration of alcohol and gradually moving the dose up to the desired final concentration. For example, we previously housed groups of zebrafish first in 0.125% v/v ethanol for 4 days, subsequently in 0.25% for 4 days, and then in 0.375% for 4 days, before moving to the final concentration of 0.50% v/v for the last 10 days of chronic alcohol exposure (Gerlai et al., 2009; Tran & Gerlai et al., 2013). Zebrafish chronically exposed to

alcohol have been shown to develop tolerance after 2 weeks as demonstrated, for example, by the apparently normal shoaling behaviour after a subsequent acute alcohol challenge (Dlugos & Rabin, 2003; Damodaran et al., 2006; Gerlai et al., 2009; Dlugos et al., 2011). Similarly, zebrafish that were chronically pre-exposed to alcohol exhibited a blunted increase of activity in response to a subsequent acute alcohol challenge (Gerlai et al., 2006; Tran & Gerlai, 2013). It is noteworthy that the development of tolerance blunts both the stimulatory and depressant effects of alcohol observed during the biphasic time-course (Tran & Gerlai, 2013). It is tempting to explain the development of tolerance as a simple increase in alcohol metabolism, but our recent work found no significant reduction in brain alcohol levels following chronic exposure (Tran & Gerlai, unpublished results), suggesting adaptation by central nervous system mechanisms rather than putative changes in alcohol excretion or metabolism by other organs.

Although the molecular mechanisms underlying the development of alcohol tolerance in zebrafish is currently unknown, several noteworthy findings have already emerged. Chronic alcohol exposure significantly attenuates the alcohol-induced increase in a number of different neurochemicals including dopamine, DOPAC, serotonin, 5HIAA, glutamate, aspartate, taurine, glycine and GABA (Gerlai et al., 2009; Chatterjee et al., 2014). The decrease in dopamine and DOPAC is likely due to the reduced activity of tyrosine hydroxylase (Chatterjee et al., 2014). Alterations of cortisol levels in zebrafish have also been reported following chronic alcohol exposure (Cachat et al., 2010; Tran et al., unpublished data).

Similar to the effects of acute alcohol exposure, the development of alcohol tolerance following chronic exposure also appears to occur in a strain dependent manner (Dlugos & Rabin, 2003; Damodaran et al., 2006; Gerlai et al., 2009; Chatterjee et al., 2014). Chronic alcohol exposure may alter signal transduction pathways downstream of neurotransmitter receptors. For example, chronic exposure has been shown to modify purinergic signaling (Rico et al., 2011a) and protein expression (Damodaran et al., 2006). A DNA microarray study recently identified close to 2000 differentially expressed genes in the zebrafish brain following chronic alcohol exposure (Pan et al., 2011), reinforcing the notion that alcohol engages a large number of diverse molecular pathways (Vengeliene, 2008). Of particular interest is the altered expression of genes encoding the solute carrier (SLC) family proteins, specifically, SLC6 which encodes a number of Na⁺ dependent transporters, including the dopamine transporter (Pan et al., 2011). The dopamine transporter gene in zebrafish may be involved in addiction and its expression has been reported to decrease following withdrawal from addictive substances such as cocaine (Lopez Patino et al., 2008). Genes involved in the metabolism of alcohol such as alcohol dehydrogenase (ADH), acetaldehyde dehydrogenase (ALDH2), and cytochrome P450 were found to be up-regulated in the zebrafish brain following chronic alcohol exposure, suggesting a homeostatic mechanism for the development of tolerance (Pan et al., 2011). ADH and ALDH2 are primarily involved in the metabolism of alcohol and its metabolite in the liver. However, alcohol is also metabolized in the brain in the absence of ADH by cytochrome P450 enzymes, specifically after chronic exposure (Zakhari, 2006). Although the behavioural adaptations demonstrated after chronic alcohol exposure are unlikely to be due to improved metabolism of alcohol (during the alcohol treatment and behavioural testing alcohol is continually supplied (bath

application) and the amount of alcohol in the brain was found unaltered), future studies will need to carefully examine metabolic processes, including for example the expression of genes related to alcohol metabolism in the zebrafish liver, before the role of central nervous system specific adaptations versus physiological adaptations occurring in other organs leading to tolerance may be dissociated.

Thus far, we have reviewed the effects of chronic alcohol exposure and the subsequent adaptation that follows. However, when exposures are spaced apart and alcohol repeatedly administered at higher doses, the substance is expected to activate the reward system and a different type of neuroplastic change may ensue.

Repeated exposure to alcohol - Sensitization and conditioned place preference

Activation of the mesolimbic dopaminergic system and the subsequent increase in dopamine in the nucleus accumbens is thought to mediate the rewarding effects of a number of drugs (Kalivas & Stewart, 1991; Phillips et al., 1997). Alcohol is thought to indirectly increase dopamine in the nucleus accumbens via a number of different pathways (Tupala & Tiihonen, 2004). Repeated exposure to alcohol has been reported to elicit sensitization, a phenomenon in which the response to the substance becomes progressively enhanced over time (Pierce & Kalivas, 1997; Kalivas & Stewart, 1991). The phenomenon is thought to mediate the development of alcohol addiction by enhancing the motivational value of the abused drug through alterations to the mesolimbic reward pathway (Phillips et al., 1994; Berridge & Robinson, 1998; Kalivas & Stewart, 1991). The most common measure of alcohol-induced sensitization is the progressive increase of locomotor activation in response to repeated administration (Kalivas & Stewart, 1991; Harrison & Nobrega, 2009). Although repeated exposure to alcohol often leads to the development of tolerance (Seeley et al., 1996; Lindsenbardt et al., 2011), alcohol sensitization may also occur under specific temporal and dose specific administration conditions (Didone et al., 2008; Philips et al., 2011).

Several studies have utilized repeated intermittent exposure to alcohol using zebrafish (Kily et al., 2008; Mathur et al., 2011; Mathur & Guo, 2011), yet few have examined sensitization and none have investigated its underlying molecular mechanism. To our knowledge, the only successful demonstration of alcohol sensitization in adult zebrafish was achieved by Blaser et al. (2010). These authors used a heterogenous wild-type (WT) population of zebrafish and exposed individuals to either 0.50 or 1.00 % v/v alcohol for 1 hour every day for 8 consecutive days (induction phase). After the induction period, individuals were challenged with the same dose used before to examine sensitization (expression phase). The results demonstrated that alcohol sensitization was more robust when the higher concentration was employed. Our own pilot work confirms these findings and shows higher concentrations of alcohol to induce a more robust sensitization effect (unpublished results). Interestingly, alcohol sensitization has also been reported to be context dependent, i.e. sensitization was only observed in the same environment in which alcohol was administered (Blaser et al., 2010). Alcohol sensitization has been reported to be dependent on the number of exposure events and the challenge dose (Didone et al., 2008). In humans, alcohol

tolerance and sensitization ensues often after months to years of alcohol exposure. In zebrafish, both alcohol tolerance and sensitization have been attempted to be induced by only a relatively short period of alcohol exposure or limited number of exposure events (spanning no more than 3 weeks of exposure). It is not known to what period of time would years of alcohol exposure in humans correspond in zebrafish. Future studies will determine if longer exposure periods and/or larger number of exposure events may be required to induce robust sensitization. Similarly, the concentration of alcohol and the bout length of exposure will also need to be explored and optimized. Other studies have investigated the effects of repeated alcohol exposure and found that it could induce conditioned place preference (CPP) (Kily et al., 2008; Mathur et al., 2011). Daily exposure to high concentrations of alcohol (e.g. 1.00% v/v) has been shown to induce a preference for the location in which previously alcohol was delivered, an effect that was found to persist for over 3 weeks in zebrafish (Kily et al., 2008). Furthermore, CPP has also been reported following a single exposure to alcohol (Mathur et al., 2011), underscoring the addictive potential of this substance. The problem with CPP paradigms, however, is that they have a significant memory component. A drug that alters any aspect of learning and memory (e.g. attention, acquisition, consolidation, retention and recall of memory) will modify CPP performance. Thus performance in a CPP task will not only dependent upon the reinforcing value of the tested drug. Paradigms that can directly measure the reinforcing value of the drug are needed. A Y-maze task that can quantify alcohol preference and alcohol avoidance in zebrafish has been proposed, but so far such tasks have not been successfully employed (Grella et al., 2010).

Our own pilot work with AB zebrafish, a commonly used and highly homozygous strain, has shown that it is difficult to induce alcohol sensitization. The reasons for the difficulties are unknown but may be several-fold. First, alcohol sensitization appears to be species and strain dependent (Hoshaw & Lewis, 2001; Phillips et al., 1994). Second, it is dose dependent (Didone et al., 2008; Philips et al., 2011). Third, it is context dependent (Blaser et al., 2010). Finally, there are individual differences in an animal's susceptibility to become sensitized, which may increase variability and reduce statistical power (Harrison & Nobrega, 2009a; Souza-Formigoni et al., 1999; Nona et al., 2013).

Although the mechanisms underlying alcohol sensitization has not been investigated in zebrafish, researchers have identified conserved neuroadaptive pathways and homologs of human alcohol abuse associated genes in zebrafish (Kily et al., 2008; Klee et al., 2012). For example, alcohol-induced CPP was shown to alter the expression of a number of genes that are known to be important for neurotransmission and signal transduction (Kily et al., 2008). Alcohol-induced sensitization is thought to have two separate components, the development of the sensitized behaviour (induction phase) and the long-term maintenance of the behaviour (expression phase) once the drug is re-administered following a period of abstinence (Kalivas & Stewart, 1991; Harrison & Nobrega, 2009a; 2009b). The development of sensitization is thought to involve transient molecular mechanisms that occur in response to repeated activation of the reward pathway (Harrison & Nobrega, 2009b). The expression of sensitization is thought to represent an enduring neuroplastic change in the reward centers of the brain that maintains the enhanced behaviour (Lessov & Phillips, 1998; Harrison & Nobrega, 2009b). Furthermore, the induction and expression of

sensitization have been suggested to involve separate molecular pathways that are engaged in different anatomical areas of the brain (Ding et al., 2009; Abrahao et al., 2011; Vezina, 2004).

The utility of zebrafish in modeling alcohol-induced neuroplasticity

The development of tolerance and sensitization to drugs of abuse has been primarily investigated in animal models due to ethical as well as practical considerations. As individuals become tolerant to the effects of alcohol, larger quantities or higher concentrations are required to achieve the same euphoric effects (King et al., 2002). The development of sensitization to the reinforcing effects of different drugs may suggest why it is so difficult to abstain. For example, the long-lasting effect of sensitization and CPP may explain the high rates of relapse for individuals who have gone through extended periods of abstinence (Breese et al., 2011).

Alcohol tolerance and sensitization are often viewed as opposing or opposite responses, yet both paradoxically reinforce the same overall behaviour, the development of addiction. This paradox may be reconciled by examining the specific responses and potential mechanisms that may underlie these two phenomena. In humans, tolerance often develops towards alcohol's hedonic effect, requiring individuals to consume larger quantities (Bennet et al., 1993). Tolerance to the sedative, ataxic, and hypothermic effects of alcohol (i.e. the aversive effects) are also commonly reported and are primarily evaluated in animal models of tolerance (Ozburn et al., 2013; Phillips et al., 1996). On the other hand, sensitization is thought to increase the motivational value or "wanting" of the drug by reinforcing drug-seeking behaviours which could increase the chance of relapse (Berridge & Robinson, 1998; Steketee & Kalivas, 2011). It has been demonstrated in humans as increases in finger pulse amplitude (Newlin & Thomson, 1991; 1999), but primarily examined in animal models as locomotor activation.

Although alcohol tolerance and sensitization appear to occur in a dose- and regiment-dependent manner, the two phenomena have been examined concurrently (Phillips et al., 1996). This brings up an interesting question, does the development of tolerance to the sedative/ataxic effects of ethanol facilitate the development of sensitization to the locomotor effect, and are the two phenomena dependent on each other? The current literature suggests this is not the case (Phillips et al., 1996; Grahame et al., 2000; Quoilin et al., 2013). For example, alcohol sensitization is increased in an artificially selected alcohol-preferring line of mice, but development of tolerance remained unaltered in these animals (Grahame et al., 2000). Furthermore, quantitative trait loci (QTL) analysis revealed no significant relationships between loci associated with alcohol tolerance and sensitization (Phillips et al., 1996), suggesting the two phenomena may occur through different mechanisms.

The zebrafish has proven to be a useful animal model for characterizing both the behavioural effects of alcohol (Gerlai et al., 2000; Echevarria et al., 2011) and some of the underlying molecular and genetic underpinnings (Kily et al., 2008; Pan et al., 2011). Recent advances in automated video tracking (e.g. 3D analysis of zebrafish locomotion) may also allow more precise measurements of alcohol-induced behavioural alterations (e.g. total

distance travelled) by obtaining data points from an xyz-coordinate plane (Cachat et al., 2011; Maaswinkel et al., 2013). More sophisticated analysis of the dynamic movements during shoaling (group forming behavior), for example, analysis of the polarization (synchronized swim direction) or short-time scale shoal cohesion changes, or excursions from and fissures of the shoal may also significantly enhance our ability to detect subtle alcohol induced changes in zebrafish (Miller et al., 2013; Miller & Gerlai, 2012; 2011; 2008). The results obtained using multiple laboratory organisms as well as humans all confirm that alcohol acts in a complex manner and engages numerous molecular targets in a dose and exposure regimen dependent manner. The advantage of zebrafish in research aimed at uncovering these mechanisms mainly lies in the fact that this species is highly amenable to high throughput screening (drug screens and mutagenesis), which in turn allows the investigator to systematically explore and uncover all the potential mechanisms (see Gerlai, 2012). In comparison to rodents, zebrafish are a cheap alternative and due to their small size and social nature, they can be housed in high densities. In fact, a standard vivarium room (40 m²) can hold up to 20,000 zebrafish, whereas the equivalent space may equate only to several hundred mice or rats. Furthermore, powerful genetic techniques have been developed for zebrafish, with the ability to manipulate specific genes (reverse genetics), as well as to carry out full-scale mutagenesis to discover novel genes (forward genetics) relevant for alcohol addiction. The identification of molecular pathways mediating the development of alcohol-induced neuroplasticity is an important step towards developing therapeutic treatments that could target vulnerable populations that are likely to experiment with drugs, as well as those who have already become addicted.

References

- Abrahao KP, Quadros IMH, Souza-Formigoni MLO. Nucleus accumbens dopamine D-1 receptors regulate the expression of alcohol-induced behavioural sensitization. *International Journal of Neuropsychopharmacology*. 2011; 14:175–185. [PubMed: 20426882]
- Barbazuk WB, Korf I, Kadavi C, Heyen H, Tate S, Wun E, et al. The syntenic relationship of the zebrafish and human genomes. *Genome Res*. 2000; 10:1351–1358. [PubMed: 10984453]
- Bennett RH, Cherek DR, Spiga R. Acute and Chronic Alcohol Tolerance in Humans: Effects of Dose and Consecutive Days of Exposure. *Alcoholism: Clinical and Experimental Research*. 1993; 17:740–745.
- Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Research Reviews*. 1998; 28:309–369. [PubMed: 9858756]
- Bilotta J, Saszik S, Givin CM, Hardesty HR, Sutherland SE. Effects of embryonic exposure to alcohol on zebrafish visual function. *Neurotoxicology and Teratology*. 2002; 24:759–766. [PubMed: 12460658]
- Blaser RE, Koid A, Poliner RM. Context-dependent sensitization to in zebrafish *Danio rerio*. *Pharmacology, Biochemistry and Behaviour*. 2010; 95:278–284.
- Blaser RE, Penalosa YM. Stimuli affecting zebrafish (*Danio rerio*) behavior in the light/dark preference test. *Physiol Behav*. 2011; 104:831–837. [PubMed: 21839758]
- Breese GR, Sinha R, Heilig M. Chronic alcohol neuroadaptation and stress contribute to susceptibility for alcohol craving and relapse. *Pharmacology & Therapeutics*. 2011; 129:149–171. [PubMed: 20951730]
- Cachat J, Canavella P, Elegante M, Bartels B, Hart P, Bergner C, et al. Modeling withdrawal syndrome in zebrafish. *Behavioural Brain Research*. 2010; 208:371–376. [PubMed: 20006651]

- Carvan MJ, Loucks E, Weber DN, Williams FE. Alcohol effects on the developing zebrafish: neurobehaviour and skeletal morphogenesis. *Neurotoxicology and Teratology*. 2004; 26:757–768. [PubMed: 15451040]
- Cachat J, Stewart A, Utterback E, Hart P, Gaikwad S, Wong K, Kyzar E, Wu N, Kalueff AV. Three-dimensional neurophenotyping of adult zebrafish behaviour. *PLoS One*. 2011; 6:e17597. [PubMed: 21408171]
- Chatterjee D, Gerlai R. High precision liquid chromatography analysis of dopaminergic and serotonergic responses to acute alcohol exposure in zebrafish. *Behavioural Brain Research*. 2009; 200:208–213. [PubMed: 19378384]
- Chatterjee D, Shams S, Gerlai R. Chronic and acute administration induced neurochemical changes in the brain: Comparison of distinct zebrafish populations. *Amino Acids*. 2013 In Press.
- Collier AD, Echevarria DJ. The utility of the zebrafish model in conditioned place preference to assess the rewarding effects of drugs. *Behav Pharmacol*. 2013; 24:375–83. [PubMed: 23811781]
- Crabbe JC, Phillips TJ, Belknap JK. The complexity of alcohol drinking: Studies in Rodent Genetic models. *Behavioural Genetics*. 2010; 40:737–750.
- Crabbe J, Phillips T. Pharmacogenetic studies of alcohol self-administration and withdrawal. *Psychopharmacology*. 2004; 174:539–560. [PubMed: 14551670]
- Damodaran S, Dlugos CA, Wood TD, Rabin RA. Effects of chronic alcohol administration on brain protein levels: a proteomic investigation using 2-D DIGE system. *Eur J Pharmacol*. 2006; 547:75–82. [PubMed: 16978605]
- Didone V, Quoilin C, Tirelli E, Quertemont. Parametric analysis of the development and expression of alcohol-induced behavioural sensitization in female Swiss mice: effects of dose, injection schedule, and test context. *Psychopharmacology*. 2008; 201:249–260. [PubMed: 18685830]
- Ding ZM, Rodd ZA, Engleman EA, McBride WJ. Sensitization of ventral tegmental area dopamine neurons to the stimulating effects of alcohol. *Alcoholism-Clinical and Experimental Research*. 2009; 33:2009.
- Dlugos CA, Rabin RA. Alcohol effects on three strains of zebrafish: model system for genetic investigations. *Pharmacology Biochemistry and Behaviour*. 2003; 74:471–480.
- Dlugos CA, Brown SJ, Rabin RA. Gender differences in alcohol-induced behavioural sensitivity in zebrafish. *Alcohol*. 2011; 45:11–18. [PubMed: 20880661]
- Echevarria DJ, Hammack CM, Jouandot DJ, Toms CN. Does acute alcohol exposure modulate aggressive behaviours in the zebrafish (*Danio rerio*), or is the bark worse than the bite? *International Journal of Comparative Psychology*. 2010; 23:62–69.
- Echevarria DJ, Toms CN, Jouandot DJ. Alcohol-induced behavioural change in zebrafish models. *Rev Neurosci*. 2011; 22:85–93. [PubMed: 21615263]
- Egan RJ, Bergner CL, Hart PC, Cachat JM, Canavella PR, Elegante MF, et al. Understanding behavioural and physiological phenotypes of stress and anxiety in zebrafish. *Behavioural Brain Research*. 2009; 205:38–44. [PubMed: 19540270]
- Elsworth JD, Roth RH. Dopamine synthesis, uptake, metabolism, and receptors: relevance to gene therapy of parkinson's disease. *Experimental Neurology*. 1997; 144:4–9. [PubMed: 9126143]
- Erblich J, Earleywine M, Erlich B, Bovbjerg DH. Biphasic stimulant and sedative effects of alcohol: Are children of alcoholics really different? *Addictive Behaviours*. 2003; 28:1129–1139.
- Gerlai R. Using zebrafish to unravel the genetics of complex brain disorders. *Curr Top Behav Neurosci*. 2012; 12:3–24. [PubMed: 22250005]
- Gerlai R, Lahav S, Rosenthal A. Drinks like a fish: zebrafish *Danio rerio* as a behaviour genetic model to study alcohol effects. *Pharmacology, Biochemistry, and Behaviour*. 2000; 67:773–782.
- Gerlai R, Chatterjee D, Pereira T, Sawashima T, Krishnannair R. Acute and chronic alcohol dose: population differences in behaviour and neurochemistry of zebrafish. *Genes, Brain and Behaviour*. 2009; 8:586–599.
- Gerlai R, Lee V, Blaser R. Effects of acute and chronic exposure on the behaviour of adult zebrafish *Danio rerio*. *Pharmacology, Biochemistry and Behaviour*. 2006; 85:752–761.
- Gerlai R, Prajapati S, Ahmad F. Differences in acute alcohol-induced behavioural responses among zebrafish populations. *Alcohol Clin Exp Res*. 2008; 32:1763–1773. [PubMed: 18652595]

- Goetzel RZ, Hawkins K, Ozminkowski RJ. The health and productivity cost burden of the “top 10” physical and mental conditions affecting six large U.S. employers in 1999. *J Occup Environ Med*. 2003; 45:5–14. [PubMed: 12553174]
- Grant BF, Dawson DA, Stinson FS, Chou SP, Dufour MC, Pickering RP. The 12-month prevalence and trends in DSM-IV alcohol abuse and dependence: United States, 1991–1992 and 2001–2002. *Drug and Alcohol Dependence*. 2004; 74:223–234. [PubMed: 15194200]
- Grahame NJ, Rodd-Henricks K, Li TK, Lumeng L. Ethanol locomotor sensitization, but not tolerance correlates with selection for alcohol preference in high- and low-alcohol preferring mice. 2000; 151:252–260.
- Grella S, Kapur N, Gerlai R. A Y-maze choice task fails to detect alcohol avoidance or alcohol preference in zebrafish. *Intl J Comp Psych*. 2010; 23:26–42.
- Grunwald JD, Eisen JS. Timeline: Headwaters of the zebrafish emergence of a new model vertebrate. *Nature Reviews Genetics*. 2003; 3:717–724.
- Haberstick BC, Young SE, Zeiger JS, Lessem JM, Hewitt JK, Hopfer CJ. Prevalence and correlates of alcohol and cannabis use disorders in the United States: Results from the national longitudinal study of adolescent health. *Drug and Alcohol Dependence*. (In Press.).
- Hahn MK, Blakely RD. The functional impact of SLC6 transporter genetic variation. *Annu Rev Pharmacol Toxicol*. 2007; 47:401–441. [PubMed: 17067279]
- Harrison SJ, Nobrega JN. Differential susceptibility to alcohol and amphetamine sensitization in dopamine D3 receptor-deficient mice. *Psychopharmacology*. 2009a; 204:49–59. [PubMed: 19096829]
- Harrison SJ, Nobrega SN. A functional role for the dopamine D3 receptor in the induction and expression of behavioural sensitization to alcohol in mice. *Psychopharmacology*. 2009b; 207:47–56. [PubMed: 19662386]
- Hoshaw B, Lewis M. Behavioural sensitization to alcohol in rats: evidence from the Sprague-Dawley strain. *Pharmacology Biochemistry and Behaviour*. 2001; 68:685–690.
- Hyman SE, Malenka RC. Addiction and the brain: The neurobiology of compulsion and its persistence. *Nature reviews neuroscience*. 2001; 210:695–703.
- Hyman SE, Malenka RC, Nestler EJ. Neural Mechanisms of Addiction: The Role of Reward-Related Learning and Memory. *Annual Review in Neuroscience*. 2006; 29:565–598.
- Kalivas PW, Stewart J. Dopamine transmission in the initiation and expression of drug-induced and stress-induced sensitization of motor-activity. *Brain Research Reviews*. 1991; 16:223–244. [PubMed: 1665095]
- Kanny D, Liu Y, Brewer RD. National Center for Chronic Disease Prevention and Health Promotion, CDC. Binge Drinking United States, 2011. *MMWR Surveill Summ*. 2013; 62:77–80. [PubMed: 24264494]
- Kaun KR, Azanchi R, Maung Z, Hirsh J, Heberlein U. A *Drosophila* model for alcohol reward. *Nature Neuroscience*. 2011; 14:612–619.
- Kily LJM, Cowe YC, Hussain O, Patel S, McElwaine S, Cotter FE, Brennan CH. Gene expression changes in a zebrafish model of drug dependency suggest conservation of neuro-adaptation pathways. *The Journal of Experimental Biology*. 2008; 211:1623–1634. [PubMed: 18456890]
- King AC, Houle T, de Wit H, Holdstock L, Schuster A. Biphasic alcohol response differs in heavy versus light drinkers. *Alcoholism: Clinical and Experimental Research*. 2002; 26:827–835.
- Klee EW, Schneider H, Clark KJ, Cousin MA, Ebbert JO, Hooten, et al. Zebrafish: a model for the study of addiction genetics. *Hum Genet*. 2012; 131:977–1008. [PubMed: 22207143]
- Koob GF. A role for GABA mechanisms in the motivational effects of alcohol. *Biochem Pharmacol*. 2004; 68:1515–1525. [PubMed: 15451394]
- Kyzar E, Stewart AM, Landsman S, Collins C, Gebhardt M, Robinson K, et al. Behavioural effects of bidirectional modulators of brain monoamines reserpine and d-amphetamine in zebrafish. *Brain Research*. 2013; 1527:108–116. [PubMed: 23827499]
- Lopez Patino MA, Yu L, Yamamoto BK, Zhdanova IV. Gender differences in zebrafish responses to cocaine withdrawal. *Physiol Behav*. 2008; 95:36–47. [PubMed: 18499199]
- Luca RM, Gerlai R. Animated bird silhouette above the tank: Acute alcohol diminishes fear responses in zebrafish. *Behavioural Brain Research*. 2012; 229:194–201. [PubMed: 22266470]

- Lessov CN, Phillips T. Duration of sensitization to locomotor stimulant effects of alcohol in mice. *Psychopharmacology*. 1998; 135:374–382. [PubMed: 9539262]
- Leyton M. Conditioned and sensitized responses to stimulant drugs in humans. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 2007; 31:1601–1613. [PubMed: 17888557]
- Lindsenbardt DN, Moore EM, Griffin KD, Gigante ED, Boehm SL 2nd. Tolerance to alcohol's ataxic effects and alterations in alcohol-induced locomotion following repeated binge-like alcohol intake using the DID model. *Alcohol Clin Exp Res*. 2011; 35:1246–1255. [PubMed: 21410484]
- Maaswinkel H, Zhu L, Weng W. Using an automated 3D-tracking system to record individual and shoals of adult zebrafish. *J Vis Exp*. 2013; 82:50681. [PubMed: 24336189]
- Mathur P, Guo S. Use of zebrafish as a model to understand mechanisms of addiction and complex neurobehavioural phenotypes. *Neurobiology of Disease*. 2010; 1:66–72. [PubMed: 20493262]
- Mathur P, Guo S. Differences of acute versus chronic exposure on anxiety-like behavioural response in zebrafish. *Behavioural Brain Research*. 2011; 219:234–239. [PubMed: 21255611]
- Mathur P, Berberoglu MA, Guo S. Preference for alcohol in zebrafish following a single exposure. *Behav Brain Res*. 2011; 217:128–133. [PubMed: 20974186]
- Miller N, Greene K, Dydzinski A, Gerlai R. Effects of nicotine and alcohol on zebrafish *Danio rerio* shoaling. *Behavioural Brain Research*. 2013; 240:192–196. [PubMed: 23219966]
- Miller N, Gerlai R. From Schooling to Shoaling: Patterns of Collective Motion in Zebrafish (*Danio rerio*). *PLOS ONE*. 2012; 7(11):e48865.10.1371/journal.pone.0048865 [PubMed: 23166599]
- Miller N, Gerlai R. Redefining membership in animal groups. *Behavior Research Methods*. 2011; 43:364–371.
- Miller N, Gerlai R. Oscillations in Shoal Cohesion in Zebrafish (*Danio rerio*) *Behav. Brain Res*. 2008; 193:148–151.
- Naimi TS, Brewer RD, Mokdad A, Denny C, Serdula MK, Marks JS. Binge drinking among US adults. *JAMA*. 2003; 289:70–75. [PubMed: 12503979]
- Newlin DB, Thomson JB. Chronic tolerance and sensitization to alcohol in sons of alcoholics. *Alcohol Clin Exp Res*. 1991; 15:399–405. [PubMed: 1877726]
- Newlin DB, Thomson JB. Chronic tolerance and sensitization to alcohol in sons of alcoholics: II. Replication and reanalysis. *Exp Clin Psychopharmacol*. 1999; 7:234–243. [PubMed: 10472511]
- Nona C, Guirguis S, Nobrega J. Susceptibility to alcohol sensitization is differentially associated with changes in pCREB, trkB and BDNF mRNA expression in the mouse brain. *Behavioural Brain Research*. 2013; 242:25–33. [PubMed: 23291223]
- Ozburn AR, Harris RA, Blednov YA. Chronic voluntary alcohol consumption results in tolerance to sedative/hypnotic and hypothermic effects of alcohol in hybrid mice. *Pharmacology Biochemistry and Behaviour*. 2013; 104:33–39.
- Pannia E, Tran S, Rampersad M, Gerlai R. Acute ethanol induces behavioural differences in two zebrafish (*Danio rerio*) strains: a time course analysis. *Behavioural Brain Research*. 2014; 259:174–185. [PubMed: 24239692]
- Pan Y, Kaiguo M, Razak Z, Westwood JT, Gerlai R. Chronic alcohol exposure induced gene expression changes in the zebrafish brain. *Behavioural Brain Research*. 2011; 216:66–76. [PubMed: 20654657]
- Pan Y, Chatterjee D, Gerlai R. Strain dependent gene expression and neurochemical levels in the brain of zebrafish: Focus on a few alcohol related targets. *Physiology & Behaviour*. 2012; 107:773–780.
- Peng J, Wagle M, Mueller T, Mathur P, Lockwood BL, Bretaude S, Guo S. Alcohol-modulated camouflage response screen in zebrafish uncovers a novel role for cAMP and extracellular signal-regulated kinase signaling in behavioural sensitivity to alcohol. *J Neurosci*. 2009; 29:8408–8418. [PubMed: 19571131]
- Phillips T, Dickinson S, Burkhart-Kasch S. Behavioural sensitization to drug stimulant effects in C57BL/6J and DBA/2J inbred Mice. *Behavioural Neuroscience*. 1994; 108:789–803.
- Phillips T, Lessove C, Harland R, Mitchell S. Evaluation of Potential Genetic Associations Between Ethanol Tolerance and Sensitization in BXD/Ty Recombinant Inbred Mice. *Pharmacology and Experimental Therapeutics*. 1996; 277:613–623.

- Phillips T, Roberts A, Lessov C. Behavioural Sensitization to Alcohol: Genetics and the Effects of Stress. *Pharmacology Biochemistry and Behaviour*. 2011; 57:487–493.
- Phillips T, Shen E. Neurochemical bases of locomotion and ethanol stimulant effects. *International Review of Neurobiology*. 1996; 39:243–282. [PubMed: 8894850]
- Pierce RC, Kalivas PW. A circuitry model of the expression of behavioural sensitization to amphetamine-like stimulants. *Brain Research Reviews*. 1997; 25:192–216. [PubMed: 9403138]
- Puttonen HAJ, Sundvik M, Rozov S, Chen Y, Panula P. Acute alcohol treatment upregulates th1, th2 and hdc in larval zebrafish in stable networks. *Frontiers in Neural Circuits*. 2013; 7:1–10. [PubMed: 23440175]
- Quoilin C, Didone V, Tirelli E, Quertemont E. Chronic tolerance to ethanol-induced sedation: Implication for age-related differences in locomotor sensitization. *Alcohol*. 2013; 47:317–322. [PubMed: 23566528]
- Rice DP. Economic costs of substance abuse. *Proc Assoc Am Physicians*. 1995; 111:119–125. [PubMed: 10220806]
- Rico EP, Rosemberg DB, da Silveira Langoni A, Souto AA, Dias RD, Bogo MR, et al. Chronic alcohol treatment alters purine nucleotide hydrolysis and nucleotidase gene expression pattern in zebrafish brain. *NeuroToxicology*. 2011a; 32:871–878. [PubMed: 21704070]
- Rico EP, Rosemberg DB, Seibt KJ, Capiotti KM, Da Silva RS, Bonan CD. Zebrafish neurotransmitter systems as potential pharmacological and toxicological targets. *Neurotoxicology and Teratology*. 2011b; 33:608–617. [PubMed: 21907791]
- Rink E, Wullimann MF. Connections of the ventral telecephalone and tyrosine hydroxylase distribution in the zebrafish *Danio rerio* lead to identification of an ascending dopaminergic system in a teleost. *Brain Research Bulletin*. 2002; 57:385–387. [PubMed: 11922994]
- Rosemberg DB, Braga MM, Rico EP, Loss CM, Cordova SD, Mussulini BH, et al. Behavioural effects of taurine pretreatment in zebrafish acutely exposed to alcohol. *Neuropharmacol*. 2012; 63:613–623.
- Rosemberg DB, da Rocha RF, Rico EP, Zanotto-Filho A, Dias RD, Bogo MR, et al. Taurine pretreatment prevents enhancement of acetylcholinesterase activity induced by acute alcohol exposure and decreases the level of markers of oxidative stress in zebrafish brain. *Neuroscience*. 2010a; 171:683–692. [PubMed: 20884336]
- Rosemberg DB, Kist LW, Etchart RH, Rico EP, Langoni AS, Dias RD, et al. Evidence that acute taurine treatment alters extracellular AMP hydrolysis and adenosine deaminase activity in zebrafish brain membranes. *Neurosci Lett*. 2010b; 481:105–109. [PubMed: 20600599]
- Sackerman J, Donegan JJ, Cunningham CS, Nguyen NN, Lawless K, Long A, et al. Zebrafish behaviour in novel environments: effects of acute exposure to anxiolytic compounds and choice of *Danio rerio* line. *Int J Comp Psychol*. 2010; 23:43–61. [PubMed: 20523756]
- Seeley RJ, Hawkins MH, Ramsay DS, Wilkinson DS, Woods SC. Learned tolerance to the corticosterone-increasing action of alcohol in rats. *Pharmacol Biochem Behav*. 1996; 55:268–273. [PubMed: 8951964]
- Sison M, Gerlai R. Associative learning in zebrafish (*Danio rerio*) in the plus maze. *Behav Brain Res*. 2010; 207:99–104. [PubMed: 19800919]
- Sison M, Gerlai R. Associative learning performance is impaired in zebrafish *Danio rerio* by the NMDA-R antagonist MK-801. *Neurobiology of Learning and Memory*. 2011; 96:230–237. [PubMed: 21596149]
- Souza-Formigoni ML, De Lucca EM, Hipolide DC, Enns SC, Oliveira SC, Nobrega JN. Sensitization to alcohol's stimulant effect is associated with region-specific increases in brain D2 receptor binding. *Psychopharmacology*. 1999; 146:262–267. [PubMed: 10541725]
- Spanagel R. Alcohol addiction research: from animal models to clinics. *Best Practice & Research in Clinical Gastroenterology*. 2003; 17:507–518.
- Spanagel R. Animal Models of Behaviour: Alcohol Addiction. *Encyclopedia of Behavioural Neuroscience*. 2010:65–70.
- Steketee JD, Kalivas PW. Drug wanting: behavioural sensitization and relapse to drug-seeking behaviour. *Pharmacol Rev*. 2011; 63:348–365. [PubMed: 21490129]

- Tran S, Gerlai R. Time-course of behavioural changes induced by alcohol in zebrafish *Danio rerio*. *Behavioural Brain Research*. 2013; 252:204–213. [PubMed: 23756142]
- Tran S, Chatterjee D, Gerlai R. An integrative analysis of alcohol tolerance and withdrawal in zebrafish. Unpublished manuscript. 2014
- Tupala E, Tiihonen J. Dopamine and alcoholism: neurobiological basis of alcohol abuse. *Progress In Neuro-Psychopharmacology & Biological Psychiatry*. 2004; 28:1221–1247. [PubMed: 15588749]
- Vengeliene V, Bilbao A, Molander A, Spanagel R. *British Journal of Pharmacology*. 2008; 154:599–315.
- Vezina P. Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. *Neuroscience and Biobehavioural Reviews*. 2004; 27:827–839.
- Wong K, Elgante M, Bartels B, Elkhayat S, Tien D, Roy S, et al. Analyzing habituation responses to novelty in zebrafish (*Danio rerio*). *Behav Brain Res*. 2010; 208:450–457. [PubMed: 20035794]
- Zakhari S. Overview: How is Alcohol Metabolized by the Body? *Alcohol Research & Health*. 2006; 29:245–254. [PubMed: 17718403]

Highlights

- The zebrafish is becoming increasingly appreciated in alcohol research
- Acute and chronic alcohol administration induced behavioral changes are seen in zebrafish
- Constant exposure to low alcohol concentrations induce tolerance in zebrafish
- Repeated exposure to high alcohol concentrations induce sensitization in zebrafish
- Biological correlates underlying alcohol induced plasticity have started to be revealed