Current Neuropharmacology, 2022, *20*, 560-578

REVIEW ARTICLE

Neurochemical and Behavioral Consequences of Ethanol and/or Caffeine Exposure: Effects in Zebrafish and Rodents

Carly L. Clayman[1] and Victoria P. Connaughton[1,*]

[1]*Department of Biology and Center for Neuroscience and Behavior, American University, Washington, DC 20016, USA*

Abstract: Zebrafish are increasingly being utilized to model the behavioral and neurochemical effects of pharmaceuticals and, more recently, pharmaceutical interactions. Zebrafish models of stress establish that both caffeine and ethanol influence anxiety, though few studies have implemented co-administration to assess the interaction of anxiety and reward-seeking. Caffeine exposure in zebrafish is teratogenic, causing developmental abnormalities in the cardiovascular, neuromuscular, and nervous systems of embryos and larvae. Ethanol is also a teratogen and, as an anxiolytic substance, may be able to offset the anxiogenic effects of caffeine. Co-exposure to caffeine and alcohol impacts neuroanatomy and behavior in adolescent animal models, suggesting stimulant substances may moderate the impact of alcohol on neural circuit development. Here, we review the literature describing neuropharmacological and behavioral consequences of caffeine and/or alcohol exposure in the zebrafish model, focusing on neurochemistry, locomotor effects, and behavioral assessments of stress/anxiety as reported in adolescent/juvenile and adult animals. The purpose of this review is twofold: (1) describe the work in zebrafish documenting the effects of ethanol and/or caffeine exposure and (2) compare these zebrafish studies with comparable experiments in rodents. We focus on specific neurochemical pathways (dopamine, serotonin, adenosine, GABA), anxiety-type behaviors (assessed with a novel tank, thigmotaxis, shoaling), and locomotor changes resulting from both individual and co-exposure. We compare findings in zebrafish with those in rodent models, revealing similarities across species and identifying conservation of mechanisms that potentially reinforce co-addiction.

ARTICLE HISTORY

Received: May 31, 2021
Revised: August 31, 2021
Accepted: September 17, 2021

DOI:
10.2174/1570159X19666211111142027

Keywords: *Danio rerio*, ethanol, caffeine, anxiety, adenosine, novel tank, scototaxis, shoaling.

1. INTRODUCTION

While there are many studies describing the acute and chronic effects of alcohol and caffeine exposure, the effects of early co-exposure to these substances on adult brain function and behavior are less thoroughly studied. Much of the original work on the effects of alcohol in animal models used rodents. More recently, zebrafish has become a prominent animal model for these studies [1, 2]. The use of zebrafish to assess the effects of ethanol and/or caffeine includes exposure regimes and laboratory-assessed behaviors that are similar to those used with rodents. In this review, we describe the work in zebrafish documenting the effects of ethanol and/or caffeine exposure and compare these studies with comparable experiments in rodents. We focus on the neurochemical pathways involved (dopamine, serotonin, adenosine, GABA), anxiety-type behaviors (assessed with a novel tank, scototaxis, thigmotaxis, shoaling), and locomotor changes resulting from caffeine, alcohol, and their combination.

*Address correspondence to this author at the Department of Biology and Center for Neuroscience and Behavior, American University, 4400 Massachusetts Avenue, NW, Washington, DC 20016, USA;
E-mail: vconn@american.edu

Zebrafish are already an existing model for fetal alcohol syndrome/fetal alcohol spectrum disorders [3-6]. Here, we focus on later life stages, examining adolescent (juvenile) and adult zebrafish exposure to ethanol and/or caffeine. Zebrafish adolescence spans the late larval stage from 14-27 days post fertilization (dpf) and the juvenile stage from 28 dpf to ~84 dpf, depending upon strain and rearing conditions [7]. This juvenile stage is characterized by the development of most adult features, including motor behaviors [8], but the absence of sexual maturity [7]. Rodent studies summarized here used rats during early adolescence (postnatal day (PD) 28-45) or late adolescence (PD 45-55) [9, 10] or mice at PD 30-60 [11]. We also discuss the long-term consequences of adolescent/early life stage exposure in adult zebrafish (aged 80-270 dpf). Adolescent rodents undergo marked cerebral maturation, which contributes to the influence of addictive substances [12-14].

Adolescence is characterized by a rise in novelty-seeking, impulsivity, and risk-taking, even though self-control, planning, and executive function capabilities are still developing [15]. The adolescent brain undergoes concurrent activational changes in hormonal and reward-seeking systems and is especially susceptible to the impact of alcohol [16-18]. Ethanol directly or indirectly modulates dopaminergic, glutamatergic,

1875-6190/22 $65.00+.00

GABAergic, and serotonergic systems in the brain [19]. Preclinical studies demonstrate that adolescent subjects that receive chronic intermittent alcohol exposure are more susceptible to the impact of alcohol when they reach adulthood [20]. Notably, the subsequent adult response to ethanol is not only different from the responses of ethanol-naïve subjects, but also mimics the adolescent response to ethanol [20].

It is important to note that, although wild-type zebrafish are typically used to assess the impact of ethanol and/or caffeine exposure, the specific wild-type strain differs across publications. While some studies use the inbred AB, TU (Tubingen), SF (shortfin), and longfin strains; outbred wildtype ('petstore') strains have also been employed. Though all are 'wildtype', these strains differ based on behavior [21-23], gene expression profiles [24-26], nucleotide diversity [27], and physiology [28]. Importantly, strain-dependent differences are also observed with regard to ethanol [25, 29, 30] and caffeine [31] sensitivity. Consequently, we provide strain information when known. Various rat and mouse strains have also been used in studies with ethanol and/or caffeine, and we highlight this information as well.

2. ETHANOL-INDUCED NEUROCHEMICAL CHANGES

In general, ethanol triggers oxidative stress [32-34], influences a variety of neurotransmitter receptor types, and inhibits voltage-sensitive calcium channels [35, 36]. HPLC analysis of zebrafish brain homogenates [29, 37-39] suggests that dopaminergic and serotonergic systems primarily mediate the rewarding effects of alcohol [40-43], with changes in compound levels occurring after acute alcohol exposure, chronic alcohol exposure, and alcohol withdrawal [38]. Ethanol, administered to zebrafish *via* tank water (mg/L or ppm), is readily taken up by the fish [16, 44], resulting in constant exposure such that even a short exposure time (1-2 hr) can cause long-lasting neurochemical changes measured days or weeks after treatment [37, 39, 40]. The LD50 concentration in adult male zebrafish is reported as 2.75% [45]. Intake of alcohol, through oral consumption, injection, or vapor inhalation (mg/kg or ppm), also alters brain function and dopaminergic circuits in rodents, especially in adolescence [46-50].

2.1. Dopamine

In humans, ethanol activates dopaminergic (DA) reward pathways in the mesolimbic system, which includes nerve cells in the ventral tegmental area (VTA) and their projections to the nucleus accumbens (NAc), amygdala, prefrontal cortex, and striatum [51-53]. In rodents, DA levels in the ventral striatum [54-56], NAc [54, 57, 58], and prefrontal cortex [54] similarly increase after alcohol exposure and are associated with increased locomotor activity [52]. The striatum in zebrafish is located in the posterior tuberculum, an area rich in dopaminergic neurons [59]. As in mammals, ethanol increases brain DA levels in zebrafish [60-62].

Tyrosine hydroxylase (TH) is the enzyme that converts L-tyrosine to L-3,4-dihydroxyphylalanine, a precursor to DA. Released DA binds to one of several G-protein coupled receptor types leading to changes in intracellular [cAMP] [63]. D1- and D2-type receptors, in particular, are implicated in ethanol-induced signaling [53, 64]. TH is thought to be activated in response to alcohol exposure, increasing DA synthesis [61]. Indeed, rodents show increased TH mRNA levels with chronic ethanol exposure [65]. In contrast, acute 1% ethanol diminished TH gene expression in zebrafish posterior tuberculum [66].

The general effects of ethanol on dopaminergic pathways in the brain seem highly conserved, though differences [53] are reported based on the age of exposure, duration of exposure, ethanol pretreatment, and/or brain region examined. In addition, strain-dependent differences, shown particularly well in zebrafish [29, 38-40, 67], suggest a genetic component to ethanol sensitivity. For example, acute ethanol exposure increases DA levels in adult AB zebrafish in a linear, concentration-dependent manner, while the dose-response curve in SF fish is an inverted U-shape, with intermediate concentrations of ethanol stimulating the highest DA levels [29, 38]. Changes in TH activity mirror those of DA [29] and levels of the dopamine metabolite DOPAC (3,4-dihydroxyacetic acid) consistently increase in both strains, though the time course of the increase varies [38]. Finally, AB fish have a more pronounced response to withdrawal [38], revealing adaptational differences to chronic alcohol exposure [29]. Ethanol-induced DA release in the NAc is more pronounced in alcohol-preferring P rats vs. heterogenous Wistar rats [58], another strain-dependent effect.

Adult zebrafish used in a binge drinking paradigm (1.4% ethanol, once a week for 3 wk) initially displayed an increase in dopamine transporter activity (0-2 d postexposure), which was followed by an increase in brain DA levels (2 d and 9 d postexposure) [60], suggesting a time-course to the cellular response to ethanol. Chronic 1% ethanol exposure also caused time-dependent effects on dopamine receptor expression. Expression of dopamine receptor 1 (*drd1*) increased in zebrafish after 12 d of chronic exposure, whereas expression of dopamine receptor II (*drd2*) initially decreased (after 4 d and 8 d of chronic exposure) but then increased after 12 d of exposure [68].

There are also age-dependent differences. Compared to the above studies in adults, zebrafish AB adolescents (40, 70, or 102 dpf) exposed to 0-1% ethanol for 2 hr when they were embryos (24 hpf) had decreased DA and DOPAC levels [39]. DOPAC levels were also significantly decreased in 40 dpf zebrafish from strain TU [39]. Similarly, in rodents, DA and DOPAC levels were decreased in the prefrontal cortex of adolescent (PD 28-30) mice pretreated with ethanol (2 g/kg i.p.) for 15 d and then challenged with ethanol 5 d later [54]. The same treatment regime used with adult (PD 68-70) mice, in contrast, increased DA and DOPAC levels in the same brain region. In the NAc, DOPAC levels decreased in adolescent mice, with no differences in DA levels, whereas DA levels increased in adult animals with no differences in DOPAC levels [54].

2.2. Serotonin

Serotonin (5-HT or 5-hydroxytryptamine) is synthesized from the amino acid L-tryptophan. Serotonergic cell bodies are primarily located in raphe nuclei of the brain stem. However, axonal processes containing 5-HT are found throughout the brain, including retina, olfactory bulb, hypothalamus,

hippocampus, and striatum [69]. In zebrafish, there are multiple serotonergic nuclei located in the pretectal complex, the paraventricular complex/posterior tuberculum/hypothalamus, and raphe nuclei regions of the midbrain and hindbrain [70, 71].

Studies in zebrafish show an interaction between alcohol consumption and serotonin signaling that parallels the trends observed for dopamine. Increased levels of 5-HT and its metabolite 5-HIAA are reported in adult fish following acute exposure to 1% ethanol [72]. These data agree with similar studies in rats [73-75] and humans [76]. In general, ethanol exposure consistently increases 5-HT levels independent of strain used, though there are differences related to (1) magnitude of the response: a larger increase in SF fish compared to AB fish [38] and (2) dose-dependency: 5-HT levels in adult AB zebrafish increase linearly with ethanol dose, while levels in SF zebrafish were most elevated in response to an immediate dose [29, 38]. Both 5-HT and 5-HIAA levels in AB fish also increase after withdrawal from chronic exposure [29, 38]. In contrast, ethanol exposure did not alter the levels of these two neurochemicals in TU fish [39].

In rodents, ethanol, administered as either a single injection (3 g/kg) or chronically, did not alter 5-HT levels in brain tissue, though levels of the serotonin metabolite 5-HIAA (5-hydroxyindoleacetic acid) increase significantly. In fact, 5-HIAA levels remained increased for several hours following ethanol exposure, an effect attributed to altered transporter activity rather than a decrease in 5-HT metabolism [77]. In a review of the effects of alcohol on serotonergic pathways, LeMarquand *et al.* [78] concluded an inverse relationship between 5-HT and alcohol intake: increased 5-HT functioning was associated with decreased alcohol intake and vice versa. However, these authors also note the high variability in results due to different methodologies used.

2.3. GABA

Ethanol's sedative properties are attributed to its effects on the GABAergic system [79, 80], consistent with GABA's role as an inhibitory neurotransmitter. GABA (γ-aminobutyric acid) is synthesized from glutamate by enzyme glutamic acid decarboxylase (GAD). Ethanol is an agonist at $GABA_A$-type receptors [81, 82], and ethanol exposure, by itself, affects the expression of different GABA subunits [68]. For example, 1% ethanol downregulated expression of the $GABA_A\alpha1$ receptor gene *gabra2a* in adult zebrafish after both acute (4 d) and chronic (16 d) exposure. In contrast, expression of the $GABA_B$ receptor gene *gabbr1a* was reduced after 8 d of exposure, but upregulated 4 d later [68]. The ethanol-induced differences in $GABA_A$ subunit expression are also reflected in zebrafish behavioral strains with different stress coping strategies. Expression of the $GABA_A$ subunits *gabra1* and *gabrg2* are upregulated in response to chronic 14 d exposure to 0.75% ethanol in both proactive (increased risk behaviors) and reactive zebrafish. However, the increased expression pattern correlated with lower stress-related behaviors only in proactive fish [83], suggesting that effects of ethanol on GABA circuitry are affected by behavioral phenotype. In addition, acute ethanol exposure reduced GABA levels in AB fish, but increased levels in SF fish [29], suggesting short-term, strain-dependent effects.

Electrophysiological recordings from chronic ethanol-treated rats showed increased GABA transmission in the amygdala in response to acute alcohol superfusion [82, 84]. Sensitivity of $GABA_A$ receptors to ethanol depends on subunit composition [81, 85], with the $\alpha_4\delta$ [85], $\alpha_4\beta_3$ [86], and/or γ2 [79] subunits conferring the greatest sensitivity to ethanol. Interestingly, however, co-expression of $GABA_A$ receptor subunits in *Xenopus* oocytes showed they were sensitive to only high (mM) concentrations of ethanol [86], which the authors suggest may be due to other factors affecting the interaction between ethanol and $GABA_A$ receptors.

2.3.1. Summary

Overall, laboratory studies clearly show ethanol-induced stimulation of DA, 5-HT, and GABA circuits in the brain (Fig. **1**). Ethanol alters gene expression and neurotransmitter synthesis/transport, indicating a diversity of targets. Specific effects of ethanol on each circuit are age-, strain-, concentration-, and time-dependent. In general, exposure at early/young ages decreases compound levels, and later/adult exposures increase compound levels. While there are clearly differential sensitivities to ethanol exposure and/or exposure regime, the similarities between zebrafish and rodent studies suggest conserved mechanisms of action.

3. ETHANOL-INDUCED BEHAVIORAL CHANGES

Chronic developmental exposure to ethanol decreases growth and causes craniofacial skeletal abnormalities in both rodents and zebrafish, with the timing of exposure determining severity and region, affected [16]. Adult zebrafish developmentally exposed to ethanol displayed learning deficits, delayed startle responses, and slower swimming speeds than controls [87].

3.1. Locomotor Activity and Coordination

Alcohol's effects on locomotor activity and movement are well characterized and are, at least partially, due to the deleterious effects of ethanol on cerebellar circuits. In zebrafish, ethanol exposure causes a loss of cerebellar granule cells [88] in addition to a reduction in axon outgrowth of spinal motor neurons and altered efficacy of the neuromuscular junction [16]. In rodents, ethanol exposure similarly causes the apoptotic loss of cerebellar Purkinje and granule cells as well as a decrease in growth factors [16].

Rodent and zebrafish models account for locomotor confounds in quantifying ethanol-induced behaviors by measuring the basal activity and comparing it to acute ethanol-induced locomotor changes. Chronic ethanol exposure alters both the baseline behavior of zebrafish and their behavioral response to ethanol [89]. Not only may these effects be observed in adolescence, but adolescent exposure to ethanol may also induce changes in baseline measurements and the subsequent response to ethanol in adulthood.

Behaviorally, alcohol has a biphasic effect on locomotor activity. Zebrafish display an inverted U-shaped dose response in an open field, with stimulant effects reported at low to moderate ethanol doses [61, 62, 90] and motor impairment and sedative effects at higher does [62]. Alcohol also decreases maximum swimming speed and increases immobility in both 'bold' and 'shy' fish [91]. Ethanol doses

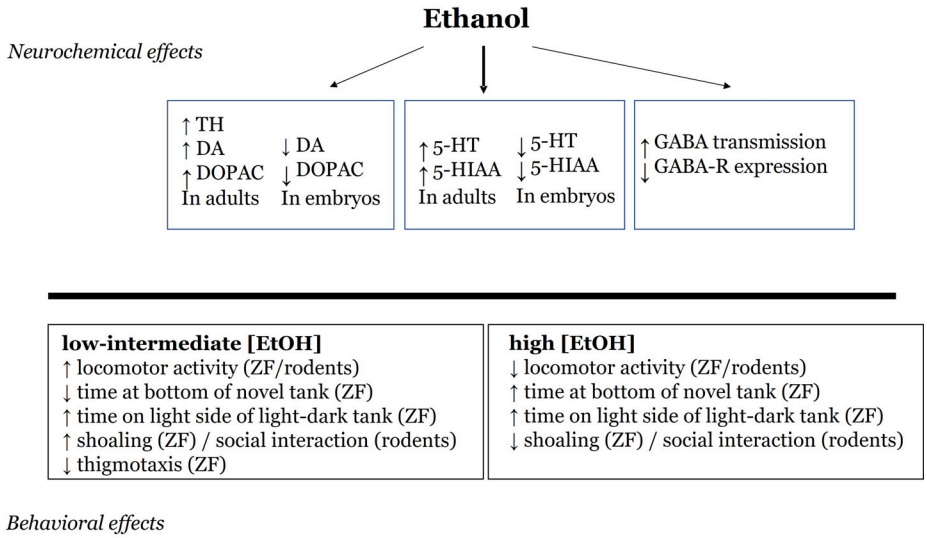

Fig. (1). Summary of ethanol-induced effects. Ethanol exposure increases dopaminergic, serotonergic, and GABAergic signaling in adult brain. Behavioral responses are dose-dependent, with low-intermediate ethanol (EtOH) doses causing stimulatory behaviors and high ethanol doses suppressing locomotion and inducing more anxiolytic behaviors. TH = tyrosine hydroxylase, DA = dopamine, DOPAC = 3,4-dihydroxyacetic acid, 5-HT = serotonin, 5-HIAA = 5-hydroxyindoleacetic acid, ↑ = enhanced or stimulated, ↓ = suppressed, ZF = zebrafish.

of 0.25% and 0.5% increase activity within 10 min of exposure compared to fish exposed to 0-1% ethanol, suggesting a time-dependent component. However, after the 10th minute, group differences in activity are less distinguished [92]. Cumulative turn angle increases as ethanol dose increases, reflecting interrupted swim paths with more direction changes [93], and angular swim velocity increases after acute ethanol withdrawal [67]. Chronically exposed fish are less responsive to an acute ethanol challenge early and late within a 60 min ethanol conditioning trial, displaying a U-shaped temporal trajectory of distance traveled [67]. Previous chronic exposure to 0.25% ethanol prevents hyperactivity induced by acute 0.25% or 0.5% ethanol treatment [94]. Though both AB and TL strain zebrafish show the same activity responses to 1% *vs* 4% ethanol administration, the response was more pronounced in the AB strain [95].

Dose- and strain-dependent effects on locomotor activity are also reported in rats [96-98]. In mice, ethanol is a locomotor stimulant at low doses [99-102], but a suppressant at high doses [97, 103, 104]. Frye and Breese [105] compared ethanol-induced changes in locomotor activity of CD-1, DBA/2J, and C57BL/6 mice with Sprague-Dawley rats. Their data revealed species-specific differences in alcohol sensitivity between rats vs. mice, as well as among mouse strains. In general, mice were more insensitive to ethanol, requiring higher doses than rats to evoke the same stimulatory response. Further, DBA/2J and CD-1 mice increased locomotor activity in response to 2 g/kg ethanol i.p. (= 2000 ppm), an effect not observed in C57BL/6 mice [105].

3.2. Behavioral Tests of Anxiety

Behavioral assays of anxiety include a variety of tests. Here, we focus on four of these tests: novel tank, scototaxis, thigmotaxis, and shoaling. These behaviors were selected because they have been quantified across a variety of paradigms exposing zebrafish to drugs of abuse and other substances. Each of these behaviors also possesses a comparable behavioral test in rodent models.

3.2.1. Novel Tank Test

Zebrafish placed into a novel tank tend to initially go to the bottom, indicating anxiety. The variable often assessed is how long the fish remain at the bottom of the tank, with less time spent on the bottom reflecting reduced anxiety [106, 107]. Ethanol dose-dependently affects novel tank test scores, with more time spent in the upper part of the novel tank with 0.5% ethanol, less time in the middle with 1% ethanol, and more time in the bottom with 0% or 1% ethanol [92]. In other words, at intermediate doses, ethanol increased time spent in the upper third of a novel tank [108] or decreased time in the bottom third [109]. This anxiolytic effect is observed in LD and longfin wildtype zebrafish, which increased distance from the bottom after 0.5% ethanol exposure [93]. In contrast, AB zebrafish spent less time in the bottom third at high (1% or 1.5%) ethanol doses [110] and increased exploration compared to controls [111]. Adult zebrafish developmentally exposed to 20 mM or 50 mM (921-2303 mg/L or ppm) ethanol also displayed decreased time on the bottom of a novel tank [112], suggesting long-term effects. However, an intermediate 0.5% ethanol dose caused AB zebrafish to decrease distance from the bottom [93]. Anxiety induced by ethanol withdrawal can also be assessed with the novel tank test [113]. In addition, female zebrafish that were previously exposed to ethanol for one week starting at 20 dpf spent more time in the bottom of the novel tank as adults, compared to males with the same ethanol exposure [114].

Habituation to a novel tank contributes to the increased time spent at the top of the tank in control fish, and anxiolytic substances can cause a similar response when administered prior to habituation trials. Although 0.3% ethanol does not contribute to altered habituation when administered acutely to zebrafish prior to the novel tank test, distinct effects emerge after chronic administration. Chronic exposure to 0.2% ethanol in the home tank for 2 wk prior to testing causes increased exploratory transitions and time spent in the

top of a novel tank, suggesting increased habituation [115]. Furthermore, chronic exposure to 0.3% ethanol for 7 d increases time spent in the top of a novel tank and increases transitions to the top; a behavior also observed following 5 min acute exposure to 0.3% ethanol [108]. Consistent with these novel tank results, there is a reduction of cortisol levels in zebrafish chronically exposed to ethanol, an anxiolytic response [106].

Adolescent rats exposed to ethanol doses ≤ 2.5 g/kg (≤ 2500 ppm) increased entries into the open arms of an elevated plus maze (EPM) [96]. In contrast, young adult (PD 70-72) and aged (18 months old) rats receiving chronic intermittent exposure spent increased time in the closed arm of the EPM [116]. Interestingly, aged rats also made fewer entries into the open arms and spent less time there during withdrawal, indicating age-dependent differences [116]. Adult rats exposed to ethanol as adolescents (drinking in the dark paradigm) displayed no significant differences in time spent in the open arm, though adult mice given an acute 2 g/kg (2000 ppm) ethanol injection increased percent time in the open arms of the maze, an anxiolytic effect [117]. Thus, in both zebrafish and rodents, ethanol exposure decreases anxiety, though species-specific differences are apparent. Anxiolytic effects of ethanol are observed across all strains and ages in zebrafish. In contrast, rodents display age-dependent differences in the ability of ethanol to decrease anxiety.

3.2.2. Light-dark Preference (Scototaxis)

Scototaxis is measured in zebrafish by placing a fish in the center of a tank that is half-dark and half-light, similar to the light-dark boxes used with rodents. Time spent on the dark side of the tank reflects increased anxiety, whereas more time on the light side reflects decreased anxiety. Though habituation of zebrafish freezing behavior is observed in a novel tank, neither freezing behavior nor light avoidance exhibit habituation in the scototaxis test, which instead shows habituation of locomotor activity [118, 119]. Freezing and erratic movement is exhibited by high-avoidant subjects when confined in the light arena, and thigmotaxis is evident in the dark compartment [118]. In addition, high avoidant subjects confined in the light compartment spend a decreased time in this area when given a free choice, while confinement of low-avoidant zebrafish contributes to more time on the light side [118].

In zebrafish, acute treatment with either 0.5% [109, 120] or 0.25% [120] ethanol has an anxiolytic effect in a modified scototaxis test, with increased time spent on the light side. Adult zebrafish developmentally exposed to ethanol also spent increased time on the light side of the tank [112]. Adult zebrafish exposed to 0.5% or 1% ethanol show more time spent on the light side compared to fish exposed to 0.25% ethanol, which is unbiased in light preference [92]. Interestingly, a daily 0.2% ethanol dose for 21 d reduced scototaxis in zebrafish when measured after 2 d of withdrawal, while weekly exposure to 1.4% ethanol did not, suggesting that dosing paradigm can affect behavior [121].

Adult rats exposed to ethanol using an adolescent intermittent exposure paradigm entered the light side of the light-dark box more often but were also more immobile than untreated adults [122]. Adolescent mice tested for drinking in the dark paradigm showed no difference in the time spent on the light side, though adult mice given an acute injection of ethanol (2 g/kg or ppm) took longer to enter the dark side and spent more time on the light side of the light-dark box [117], consistent with anxiolytic effects. In contrast, adult ethanol-exposed rats in withdrawal spent less time on, and moved less in, the light side [123], an anxiogenic response.

3.2.3. Thigmotaxis

Thigmotaxis is characterized by a preference for the edge/periphery of an open field. Though a 1 hr exposure to 0.25% - 1% ethanol prior to testing did not induce circling or thigmotaxis in adult zebrafish [124, 125], decreased, thigmotaxis was observed in 23 dpf juvenile zebrafish exposed to 20 mM and 50 mM ethanol [112].

In rodents, female PVG/c rats (which display lower anxiety than other strains [126]) exposed to 15 mg/kg/d or 30 mg/kg/d ethanol for 11 d during adolescence (PD 45-55) spent less time in the corner, and more time in the center, of an open field compared to males [127]. Time at the edge of either an open field or the Morris water maze was not significantly affected in young adult (PD 70-72) or aged (18 months) male Sprague-Dawley rats given a liquid ethanol diet [116]. Chronic (26 d) ethanol exposure followed by a long abstinence period, however, increased thigmotaxis in male Sprague-Dawley rats [128], consistent with increased anxiety during withdrawal.

3.2.4. Interactions with Conspecifics (Shoaling)

Shoaling, the loose social grouping of fish, is robust in control zebrafish, with lone individuals responding to either a live group of conspecific fish or a computer-animated shoal by decreasing their distance to the shoal relative to baseline [92, 129]. This is likely due to the sight of conspecifics eliciting a reward-response [130].

As observed for other behavioral tests, ethanol has a dose-dependent effect on shoaling. Reduced nearest neighbor distance and shoal area (or increased shoaling/interaction) occur at low-intermediate doses (0-0.5%); while an increased nearest neighbor distance and shoal area (or decreased shoaling/interaction) are observed at a high dose (1%) when fish are assessed in groups [131, 132]. For example, adult longfin striped zebrafish exposed to 0.25-1% ethanol and wild type fish exposed to 0.5-1% ethanol dose-dependently increase nearest neighbor distance [44] and decrease time spent near the stimulus fish [133]. Inter-fish distance and area occupied by the group are also increased following either acute or chronic treatment with 0.5% ethanol [44]. Adult zebrafish tested using a 3-chamber choice paradigm with a shoal reward have impaired social memory following 1% acute (30 min) ethanol treatment [134]. Thus, when tested in groups [135, 136], aggression-like response and locomotor activity are dose-dependently enhanced by intermediate ethanol doses and inhibited by a high ethanol dose [137]. Nearest neighbor distance and inter-individual distance are increased slightly, and swimming speed is decreased, though departures from the shoal are unchanged [124, 132]. These effects are suggested to emerge through mechanisms of anxiolysis at low doses of ethanol, motor impairment at high doses, and increased agonistic behavior at intermediate doses [132].

When fish are assessed individually, 0.25-1% ethanol decreases thrashing towards a shoal in AB, Long Fin, and SF adult (4 months old) zebrafish, though the distance to the stimulus is unaltered [93]. However, shoaling decreases in ethanol-naïve AB zebrafish acutely treated with 1% ethanol and SF zebrafish acutely treated with 0.25% ethanol [38], suggesting strain differences. Further, though the effects of acute alcohol challenge were reduced following chronic exposure in both AB and SF strains, withdrawal from ethanol abolished shoaling only in AB fish [38]. Zebrafish adults separated as 'bold' vs. 'shy' display opposite effects to acute ethanol exposure: 0.1% and 0.5% ethanol decrease shoaling and increase exploration in 'shy' fish; while 0.5% ethanol reduced exploration and increased socialness in 'bold' fish [91]. Others report that activity parameters are consistently decreased, though the motor function is intact, in response to the presentation of a shoal at all ethanol doses [138]. Exposure to 0.25% or 0.5% ethanol for 2 hr starting at 24 hpf alters shoal cohesion when assessed in adulthood (70 dpf), with increased inter-individual distance among members of the shoal [139].

Social interaction in male rats treated with an adolescent (PD 25-45) intermittent exposure paradigm (4 g/kg ethanol, intragastric, every other day for 11 d) reduced social preference and had fewer social investigations [140]. Compared to adults, social interaction in a familiar environment by adolescent Sprague-Dawley rats was facilitated following 0.5 g/kg (500 ppm) i.p. ethanol administration, while a higher (1 g/kg or 1000 ppm) dose reduced social activity [141], indicating age-related differences in ethanol-induced effects in this context. Thus, as in zebrafish, low doses of ethanol enhance social interactions, while high doses are inhibitory.

3.2.4.1. Summary

Overall, ethanol treatment with low/moderate doses (~0.5% or less) induces anxiolytic behavior in zebrafish (Fig. **1**) characterized by increased motor activity, reduced time at the bottom of a novel tank, increased time spent on the light side of a scototaxis chamber, decreased edge preference, and increased social interaction/shoaling. Similar behaviors are observed in rodents following low-dose ethanol administration.

4. CAFFEINE-INDUCED NEUROCHEMICAL CHANGES

Compared to ethanol, caffeine only weakly stimulates reward pathways [142]. Caffeine exposure has not been shown to change levels of DA or DOPAC in the zebrafish brain [143], nor does it alter the developmental expression of dopamine transporters (*dat*) [144], suggesting a neurochemical mechanism that is distinct from ethanol. Caffeine evokes a biphasic dose-response in rodents displayed as an initial stimulatory response, followed by a depressant response [145]. Dose-dependent behavioral effects are reported in both mammals [146-148] and zebrafish [120, 149-151]. In adult zebrafish, caffeine triggers oxidative stress [152] and increases 5-HT and 5-HIAA levels [143] in the brain. Caffeine is an acetylcholinesterase inhibitor [153] and adenosine receptor antagonist [108, 142, 154, 155], though GABAergic circuits may also be activated [148]. In agreement with findings in zebrafish, DA levels in the NAc were not elevated in rats administered caffeine *via* either i.p. or i.v.; though DA levels were increased in the medial prefrontal cortex [156]. Despite the different and opposing neurochemical effects of caffeine and ethanol, both interact through adenosine signaling pathways [1], which is discussed in a later section.

5. CAFFEINE-INDUCED BEHAVIORAL CHANGES

High doses of caffeine can induce anxiety-like responses in zebrafish [157] and, in some cases, seizure-like activity [31, 158]. Low doses, on the other hand, are reported to be beneficial, increasing the responsiveness of adult zebrafish to a visual cue [159] and increasing memory retention in adult male Wistar rats if administered either after training or just before testing on the Morris Water Maze [160]. Low dose caffeine exposure in rats also causes 'behavioral activation' characterized by significant differences in sniffing, locomotion, resting, and grooming [156]. Impulsivity in zebrafish, measured as premature responses in a 5-choice serial reaction time task, is increased following exposure to 50 or 100 μM (9.7 -19 mg/L or ppm) caffeine [143] and acute 100 μM of exposure decreased aggression in both juveniles and adults [159].

Interestingly, in some studies, caffeine's effect(s) are specific to anxiety-like behaviors, with no locomotor differences observed [31, 143, 152, 161]. Other studies report that caffeine induces both anxiety-based and locomotor changes [157, 162], and still, others report only locomotor changes [163, 164]. It is important to recognize these varied effects to help determine whether caffeine is directly affecting anxiety/stress pathways, or if the anxiety-related behavior may be confounded by locomotor changes [165].

5.1. Locomotor Tasks and Coordination

As with ethanol, there are many factors, such as the method of administration, strain, and duration of exposure, that contribute to caffeine's effects on locomotor activity. A recent study reported that i.p., injection of adult zebrafish with 100 mg/kg (100 ppm) caffeine did not affect overall locomotor activity, though erratic swimming was noted during behavioral tests [152]. Other studies report enhanced locomotor activity following 100 mg/kg injection [120], but inhibited locomotor activity when 5-50 mg/L (5-50 ppm) caffeine was dissolved in tank water [150]. Even higher caffeine doses of 1280 μM (248 mg/L) [30] or 70 mg/L (360 μM) [166] also administered in tank water reduced the total distance traveled. Caffeine exposure through tank water affects muscle fibers and axon projections of primary and secondary motor neurons, reducing zebrafish sensitivity to movement induced by touch [167] and, potentially, affecting swimming and locomotor behaviors and contributing to increased freezing behaviors.

The examples above support a biphasic effect of caffeine on locomotion which, in zebrafish, is an inverted U-shaped response [157]. Low doses of caffeine contribute to behavior indicative of acute stimulant effects, such as increased distance and speed, while at a high dose, these parameters are decreased, demonstrating depressant effects [149-151, 157, 166]. Dose-responses in rodents also reveal a biphasic profile [168, 169]. Low (30 and 100 μmol/kg i.p. = 0.58 mg/kg and 19.4 mg/kg) or moderate (3-25 mg/kg i.p.) caffeine doses

enhance locomotor activity in CD1 mice [100, 168, 170-172] while high doses (100 mg/kg i.p.) diminish activity [168]. Caffeine (50-100 μM = 9.7 – 19 mg/L) applied directly to the excised postnatal spinal cord from ICR mice also stimulates locomotor activity [64]. In rats, low caffeine doses similarly stimulate activity, and higher doses suppress activity [173]. Interestingly, the high-dose suppression of activity was observed in adult rats not habituated to the testing arena; habituated adolescent and adult rats, in contrast, displayed increased locomotor activity with increased caffeine doses [173].

Dose-dependent effects of caffeine may be confounded by strain-dependent differences. For example, low dose (1-10 μM or 0.19 – 1.9 mg/L), of caffeine does not alter the activity of adult AB zebrafish, higher doses (≥ 100 μM or ≥ 19 mg/L) increase absolute turn angle and decrease distance traveled [30]. In contrast, *leopard (leo)* zebrafish decrease total distance traveled in response to 200 mg/L caffeine, though freezing activity increased after low (25 mg/L) dose exposure and erratic swimming increased following 50 mg/L exposure [31]. In rodents, acute 50 mg/kg exposure, which was given 20 min before testing, increased ambulation and walking by PVG/c rats in the open field test, but decreased ambulation in Long-Evans and Wistar strains [126]. Chronic caffeine, on the other hand, decreased ambulation and walking in PVG/c rats [174], suggesting a chronic vs. acute effect.

5.2. Behavioral Tests of Anxiety

5.2.1. Novel Tank Test

Zebrafish adults exposed to ≥ 50 mg/L caffeine increased time spent in the bottom of a novel tank [150, 157], decreased transitions and entries into the top area, decreased time spent in the top area, and increased latency to the top [31], consistent with an anxiogenic response. Similarly, acute exposure to 100 mg/L (100 ppm) caffeine in tank water [31, 162] or 10 mg/kg (10 ppm) caffeine i.p. decreased the tendency for adults to explore the top and, for the fish that did venture to the top of the tank, it took them longer to do so. In contrast, adults exposed to 0.5-25 mg/L caffeine decreased time spent at the bottom [157], consistent with an anxiolytic response. These studies underscore the dose-dependency of caffeine's effects, with anxiety/stress responses observed in zebrafish in response to high doses.

Acute 15 min treatment with 100 mg/L caffeine does not alter habituation in adult zebrafish in terms of change in the number of transitions to the top of a novel tank and time spent at the top [115, 175]. However, erratic movements and hyperactivity increased, and exploratory behavior decreased, in caffeine-exposed fish over time, suggesting less habituation compared to controls [115, 175]. Adolescent rats assessed in an open-field (PD 48-49) also show decreased habituation with chronic caffeine intake (1 mg/mL, oral, *ad libitum*) [176].

Acute caffeine treatment decreased the number of entries and time spent by male Wistar rats in the open arms of an EPM, consistent with increased anxiety, an effect also observed during caffeine withdrawal. Chronic 21 d caffeine exposure, on the other hand, resulted in tolerance, particularly after longer exposure times (14 d and 21 d) [177]. A comparison across 3 rat strains showed that 20 min after injection with 50 mg/kg caffeine, all rats entered the open arms of the EPM more often, but decreased rearing in both open and closed arms [126], indicative of anxiogenesis. Further, chronic caffeine exposure (50 mg/kg/d) administered through drinking water significantly reduced entries into the closed arms by PVG/c rats, though open arm occupancy was increased in males. However, acute caffeine injection, with or without previous caffeine exposure, had no effect on entries or observations in the open arm [174]. PVG/c rats receiving i.p. injection of 25 mg/kg caffeine increased entries to the open arm, an anxiolytic effect [165], and suggesting that route of administration may affect caffeine-induced responses, as observed in zebrafish.

5.2.2. Light-dark Preference (Scototaxis)

Caffeine exhibits anxiogenic effects in light-dark preference tests with rodents [177-180] and zebrafish [108, 120, 151, 181]. Zebrafish injected with 100 mg/kg caffeine [120, 152] and mice injected with 50 or 100 mg/kg caffeine [178] decrease the amount of time spent in the light zone of a rectangular chamber. Additional anxiogenic effects of high-dose caffeine are observed in zebrafish adolescents and adults. These include increased freezing in the light zone, increased latency to enter the light zone, and erratic swimming [108, 120, 152, 182]. Overall, caffeine-exposed fish displayed increased scototaxis with fewer entries to the light side of the tank, less time spent on the light side, and overall reduced exploratory behavior [162, 183].

Comparing three different rodent strains revealed that caffeine (50 mg/kg i.p.) did not affect the time it took any rat strain to emerge from the light side of a light-dark box; however, there was an increase in head pokes (followed by withdrawal) into the light side. In Long-Evans rats, specifically, caffeine decreased entries to the light side overall, as well as the number of times a treated rat was observed on the light side, a caffeine-induced effect not observed in either Wistar or PVG/c rats [126].

5.2.3. Thigmotaxis

When fish are tested in groups of two, 100 mg/L caffeine increases edge preference in the absence of a visual stimulus [151]. Adult wildtype zebrafish injected with 10 mg/kg caffeine display thigmotaxis [152]. However, acute 15 min exposures to ≤ 200 mg/L caffeine in tank water had no effect on thigmotaxis in either wildtype or *leopard* zebrafish [31].

Caffeine (50 mg/kg) chronically administered through drinking water to PVG/c rats decreased center occupancy and increased immobility in an open field. However, if the same animals underwent withdrawal for 24 hr followed by an acute (50 mg/kg) caffeine injection, the rats were immobile less often, and they spent more time in the center area [174]. A direct comparison of caffeine-induced effects among PVG/c vs. Long-Evans vs. Wistar rats identified some strain-dependent differences, with many of the results indicating increased anxiety [126]. Another study, however, reported no effect of caffeine on thigmotaxis in female rats [184].

5.2.4. Interactions with Conspecifics (Shoaling)

Adult zebrafish acutely exposed to 5, 25, or 50 mg/L caffeine have spatial proximity to a robotic shoal similar to

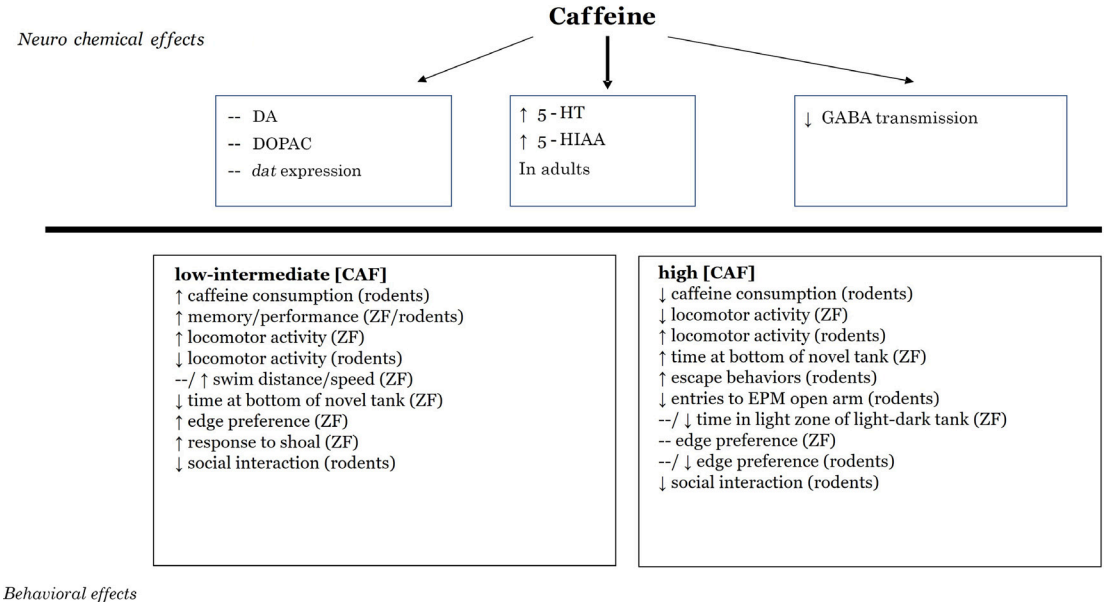

Fig. (2). Summary of caffeine-induced effects. Caffeine exposure does not alter dopamine levels, however serotonin signaling is increased and GABA transmission is decreased. Behavioral responses are dose-dependent, with low-intermediate caffeine (CAF) doses enhancing memory performance, exploratory behavior, thigmotaxis, and response to conspecifics, while high doses suppress these responses. DA = dopamine, DOPAC = 3,4-dihydroxyacetic acid, *dat* = dopamine transporter, 5-HT = serotonin, 5-HIAA = 5-hydroxyindoleacetic acid, ↑ = enhanced or stimulated, ↓ = suppressed, -- = mixed evidence, ZF = zebrafish.

controls. However, fish treated with either 25 or 50 mg/L caffeine were more responsive to movement of the robotic shoal, compared to 5 mg/L caffeine-treated fish and controls [150]. 3D neurophenotyping of zebrafish behavior identified altered shoaling after administration of 100 mg/L caffeine for 15 min. In this situation, caffeine-exposed adult fish decreased shoal volume, shoal area, and inter-fish distance. When assessed for specific shoal location, caffeine-exposed fish tended to be along the bottom of the tank and near the periphery [185]. Male Wistar rats administered 20 or 40 g/kg caffeine (i.p.) spent a decreased time on social interaction [161]. Male Wistar rats also decreased social interaction after receiving a similar caffeine dose [177]. Thus, caffeine increases anxiety and shoaling in zebrafish, but decreases social interactions in rodents.

5.2.4.1. Summary

Behavioral effects of caffeine (Fig. **2**) are biphasic with strain- and species-specific effects. Low doses of caffeine (*i.e.*, < 50 mg/kg or < 50 mg/L or < 50 ppm) increase locomotor activity, increase shoaling, and decrease time at the bottom of a novel tank. High caffeine doses decrease zebrafish locomotor activity, increase thigmotaxis, increase scototaxis, and increase anxiety in a novel tank, consistent with an angiogenic response. In rodents, high caffeine increases escape behaviors, decreases entries in to EPM open arms, and decreases social interactions, indicative of anxiogenic responses.

6. CO-EXPOSURE TO ALCOHOL AND CAFFEINE: THE 'VODKA AND RED BULL EFFECT'

In humans, adolescent alcohol consumption is increasingly accompanied by consumption of caffeine, often in the form of caffeinated energy drinks (typically 70-80 mg caffeine/ 8oz serving, but up to 242 mg caffeine/ 1.9oz serving) [186, 187], coffee (77-150 mg/ 6oz serving) and soft drinks (~34-54 mg/ 12oz serving) [188, 189]. Intake of caffeine along with alcohol brings additional implications resulting from acute effects of these substances and long-term effects of co-consumption. Alcohol mixed with caffeinated energy drinks facilitates drinking through increased stimulation and alertness and reduced fatigue and sedation, though the perception of intoxication appears to be unaltered [190]. In general, consumption of caffeinated energy drinks with alcohol is correlated with an increased risk of binge alcohol consumption [191] and an increased frequency of alcohol use [192, 193] and alcohol-related harms, such as driving under the influence [194] and aggression [195].

As detailed above, caffeine and ethanol, when administered individually, have opposite effects on the same behavioral parameters. What happens to these behaviors following co-exposure to these compounds? Though caffeine intake does not alter implicit learning that takes place in the context of ethanol exposure, caffeine can attenuate ethanol-induced explicit retrograde memory deficits [196] and 10 mg/kg caffeine co-administered with 0.65 g/kg ethanol improves trauma-induced working memory deficits in the Morris Water Maze [197]. Ethanol's effect on spatial memory correlates with a decrease in acetylcholine release and acetylcholinesterase activity in the hippocampus [198], but not the cortex [198, 199]. Ethanol induces concentration-dependent effects on acetylcholine release in the prefrontal cortex, suggesting an effect on working memory [200]. Low doses of ethanol reversed social impairment induced by moderate/high caffeine doses in rodents tested with a three-chamber box [1]. Further, studies in which caffeine and ethanol are administered sequentially, not concurrently, reveal that order of compound administration is important. For example, while

discriminative learning and exploration decreased in zebrafish during withdrawal from 0.5% ethanol; subsequent administration of 50 mg/L caffeine restored locomotor patterns and object discrimination to control levels [201]. On the other hand, chronic 50 mg/L caffeine exposure followed by acute 1% ethanol challenge during caffeine withdrawal prevented discrimination of a new object [201]. The individual and interactive effects of caffeine and ethanol are attributed to the opposite, but interactive, effects on adenosine signaling [1, 155].

6.1. The Role of Adenosine

Adenosine is a neuromodulator formed by the breakdown of ATP or cAMP that is released from neurons and glia [202-204]. Increased adenosine levels resulting from alcohol exposure activate A_1- or A_{2A}-type receptors, leading to inhibitory or stimulatory effects, respectively [202]. Unlike caffeine, ethanol does not directly bind to adenosine receptors [155]. Rather, ethanol increases extracellular adenosine levels by inhibiting ENT-1 transporters [205-208], though inhibition of catalytic enzymes that break down released adenosine [209-211] and/or the generation of acetate, which enhances adenosine synthesis [212] . In rodents, A_1 receptors are widespread in brain; A_{2A} receptors are localized to the striatum, thalamus, and olfactory tubercle [1]. Zebrafish express three A_2 receptor genes with strong homology and developmental expression patterns to mammals [144]. Given the location of these receptors, it is not surprising that adenosine modulates a variety of neurotransmitter systems, including 5-HT, DA, and GABA [203, 213].

Caffeine's pharmacological profile related to adenosine signaling is more consistent with that of stimulant drugs at lower doses [181], while anxiogenic effects are more evident at high doses [107, 108, 179, 214]. This profile is based on the differential sensitivities of adenosine receptors to caffeine: antagonism of A_{2A} receptors with low caffeine doses stimulates motor output while high caffeine doses antagonize A_1 receptors, suppressing motor output [149, 204, 215]. Though both receptor types are implicated in the locomotor-activating and reinforcing effects of caffeine in rodent models [154, 168], the A_1 receptor seems to be more involved in caffeine's anxiogenic effects [147, 168, 216, 217]. Antagonism of zebrafish A_1 receptors increased anxiety while antagonism of A_{2A} receptors caused hyperlocomotor effects [120]. Increased anxiety-like states are also observed in A_1 receptor-knockout mice [218-220]. Similarly, mice co-exposed to ethanol (1.2 g/kg i.p.) and either caffeine (30 mg/kg, i.p.) or an A_1 antagonist (DPCPX; 6 mg/kg, i.p.) displayed anxiogenic effects in the EPM [217]. Both adenosine receptor types are involved in ethanol's rewarding properties [204], though specific receptor antagonists have distinct effects on ethanol-induced behavioral responses of rodents [221-224].

During co-exposure, A_{2A} receptor antagonists, such as caffeine [225], enhance DA release [226, 227], increasing alcohol intake [224], potentiating the reinforcing effects of alcohol [108] and contributing to ethanol-seeking [154, 228]. In contrast, A_{2A} receptor activation, as would occur following ethanol exposure, inhibits DA release [226, 227]. The opposing effects of caffeine (blocker) and ethanol (activator) on adenosine receptors likely contribute to the dose-dependent effects observed following co-exposure and are mediated by the integrative effects of the A_{2A}-D_2 receptor heterodimer [142]. This heterodimer, located on GABAergic striatal neurons, is sensitive to external adenosine and dopamine levels and is able to adjust neuronal responses based on chemical concentrations and an intracellular interaction between receptor types. When a reward stimulus (ethanol) is present, the balance shifts toward D_2-receptor activation in psychomotor pathways. In contrast, activation of A_{2A} receptors by caffeine decreases DA receptor activity, but increases neuronal activity resulting in DA release [142]. A caffeine-induced increase in locomotor activity in spinal cord requires inhibition of A_1 receptors and activation of D_1 receptors through a PKA-dependent mechanism [64], similar to the A_{2A}-D_2 heterodimer complexes in the striatum [142]. Thus, there is a mechanistic link between caffeine, ethanol, and dopamine signaling.

6.1.1. Summary

The adenosine system is implicated in reward, wakefulness, and anxiety-like behaviors. This system is altered by ethanol and/or caffeine exposure. Both A_1 and A_{2A} receptors are involved in these responses, despite the opposite actions of caffeine and ethanol on these receptor types. While A_{2A} receptors appear more associated with ethanol's rewarding effects, A_1 receptors seem to mediate the anxiety and locomotory affects [155]. A commonality is that ethanol stimulates DA release and antagonism of adenosine receptors by caffeine stimulates DA release, the latter enhancing/potentiating ethanol's effects.

6.2. Co-exposure to Caffeine and Ethanol

Studies quantifying consumption of caffeine and/or ethanol use rodent models, since the animal has a clear choice of beverage and volumes consumed can be accurately measured. In zebrafish, these tests are experimentally difficult as chemicals are administered through tank water. Zebrafish may be trained to consume stable levels of 0.1%, 10% [229], or 20% ethanol mixed with gelatin, which leads to increased blood alcohol levels, increased locomotion, and reduced anxiety in a novel tank test [230]. Zebrafish will also consume caffeine powder mixed with food [231]; however, zebrafish have not yet been trained to co-consume ethanol and caffeine.

Studies in rodents have revealed that timing of ethanol *vs*. caffeine administration, dose used, and previous experience with ethanol are variables that affect subsequent ethanol intake. For example, chronic treatment with either 10 or 20 mg/kg caffeine decreases ethanol intake in rats [228] and mice [232], a result also observed in rats treated with 50 mg/kg caffeine (i.p.) [233, 234]. In contrast, most studies report that caffeine increases alcohol consumption. This has been observed (1) in male Wistar rats initially given sweetened 10% ethanol containing 1 g/L caffeine and water in a free choice paradigm and tested after 7 d of alcohol deprivation [235], (2) in C57BL/6J male mice classified as 'moderate consumers' when allowed unrestricted access to ethanol and water [232], (3) in adult P rats [228] and Wistar rats [236] treated with a low dose of caffeine (5 mg/kg) and in ethanol-experienced male Wistar rats pre-treated with 5 mg/kg caffeine [237], (4) in adolescent and adult C57BL/6J

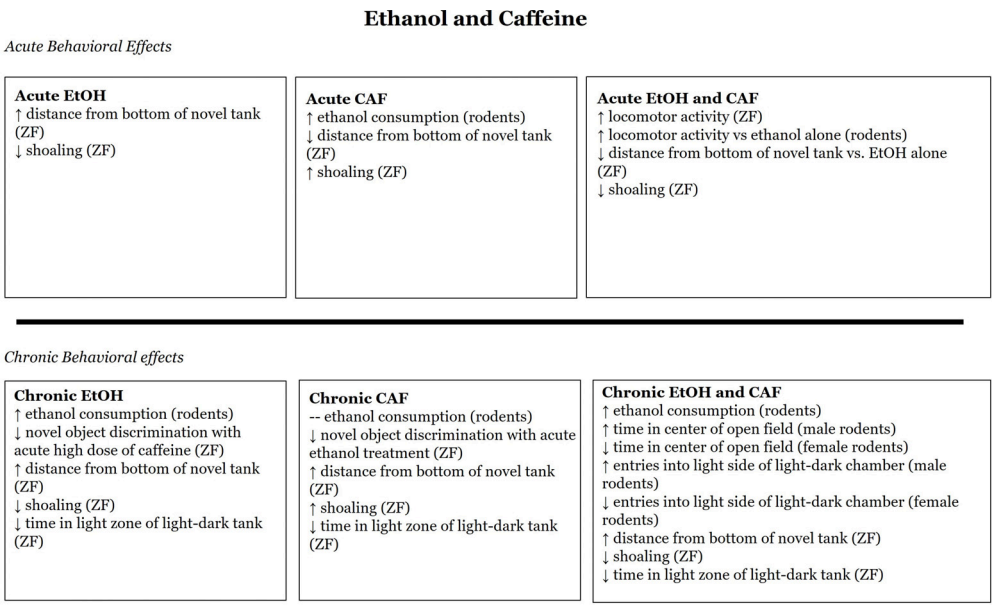

Fig. (3). Behavioral differences resulting from caffeine + ethanol co-exposure. Acute and chronic doses of ethanol (EtOH) reduce anxiety-like behaviors in zebrafish. Acute and chronic caffeine (CAF) exposure increase shoaling in zebrafish, while anxiety-related behavior in the novel tank shows opposing effects. Ethanol consumption in rodents is increased with acute caffeine and chronic ethanol exposure. Acute ethanol and caffeine exposure in zebrafish and rodents show similar effects to caffeine alone. Chronic ethanol and caffeine exposure show similar effects to chronic ethanol alone. Time in the light zone of a light dark chamber is reduced in zebrafish with chronic ethanol and caffeine exposure. ↑ = enhanced or stimulated, ↓ = suppressed, -- = mixed evidence, ZF = zebrafish.

mice with access to either caffeine, ethanol, or ethanol + caffeine for 14 d, followed by 7 d of continuous access to a choice of 10% ethanol or water [238], and (5) in alcohol-preferring female rats exposed to caffeine in adulthood [188].

6.3. Behavioral Changes and Ethanol-caffeine Co-exposure

Though there are a wealth of studies examining the individual effects of ethanol or caffeine exposure during adolescence and adulthood, there are only a few that examine the effects of co-exposure.

6.3.1. Locomotor Tasks and Coordination

Adult zebrafish co-exposed to caffeine and ethanol show effects on distance travelled and absolute turn angle that are similar to those seen with caffeine. [30]. Swim velocity, swim bout, frequency and length of freezing also increased in co-exposed zebrafish [239]. In contrast, in male CD1 mice, 25 mg/kg (i.p.) caffeine prevents motor suppression induced by 3.5-4 g/kg (i.p.) ethanol [240].

6.3.2. Behavioral Tests of Anxiety

In adult zebrafish, either caffeine alone (100 – 1280 μM or 19 – 249 mg/L) or caffeine + ethanol decreases distance and variance of distance from the bottom of a novel tank [30]. Consistent with this, when acute ethanol exposure is followed by 100 mg/L caffeine treatment immediately prior to the novel tank test, there is a greater latency to enter the top of the novel tank and fewer transitions to the top compared to controls [241]. Co-exposed zebrafish also increased latency to the top of a novel tank [239]. Thus, in zebrafish, co-exposure results in anxiety-like behavior similar to a caffeine-induced response. Using a novel object discrimination task, adult zebrafish chronically exposed to caffeine and then acutely exposed to ethanol were unable to discriminate the new object, a result also observed in fish receiving the opposite treatment (*i.e.*, chronic ethanol exposure followed by a high acute caffeine dose). However, if the chronic ethanol exposure was followed by a lower caffeine dose, the fish were able to complete the task, an effect attributed to caffeine attenuating ethanol withdrawal [201].

Our group has found similar results [242]. Acute adolescent exposure to ethanol or caffeine causes opposing effects on anxiety-like behaviors in the novel tank and shoaling tests. Ethanol-exposed zebrafish increase their distance from the bottom of a novel tank and reduce shoaling, while caffeine-exposed fish decrease their distance from the bottom and increase shoaling. Acute co-exposure decreased shoaling, similar to ethanol alone, though the distance from the bottom of a novel tank was not as increased as ethanol-exposed juveniles.

Adult zebrafish chronically exposed to ethanol during adolescence respond in the same manner as acutely exposed adolescent animals for both the novel tank and shoaling tests. Similarly, adult fish chronically exposed to caffeine during adolescence displayed shoaling behaviors similar to acutely exposed juveniles, though the distance from the bottom of a novel tank was increased in adults. Adults chronically co-exposed to ethanol + caffeine during adolescence demonstrated an increased distance from the bottom of the novel tank and decreased shoaling. In the scototaxis test, adults exposed to either chronic ethanol, chronic caffeine, or chronic ethanol + caffeine during adolescence reduced their time in the light zone of a light-dark tank ([242]; Clayman and Connaughton, manuscript in preparation).

In rodents, exposure of male rats to 1.2-1.34 g/kg/d alcohol + 24-26 mg/kg/d caffeine in their drinking water in late adolescence (PD 45-55) enhances ambulation compared to alcohol alone and displays a greater anxiolytic effect of increased time in the center of the open field in mid-adulthood (PD 120) [243]. These authors also report that the use of a slightly higher caffeine dose increased entries into the light half of the light-dark transition test and increased open field ambulation in males, while females show decreased entries of and time spent in the light half and decreased open field ambulation [243].

6.3.2.1. Summary

Behavioral responses resulting from ethanol and/or caffeine exposure depend upon whether exposure is acute vs. chronic and, in some cases, the order of compound administration (Fig. **3**). Acute and chronic doses of ethanol reduce anxiety-like behaviors in zebrafish, with chronic ethanol also impairing memory performance. Acute and chronic caffeine exposure increase shoaling in zebrafish, while anxiety-related behavior in the novel tank shows opposing effects, with decreased distance from the bottom with acute caffeine and increased distance from the bottom with chronic caffeine. Ethanol consumption in rodents increases with acute caffeine and chronic ethanol exposure. Acute ethanol + caffeine co-exposure in zebrafish and rodents show similar effects to caffeine alone, though shoaling is reduced. Chronic ethanol + caffeine co-exposure shows similar effects to chronic ethanol alone, with increased ethanol consumption in rodents and reduced anxiety-like responses in the novel tank and shoaling tests in zebrafish. Time in the light zone of a light-dark chamber is reduced in zebrafish following chronic ethanol + caffeine co-exposure, while this behavior shows a sex-difference in rodents after chronic exposure to both ethanol and caffeine, and females showing greater anxiety-related behaviors than males.

CONCLUSION

In mammals, ethanol and caffeine increase dopaminergic signaling *via* adenosine and DA receptors in the dorsal striatum and NAc [244]. Pre-exposure to caffeine in larval zebrafish and adolescent rodents influences the adult response to ethanol [245-247], with caffeine contributing to altered serotonin and DA neurotransmission [147]. The locomotor effects of ethanol and caffeine in both zebrafish and rodents are dependent on several parameters, including dose used, age of testing, and previous age of drug exposure. The locomotor effects of both substances also vary within a given exposure session and between sessions of intermittent exposure, as tolerance develops. These effects of ethanol are modulated by simultaneous treatment with caffeine in both zebrafish and rodents.

The consistent findings across species suggest that zebrafish are an effective model to further characterize the neurobiological and behavioral effects of ethanol and/or caffeine exposure. Zebrafish are amenable to high-throughput analyses involving behavioral assays for assessing anxiety-like and reward seeking behaviors, pharmacological analysis of receptor types, molecular analysis of gene expression, and anatomical consequences of exposure. These techniques, which have also been applied to similar treatment paradigms in rodents, coupled with the fast generation time, smaller size, and ability to test multiple individuals at the same time, further suggest zebrafish are an ideal model to assess pharmaceuticals, especially those which influence anxiety-related behaviors.

These various considerations are essential not only to establish a consensus about the influence of alcohol and stimulant co-exposure in animal models, but for translating the findings to humans. This is especially relevant in the context of alcohol and caffeine co-consumption in human adolescents, as this has implications for the development of addiction in adulthood. Since the previous drug exposure history of animal models may be controlled, this approach should be utilized to examine the influence of various patterns of treatments and combinations of drug doses. Through further analysis of acute and chronic treatments across a variety of treatment regimens and time-points in development, a clearer picture will emerge of the influence of ethanol and caffeine in adolescence, along with its implications for adult neural and behavioral responses.

AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to analysis, interpretation, and summarization of the literature on the topic of alcohol and caffeine exposure in zebrafish and rodent models, and on formatting, writing, and revising the manuscript prior to submission.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors wish to thank L. Juliano for helpful comments on previous versions of the manuscript.

REFERENCES

[1] Correa, M.; Lopez-Cruz, L.; Porru, S.; Salamone, J. The impact of ethanol plus caffeine exposure on cognitive, emotional, and motivational effects related to social functioning. Neuroscience of Alcohol*;* Elsevier, **2019**, pp. 545-554.

[2] Tran, S. Acute and chronic alcohol effects in zebrafish. Behavioral and Neural Genetics of Zebrafish*;* Elsevier, **2020**, pp. 325-341. http://dx.doi.org/10.1016/B978-0-12-817528-6.00020-6

[3] Abozaid, A.; Trzuskot, L.; Najmi, Z.; Paul, I.; Tsang, B.; Gerlai, R. Developmental stage and genotype dependent behavioral effects of embryonic alcohol exposure in zebrafish larvae. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **2020**, *97*, 109774. http://dx.doi.org/10.1016/j.pnpbp.2019.109774 PMID: 31655157

[4] Fernandes, Y.; Buckley, D.M.; Eberhart, J.K. Diving into the world of alcohol teratogenesis: a review of zebrafish models of fetal alcohol spectrum disorder. *Biochem. Cell Biol.,* **2018**, *96*(2), 88-97. http://dx.doi.org/10.1139/bcb-2017-0122 PMID: 28817785

[5] Lovely, C.B.; Fernandes, Y.; Eberhart, J.K. Fishing for fetal alcohol spectrum disorders: zebrafish as a model for ethanol teratogenesis. *Zebrafish,* **2016**, *13*(5), 391-398. http://dx.doi.org/10.1089/zeb.2016.1270 PMID: 27186793

[6] Pinheiro-da-Silva, J.; Luchiari, A.C. Embryonic ethanol exposure on zebrafish early development. *Brain Behav.,* **2021**, *11*(6), e02062. http://dx.doi.org/10.1002/brb3.2062 PMID: 33939334

[7] Parichy, D.M.; Elizondo, M.R.; Mills, M.G.; Gordon, T.N.; Engeszer, R.E. Normal table of postembryonic zebrafish development: staging by externally visible anatomy of the living fish. *Dev. Dyn.,* **2009**, *238*(12), 2975-3015. http://dx.doi.org/10.1002/dvdy.22113 PMID: 19891001

[8] Westphal, R.E.; O'Malley, D.M. Fusion of locomotor maneuvers, and improving sensory capabilities, give rise to the flexible homing strikes of juvenile zebrafish. *Front. Neural Circuits,* **2013**, *7*, 108. http://dx.doi.org/10.3389/fncir.2013.00108 PMID: 23761739

[9] O'Neill, C.E.; Levis, S.C.; Schreiner, D.C.; Amat, J.; Maier, S.F.; Bachtell, R.K. Effects of adolescent caffeine consumption on cocaine sensitivity. *Neuropsychopharmacology,* **2015**, *40*(4), 813-821. http://dx.doi.org/10.1038/npp.2014.278 PMID: 25328052

[10] Vorhees, C.V.; Reed, T.M.; Morford, L.L.; Fukumura, M.; Wood, S.L.; Brown, C.A.; Skelton, M.R.; McCrea, A.E.; Rock, S.L.; Williams, M.T. Periadolescent rats (P41-50) exhibit increased susceptibility to D-methamphetamine-induced long-term spatial and sequential learning deficits compared to juvenile (P21-30 or P31-40) or adult rats (P51-60). *Neurotoxicol. Teratol.,* **2005**, *27*(1), 117-134. http://dx.doi.org/10.1016/j.ntt.2004.09.005 PMID: 15681126

[11] Robins, M.T.; Lu, J.; van Rijn, R.M. Unique behavioral and neurochemical effects induced by repeated adolescent consumption of caffeine-mixed alcohol in C57B/6 mice. *PLoS One,* **2016**, *11*(7), e0158189. http://dx.doi.org/10.1371/journal.pone.0158189 PMID: 27380261

[12] Arain, M.; Haque, M.; Johal, L.; Mathur, P.; Nel, W.; Rais, A.; Sandhu, R.; Sharma, S. Maturation of the adolescent brain. *Neuropsychiatr. Dis. Treat.,* **2013**, *9*, 449-461. PMID: 23579318

[13] Casey, B.J.; Getz, S.; Galvan, A. The adolescent brain. *Dev. Rev.,* **2008**, *28*(1), 62-77. http://dx.doi.org/10.1016/j.dr.2007.08.003 PMID: 18688292

[14] Smith, R.F. Animal models of periadolescent substance abuse. *Neurotoxicol. Teratol.,* **2003**, *25*(3), 291-301. http://dx.doi.org/10.1016/S0892-0362(02)00349-5 PMID: 12757826

[15] Crews, F.T.; Vetreno, R.P.; Broadwater, M.A.; Robinson, D.L. Adolescent alcohol exposure persistently impacts adult neurobiology and behavior. *Pharmacol. Rev.,* **2016**, *68*(4), 1074-1109. http://dx.doi.org/10.1124/pr.115.012138 PMID: 27677720

[16] Cole, G.J.; Zhang, C.; Ojiaku, P.; Bell, V.; Devkota, S.; Mukhopadhyay, S. Effects of ethanol exposure on nervous system development in zebrafish. *Int. Rev. Cell Mol. Biol.,* **2012**, *299*, 255-315. http://dx.doi.org/10.1016/B978-0-12-394310-1.00007-2 PMID: 22959306

[17] De Bellis, M.D.; Van Voorhees, E.; Hooper, S.R.; Gibler, N.; Nelson, L.; Hege, S.G.; Payne, M.E.; MacFall, J. Diffusion tensor measures of the corpus callosum in adolescents with adolescent onset alcohol use disorders. *Alcohol. Clin. Exp. Res.,* **2008**, *32*(3), 395-404. http://dx.doi.org/10.1111/j.1530-0277.2007.00603.x PMID: 18241319

[18] Silveri, M.M.; Rohan, M.L.; Pimentel, P.J.; Gruber, S.A.; Rosso, I.M.; Yurgelun-Todd, D.A. Sex differences in the relationship between white matter microstructure and impulsivity in adolescents. *Magn. Reson. Imaging,* **2006**, *24*(7), 833-841. http://dx.doi.org/10.1016/j.mri.2006.03.012 PMID: 16916700

[19] Spear, L. Modeling adolescent development and alcohol use in animals. *Alcohol Res. Health,* **2000**, *24*(2), 115-123. PMID: 11199278

[20] Spear, L.P.; Swartzwelder, H.S. Adolescent alcohol exposure and persistence of adolescent-typical phenotypes into adulthood: a mini-review. *Neurosci. Biobehav. Rev.,* **2014**, *45*, 1-8. http://dx.doi.org/10.1016/j.neubiorev.2014.04.012 PMID: 24813805

[21] Séguret, A.; Collignon, B.; Halloy, J. Strain differences in the collective behaviour of zebrafish (*Danio rerio*) in heterogeneous environment. *R. Soc. Open Sci.,* **2016**, *3*(10), 160451. http://dx.doi.org/10.1098/rsos.160451 PMID: 27853558

[22] Spinello, C.; Macrì, S.; Porfiri, M. Acute ethanol administration affects zebrafish preference for a biologically inspired robot. *Alcohol,* **2013**, *47*(5), 391-398. http://dx.doi.org/10.1016/j.alcohol.2013.04.003 PMID: 23725654

[23] Vignet, C.; Bégout, M-L.; Péan, S.; Lyphout, L.; Leguay, D.; Cousin, X. Systematic screening of behavioral responses in two zebrafish strains. *Zebrafish,* **2013**, *10*(3), 365-375. http://dx.doi.org/10.1089/zeb.2013.0871 PMID: 23738739

[24] Holden, L.A.; Brown, K.H. Baseline mRNA expression differs widely between common laboratory strains of zebrafish. *Sci. Rep.,* **2018**, *8*(1), 4780. http://dx.doi.org/10.1038/s41598-018-23129-4 PMID: 29555936

[25] 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. *Physiol. Behav.,* **2012**, *107*(5), 773-780. http://dx.doi.org/10.1016/j.physbeh.2012.01.017 PMID: 22313674

[26] van den Bos, R.; Mes, W.; Galligani, P.; Heil, A.; Zethof, J.; Flik, G.; Gorissen, M. Further characterisation of differences between TL and AB zebrafish (*Danio rerio*): Gene expression, physiology and behaviour at day 5 of the larval stage. *PLoS One,* **2017**, *12*(4), e0175420. http://dx.doi.org/10.1371/journal.pone.0175420 PMID: 28419104

[27] Guryev, V.; Koudijs, M.J.; Berezikov, E.; Johnson, S.L.; Plasterk, R.H.; van Eeden, F.J.; Cuppen, E. Genetic variation in the zebrafish. *Genome Res.,* **2006**, *16*(4), 491-497. http://dx.doi.org/10.1101/gr.4791006 PMID: 16533913

[28] van den Bos, R.; Althuizen, J.; Tschigg, K.; Bomert, M.; Zethof, J.; Filk, G.; Gorissen, M. Early life exposure to cortisol in zebrafish (*Danio rerio*): similarities and differences in behaviour and physiology between larvae of the AB and TL strains. *Behav. Pharmacol.,* **2019**, *30*(2 and 3-Spec Issue), 260-271. http://dx.doi.org/10.1097/FBP.0000000000000470 PMID: 30724799

[29] Chatterjee, D.; Shams, S.; Gerlai, R. Chronic and acute alcohol administration induced neurochemical changes in the brain: comparison of distinct zebrafish populations. *Amino Acids,* **2014**, *46*(4), 921-930. http://dx.doi.org/10.1007/s00726-013-1658-y PMID: 24381007

[30] Tran, S.; Fulcher, N.; Nowicki, M.; Desai, P.; Tsang, B.; Facciol, A.; Chow, H.; Gerlai, R. Time-dependent interacting effects of caffeine, diazepam, and ethanol on zebrafish behaviour. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **2017**, *75*, 16-27. b http://dx.doi.org/10.1016/j.pnpbp.2016.12.004 PMID: 28025019

[31] Rosa, L.V.; Ardais, A.P.; Costa, F.V.; Fontana, B.D.; Quadros, V.A.; Porciúncula, L.O.; Rosemberg, D.B. Different effects of caffeine on behavioral neurophenotypes of two zebrafish populations. *Pharmacol. Biochem. Behav.,* **2018**, *165*, 1-8. http://dx.doi.org/10.1016/j.pbb.2017.12.002 PMID: 29241648

[32] Das, S.K.; Vasudevan, D.M. Alcohol-induced oxidative stress. *Life Sci.,* **2007**, *81*(3), 177-187. http://dx.doi.org/10.1016/j.lfs.2007.05.005 PMID: 17570440

[33] Zima, T.; Fialová, L.; Mestek, O.; Janebová, M.; Crkovská, J.; Malbohan, I.; Stípek, S.; Mikulíková, L.; Popov, P. Oxidative stress, metabolism of ethanol and alcohol-related diseases. *J. Biomed. Sci.,* **2001**, *8*(1), 59-70. http://dx.doi.org/10.1007/BF02255972 PMID: 11173977

[34] Comporti, M.; Signorini, C.; Leoncini, S.; Gardi, C.; Ciccoli, L.; Giardini, A.; Vecchio, D.; Arezzini, B. Ethanol-induced oxidative stress: basic knowledge. *Genes Nutr.,* **2010**, *5*(2), 101-109. http://dx.doi.org/10.1007/s12263-009-0159-9 PMID: 20606811

[35] Krystal, J.; Tabakoff, B. *Ethanol abuse, dependence, and withdrawal: neurobiology and clinical implications. Psychopharmacol A Fifth Gener Progress*; Lippincott Williams & Wilkins: Philadelphia, PA, **2002**, pp. 1425-1443.

[36] Walter, H.J.; Messing, R.O. Regulation of neuronal voltage-gated calcium channels by ethanol. *Neurochem. Int.,* **1999**, *35*(2), 95-101. http://dx.doi.org/10.1016/S0197-0186(99)00050-9 PMID: 10405992

[37] Facciol, A.; Bailleul, C.; Nguyen, S.; Chatterjee, D.; Gerlai, R. Developmental stage-dependent deficits induced by embryonic ethanol exposure in zebrafish: A neurochemical analysis. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **2020**, *99*, 109859. http://dx.doi.org/10.1016/j.pnpbp.2020.109859 PMID: 31917146

[38] Gerlai, R.; Chatterjee, D.; Pereira, T.; Sawashima, T.; Krishnannair, R. Acute and chronic alcohol dose: population differences in behavior and neurochemistry of zebrafish. *Genes Brain Behav.,* **2009**, *8*(6), 586-599. http://dx.doi.org/10.1111/j.1601-183X.2009.00488.x PMID: 19243447

[39] Mahabir, S.; Chatterjee, D.; Gerlai, R. Strain dependent neurochemical changes induced by embryonic alcohol exposure in zebrafish. *Neurotoxicol. Teratol.,* **2014**, *41*, 1-7. http://dx.doi.org/10.1016/j.ntt.2013.11.001 PMID: 24225385

[40] Mahabir, S.; Chatterjee, D.; Gerlai, R. Short exposure to low concentrations of alcohol during embryonic development has only subtle and strain- dependent effect on the levels of five amino acid neurotransmitters in zebrafish. *Neurotoxicol. Teratol.,* **2018**, *68*, 91-96. http://dx.doi.org/10.1016/j.ntt.2018.05.005 PMID: 29886245

[41] Maximino, C.; Herculano, A.M. A review of monoaminergic neuropsychopharmacology in zebrafish. *Zebrafish,* **2010**, *7*(4), 359-378. http://dx.doi.org/10.1089/zeb.2010.0669 PMID: 21158565

[42] Renier, C.; Faraco, J.H.; Bourgin, P.; Motley, T.; Bonaventure, P.; Rosa, F.; Mignot, E. Genomic and functional conservation of sedative-hypnotic targets in the zebrafish. *Pharmacogenet. Genomics,* **2007**, *17*(4), 237-253. http://dx.doi.org/10.1097/FPC.0b013e3280119d62 PMID: 17496723

[43] Schweitzer, J.; Driever, W. Development of the dopamine systems in zebrafish. *Adv. Exp. Med. Biol.,* **2009**, *651*, 1-14. http://dx.doi.org/10.1007/978-1-4419-0322-8_1 PMID: 19731546

[44] Dlugos, C.A.; Rabin, R.A. Ethanol effects on three strains of zebrafish: model system for genetic investigations. *Pharmacol. Biochem. Behav.,* **2003**, *74*(2), 471-480. http://dx.doi.org/10.1016/S0091-3057(02)01026-2 PMID: 12479969

[45] Lin, J-N.; Chang, L-L.; Lai, C-H.; Lin, K-J.; Lin, M-F.; Yang, C-H.; Lin, H.H.; Chen, Y.H. Development of an animal model for alcoholic liver disease in zebrafish. *Zebrafish,* **2015**, *12*(4), 271-280. http://dx.doi.org/10.1089/zeb.2014.1054 PMID: 25923904

[46] Crews, F.T.; Braun, C.J.; Hoplight, B.; Switzer, R.C., III; Knapp, D.J. Binge ethanol consumption causes differential brain damage in young adolescent rats compared with adult rats. *Alcohol. Clin. Exp. Res.,* **2000**, *24*(11), 1712-1723. http://dx.doi.org/10.1111/j.1530-0277.2000.tb01973.x PMID: 11104119

[47] Ehlers, C.L.; Criado, J.R. Adolescent ethanol exposure: does it produce long-lasting electrophysiological effects? *Alcohol,* **2010**, *44*(1), 27-37. http://dx.doi.org/10.1016/j.alcohol.2009.09.033 PMID: 20113872

[48] Maldonado-Devincci, A.M.; Badanich, K.A.; Kirstein, C.L. Alcohol during adolescence selectively alters immediate and long-term behavior and neurochemistry. *Alcohol,* **2010**, *44*(1), 57-66. http://dx.doi.org/10.1016/j.alcohol.2009.09.035 PMID: 20113874

[49] Nagel, B.J.; Schweinsburg, A.D.; Phan, V.; Tapert, S.F. Reduced hippocampal volume among adolescents with alcohol use disorders without psychiatric comorbidity. *Psychiatry Res.,* **2005**, *139*(3), 181-190. http://dx.doi.org/10.1016/j.pscychresns.2005.05.008 PMID: 16054344

[50] Witt, E.D. Research on alcohol and adolescent brain development: opportunities and future directions. *Alcohol,* **2010**, *44*(1), 119-124. http://dx.doi.org/10.1016/j.alcohol.2009.08.011 PMID: 20113880

[51] Arias-Carrión, O.; Stamelou, M.; Murillo-Rodríguez, E.; Menéndez-González, M.; Pöppel, E. Dopaminergic reward system: a short integrative review. *Int. Arch. Med.,* **2010**, *3*, 24. http://dx.doi.org/10.1186/1755-7682-3-24 PMID: 20925949

[52] Ayano, G. Dopamine: receptors, functions, synthesis, pathways, locations and mental disorders: review of literatures. *J Ment Disord Treat.,* **2016**, *2*, 10000120. http://dx.doi.org/10.4172/2471-271X.1000120

[53] Ma, H.; Zhu, G. The dopamine system and alcohol dependence. *Shanghai Jingshen Yixue,* **2014**, *26*(2), 61-68. PMID: 25092951

[54] Carrara-Nascimento, P.F.; Hoffmann, L.B.; Flório, J.C.; Planeta, C.S.; Camarini, R. Effects of ethanol exposure during adolescence or adulthood on locomotor sensitization and dopamine levels in the reward system. *Front. Behav. Neurosci.,* **2020**, *14*, 31. http://dx.doi.org/10.3389/fnbeh.2020.00031 PMID: 32210774

[55] Ramachandra, V.; Phuc, S.; Franco, A.C.; Gonzales, R.A. Ethanol preference is inversely correlated with ethanol-induced dopamine release in 2 substrains of C57BL/6 mice. *Alcohol. Clin. Exp. Res.,* **2007**, *31*(10), 1669-1676. http://dx.doi.org/10.1111/j.1530-0277.2007.00463.x PMID: 17651469

[56] Tang, A.; George, M.A.; Randall, J.A.; Gonzales, R.A. Ethanol increases extracellular dopamine concentration in the ventral striatum in C57BL/6 mice. *Alcohol. Clin. Exp. Res.,* **2003**, *27*(7), 1083-1089. http://dx.doi.org/10.1097/01.ALC.0000075825.14331.65 PMID: 12878914

[57] Bassareo, V.; Cucca, F.; Frau, R.; Di Chiara, G. Changes in dopamine transmission in the nucleus accumbens shell and core during ethanol and sucrose self-administration. *Front. Behav. Neurosci.,* **2017**, *11*, 71. http://dx.doi.org/10.3389/fnbeh.2017.00071 PMID: 28507512

[58] Weiss, F.; Lorang, M.T.; Bloom, F.E.; Koob, G.F. Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. *J. Pharmacol. Exp. Ther.,* **1993**, *267*(1), 250-258. PMID: 8229752

[59] Rink, E.; Wullimann, M.F. The teleostean (zebrafish) dopaminergic system ascending to the subpallium (striatum) is located in the basal diencephalon (posterior tuberculum). *Brain Res.,* **2001**, *889*(1-2), 316-330. http://dx.doi.org/10.1016/S0006-8993(00)03174-7 PMID: 11166725

[60] Alexandre, M.C.M.; Mendes, N.V.; Torres, C.A.; Baldin, S.L.; Bernardo, H.T.; Scussel, R.; Baggio, S.; Mussulini, B.H.M.; Zenki, K.C.; da Rosa, M.I.; Rico, E.P. Weekly ethanol exposure alters dopaminergic parameters in zebrafish brain. *Neurotoxicol. Teratol.,* **2019**, *75*, 106822. http://dx.doi.org/10.1016/j.ntt.2019.106822 PMID: 31421226

[61] Nowicki, M.; Tran, S.; Chatterjee, D.; Gerlai, R. Inhibition of phosphorylated tyrosine hydroxylase attenuates ethanol-induced hyperactivity in adult zebrafish (*Danio rerio*). *Pharmacol. Biochem. Behav.,* **2015**, *138*, 32-39. http://dx.doi.org/10.1016/j.pbb.2015.09.008 PMID: 26366782

[62] Rosemberg, D.B.; Braga, M.M.; Rico, E.P.; Loss, C.M.; Córdova, S.D.; Mussulini, B.H.; Blaser, R.E.; Leite, C.E.; Campos, M.M.; Dias, R.D.; Calcagnotto, M.E.; de Oliveira, D.L.; Souza, D.O. Behavioral effects of taurine pretreatment in zebrafish acutely exposed to ethanol. *Neuropharmacology,* **2012**, *63*(4), 613-623. http://dx.doi.org/10.1016/j.neuropharm.2012.05.009 PMID: 22634362

[63] Weiner, N.; Molinoff, P. Catecholamines.*Basic Neurochemistry,* 5th ed; Siegel, G.; Agranoff, B.; Albers, R.; Molinoff, P., Eds.; Raven Press: New York, **1994**, pp. 261-281.

[64] Acevedo, J.; Santana-Almansa, A.; Matos-Vergara, N.; Marrero-Cordero, L.R.; Cabezas-Bou, E.; Díaz-Ríos, M. Caffeine stimulates locomotor activity in the mammalian spinal cord via adenosine A1 receptor-dopamine D1 receptor interaction and PKA-dependent mechanisms. *Neuropharmacology,* **2016**, *101*, 490-505. http://dx.doi.org/10.1016/j.neuropharm.2015.10.020 PMID: 26493631

[65] Navarrete, F.; Rubio, G.; Manzanares, J. Effects of naltrexone plus topiramate on ethanol self-administration and tyrosine hydroxylase gene expression changes. *Addict. Biol.,* **2014**, *19*(5), 862-873. http://dx.doi.org/10.1111/adb.12058 PMID: 23573810

[66] Li, X.; Li, X.; Li, Y-X.; Zhang, Y.; Chen, D.; Sun, M-Z.; Zhao, X.; Chen, D.Y.; Feng, X.Z. The difference between anxiolytic and anxiogenic effects induced by acute and chronic alcohol exposure and changes in associative learning and memory based on color preference and the cause of Parkinson-like behaviors in zebrafish. *PLoS One,* **2015**, *10*(11), e0141134.

http://dx.doi.org/10.1371/journal.pone.0141134 PMID: 26558894

[67] Tran, S.; Gerlai, R. Time-course of behavioural changes induced by ethanol in zebrafish (*Danio rerio*). *Behav. Brain Res.,* **2013**, *252*, 204-213. http://dx.doi.org/10.1016/j.bbr.2013.05.065 PMID: 23756142

[68] Paiva, I.M.; Sartori, B.M.; Castro, T.F.D.; Lunkes, L.C.; Virote, B.D.C.R.; Murgas, L.D.S.; de Souza, R.P.; Brunialti-Godard, A.L. Behavioral plasticity and gene regulation in the brain during an intermittent ethanol exposure in adult zebrafish population. *Pharmacol. Biochem. Behav.,* **2020**, *192*, 172909. http://dx.doi.org/10.1016/j.pbb.2020.172909 PMID: 32194086

[69] Frazer, A.; Hensler, J. Serotonin.*Basic Neurochemistry,* 5th ed; Siegel, G.; Agranoff, B.; Albers, R.; Molinoff, P., Eds.; Raven Press: New York, **1994**, pp. 283-308.

[70] Herculano, A.M.; Maximino, C. Serotonergic modulation of zebrafish behavior: towards a paradox. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **2014**, *55*, 50-66. http://dx.doi.org/10.1016/j.pnpbp.2014.03.008 PMID: 24681196

[71] Maximino, C.; Lima, M.; Araujo, J.; Oliveira, K.; Herculano, A.; Stewart, A. The serotonergic system of zebrafish: genomics, neuroanatomy, and neuropharmacology. Serotonin Biosynthesis, Regul Heal Implic. New York, NY. *Nova Sci.,* **2013**, 53-67.

[72] Chatterjee, D.; Gerlai, R. High precision liquid chromatography analysis of dopaminergic and serotoninergic responses to acute alcohol exposure in zebrafish. *Behav. Brain Res.,* **2009**, *200*(1), 208-213. http://dx.doi.org/10.1016/j.bbr.2009.01.016 PMID: 19378384

[73] Langen, B.; Dietze, S.; Fink, H. Acute effect of ethanol on anxiety and 5-HT in the prefrontal cortex of rats. *Alcohol,* **2002**, *27*(2), 135-141. http://dx.doi.org/10.1016/S0741-8329(02)00219-7 PMID: 12106833

[74] Thielen, R.J.; Bare, D.J.; McBride, W.J.; Lumeng, L.; Li, T.K. Ethanol-stimulated serotonin release in the ventral hippocampus: an absence of rapid tolerance for the alcohol-preferring P rat and insensitivity in the alcohol-nonpreferring NP rat. *Pharmacol. Biochem. Behav.,* **2002**, *71*(1-2), 111-117. http://dx.doi.org/10.1016/S0091-3057(01)00633-5 PMID: 11812514

[75] Yan, Q.S. Extracellular dopamine and serotonin after ethanol monitored with 5-minute microdialysis. *Alcohol,* **1999**, *19*(1), 1-7. http://dx.doi.org/10.1016/S0741-8329(99)00006-3 PMID: 10487381

[76] Lovinger, D.M. Serotonin's role in alcohol's effects on the brain. *Alcohol Health Res. World,* **1997**, *21*(2), 114-120. PMID: 15704346

[77] Tabakoff, B.; Boggan, W.O. Effects of ethanol on serotonin metabolism in brain. *J. Neurochem.,* **1974**, *22*(5), 759-764. http://dx.doi.org/10.1111/j.1471-4159.1974.tb04291.x PMID: 4407097

[78] LeMarquand, D.; Pihl, R.O.; Benkelfat, C. Serotonin and alcohol intake, abuse, and dependence: findings of animal studies. *Biol. Psychiatry,* **1994**, *36*(6), 395-421. b http://dx.doi.org/10.1016/0006-3223(94)91215-7 PMID: 7803601

[79] Naito, A.; Muchhala, K.H.; Asatryan, L.; Trudell, J.R.; Homanics, G.E.; Perkins, D.I.; Davies, D.L.; Alkana, R.L. Glycine and GABA(A) ultra-sensitive ethanol receptors as novel tools for alcohol and brain research. *Mol. Pharmacol.,* **2014**, *86*(6), 635-646. http://dx.doi.org/10.1124/mol.114.093773 PMID: 25245406

[80] Wu, G.; Liu, H.; Jin, J.; Hong, L.; Lan, Y.; Chu, C.P.; Qiu, D.L. Ethanol attenuates sensory stimulus-evoked responses in cerebellar granule cells *via* activation of GABA(A) receptors *in vivo* in mice. *Neurosci. Lett.,* **2014**, *561*, 107-111. http://dx.doi.org/10.1016/j.neulet.2013.12.049 PMID: 24388841

[81] Paul, S.M. Alcohol-sensitive GABA receptors and alcohol antagonists. *Proc. Natl. Acad. Sci. USA,* **2006**, *103*(22), 8307-8308. http://dx.doi.org/10.1073/pnas.0602862103 PMID: 16717187

[82] Roberto, M.; Madamba, S.G.; Moore, S.D.; Tallent, M.K.; Siggins, G.R. Ethanol increases GABAergic transmission at both pre- and postsynaptic sites in rat central amygdala neurons. *Proc. Natl. Acad. Sci. USA,* **2003**, *100*(4), 2053-2058. http://dx.doi.org/10.1073/pnas.0437926100 PMID: 12566570

[83] Goodman, A.C.; Wong, R.Y. Differential effects of ethanol on behavior and $GABA_A$ receptor expression in adult zebrafish (*Danio rerio*) with alternative stress coping styles. *Sci. Rep.,* **2020**, *10*(1), 13076. http://dx.doi.org/10.1038/s41598-020-69980-2 PMID: 32753576

[84] Roberto, M.; Madamba, S.G.; Stouffer, D.G.; Parsons, L.H.; Siggins, G.R. Increased GABA release in the central amygdala of ethanol-dependent rats. *J. Neurosci.,* **2004**, *24*(45), 10159-10166. http://dx.doi.org/10.1523/JNEUROSCI.3004-04.2004 PMID: 15537886

[85] Sundstrom-Poromaa, I.; Smith, D.H.; Gong, Q.H.; Sabado, T.N.; Li, X.; Light, A.; Wiedmann, M.; Williams, K.; Smith, S.S. Hormonally regulated alpha(4)beta(2)delta GABA(A) receptors are a target for alcohol. *Nat. Neurosci.,* **2002**, *5*(8), 721-722. http://dx.doi.org/10.1038/nn888 PMID: 12118257

[86] Borghese, C.M.; Stórustovu, Sí.; Ebert, B.; Herd, M.B.; Belelli, D.; Lambert, J.J.; Marshall, G.; Wafford, K.A.; Harris, R.A. The delta subunit of gamma-aminobutyric acid type A receptors does not confer sensitivity to low concentrations of ethanol. *J. Pharmacol. Exp. Ther.,* **2006**, *316*(3), 1360-1368. http://dx.doi.org/10.1124/jpet.105.092452 PMID: 16272217

[87] Carvan, M.J., III; Loucks, E.; Weber, D.N.; Williams, F.E. Ethanol effects on the developing zebrafish: neurobehavior and skeletal morphogenesis. *Neurotoxicol. Teratol.,* **2004**, *26*(6), 757-768. http://dx.doi.org/10.1016/j.ntt.2004.06.016 PMID: 15451040

[88] Zhang, C.; Ojiaku, P.; Cole, G.J. Forebrain and hindbrain development in zebrafish is sensitive to ethanol exposure involving agrin, Fgf, and sonic hedgehog function. *Birth Defects Res. A Clin. Mol. Teratol.,* **2013**, *97*(1), 8-27. [Part A]. http://dx.doi.org/10.1002/bdra.23099 PMID: 23184466

[89] Mathur, P.; Berberoglu, M.A.; Guo, S. Preference for ethanol in zebrafish following a single exposure. *Brain Behav Res.,* **2011**, *217*(1), 128-133. http://dx.doi.org/10.1016/j.bbr.2010.10.015 PMID: 20974186

[90] Blaser, R.E.; Rosemberg, D.B. Measures of anxiety in zebrafish (*Danio rerio*): dissociation of black/white preference and novel tank test. *PLoS One,* **2012**, *7*(5), e36931. http://dx.doi.org/10.1371/journal.pone.0036931 PMID: 22615849

[91] Araujo-Silva, H.; Pinheiro-da-Silva, J.; Silva, P.F.; Luchiari, A.C. Individual differences in response to alcohol exposure in zebrafish (*Danio rerio*). *PLoS One,* **2018**, *13*(6), e0198856. http://dx.doi.org/10.1371/journal.pone.0198856 PMID: 29879208

[92] Gerlai, R.; Lahav, M.; Guo, S.; Rosenthal, A. Drinks like a fish: zebra fish (*Danio rerio*) as a behavior genetic model to study alcohol effects. *Pharmacol. Biochem. Behav.,* **2000**, *67*(4), 773-782. http://dx.doi.org/10.1016/S0091-3057(00)00422-6 PMID: 11166068

[93] Gerlai, R.; Ahmad, F.; Prajapati, S. Differences in acute alcohol-induced behavioral responses among zebrafish populations. *Alcohol. Clin. Exp. Res.,* **2008**, *32*(10), 1763-1773. http://dx.doi.org/10.1111/j.1530-0277.2008.00761.x PMID: 18652595

[94] Gerlai, R.; Lee, V.; Blaser, R. Effects of acute and chronic ethanol exposure on the behavior of adult zebrafish (*Danio rerio*). *Pharmacol. Biochem. Behav.,* **2006**, *85*(4), 752-761. http://dx.doi.org/10.1016/j.pbb.2006.11.010 PMID: 17196640

[95] de Esch, C.; van der Linde, H.; Slieker, R.; Willemsen, R.; Wolterbeek, A.; Woutersen, R.; De Groot, D. Locomotor activity assay in zebrafish larvae: influence of age, strain and ethanol. *Neurotoxicol. Teratol.,* **2012**, *34*(4), 425-433. http://dx.doi.org/10.1016/j.ntt.2012.03.002 PMID: 22484456

[96] Acevedo, M.B.; Nizhnikov, M.E.; Molina, J.C.; Pautassi, R.M. Relationship between ethanol-induced activity and anxiolysis in the open field, elevated plus maze, light-dark box, and ethanol intake in adolescent rats. *Behav. Brain Res.,* **2014**, *265*, 203-215. http://dx.doi.org/10.1016/j.bbr.2014.02.032 PMID: 24583190

[97] Correa, M.; Arizzi, M.N.; Betz, A.; Mingote, S.; Salamone, J.D. Open field locomotor effects in rats after intraventricular injections of ethanol and the ethanol metabolites acetaldehyde and acetate. *Brain Res. Bull.,* **2003**, *62*(3), 197-202. http://dx.doi.org/10.1016/j.brainresbull.2003.09.013 PMID: 14698353

[98] Nadal, R.; Armario, A.; Janak, P.H. Positive relationship between activity in a novel environment and operant ethanol self-administration in rats. *Psychopharmacology (Berl.),* **2002**, *162*(3), 333-338.

http://dx.doi.org/10.1007/s00213-002-1091-5 PMID: 12122492

[99] Griffin, W.C., III; Novak, A.J.; Middaugh, L.D.; Patrick, K.S. The interactive effects of methylphenidate and ethanol on ethanol consumption and locomotor activity in mice. *Pharmacol. Biochem. Behav.,* **2010**, *95*(3), 267-272.
http://dx.doi.org/10.1016/j.pbb.2010.01.009 PMID: 20122954

[100] Hilbert, M.L.; May, C.E.; Griffin, W.C., III Conditioned reinforcement and locomotor activating effects of caffeine and ethanol combinations in mice. *Pharmacol. Biochem. Behav.,* **2013**, *110*, 168-173.
http://dx.doi.org/10.1016/j.pbb.2013.07.008 PMID: 23872371

[101] Jerlhag, E. The antipsychotic aripiprazole antagonizes the ethanol- and amphetamine-induced locomotor stimulation in mice. *Alcohol,* **2008**, *42*(2), 123-127.
http://dx.doi.org/10.1016/j.alcohol.2007.11.004 PMID: 18358991

[102] Middaugh, L.D.; Bao, K.; Shepherd, C.L. Comparative effects of ethanol on motor activity and operant behavior. *Pharmacol. Biochem. Behav.,* **1992**, *43*(2), 625-629.
http://dx.doi.org/10.1016/0091-3057(92)90202-Q PMID: 1438501

[103] Correa, M.; Sanchis-Segura, C.; Pastor, R.; Aragon, C.M. Ethanol intake and motor sensitization: the role of brain catalase activity in mice with different genotypes. *Physiol. Behav.,* **2004**, *82*(2-3), 231-240.
http://dx.doi.org/10.1016/j.physbeh.2004.03.033 PMID: 15276784

[104] Phillips, T.J.; Shen, E.H.; Shent, E. Neurochemical bases of locomotion and ethanol stimulant effects. *Int. Rev. Neurobiol.,* **1996**, *39*, 243-282.
http://dx.doi.org/10.1016/S0074-7742(08)60669-8 PMID: 8894850

[105] Frye, G.D.; Breese, G.R. An evaluation of the locomotor stimulating action of ethanol in rats and mice. *Psychopharmacology (Berl.),* **1981**, *75*(4), 372-379.
http://dx.doi.org/10.1007/BF00435856 PMID: 6803283

[106] Cachat, J.; Canavello, P.; Elegante, M.; Bartels, B.; Elkhayat, S.; Hart, P. *Modeling stress and anxiety in zebrafish. Zebrafish Models of Neurobehavioral Research*; Humana Press, **2011**, pp. 211-222.

[107] Cachat, J.; Stewart, A.; Grossman, L.; Gaikwad, S.; Kadri, F.; Chung, K.M.; Wu, N.; Wong, K.; Roy, S.; Suciu, C.; Goodspeed, J.; Elegante, M.; Bartels, B.; Elkhayat, S.; Tien, D.; Tan, J.; Denmark, A.; Gilder, T.; Kyzar, E.; Dileo, J.; Frank, K.; Chang, K.; Utterback, E.; Hart, P.; Kalueff, A.V. Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. *Nat. Protoc.,* **2010**, *5*(11), 1786-1799.
http://dx.doi.org/10.1038/nprot.2010.140 PMID: 21030954

[108] Egan, R.J.; Bergner, C.L.; Hart, P.C.; Cachat, J.M.; Canavello, P.R.; Elegante, M.F.; Elkhayat, S.I.; Bartels, B.K.; Tien, A.K.; Tien, D.H.; Mohnot, S.; Beeson, E.; Glasgow, E.; Amri, H.; Zukowska, Z.; Kalueff, A.V. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. *Behav. Brain Res.,* **2009**, *205*(1), 38-44.
http://dx.doi.org/10.1016/j.bbr.2009.06.022 PMID: 19540270

[109] Sackerman, J.; Donegan, J.J.; Cunningham, C.S.; Nguyen, N.N.; Lawless, K.; Long, A.; Benno, R.H.; Gould, G.G. Zebrafish behavior in novel environments: effects of acute exposure to anxiolytic compounds and choice of *Danio rerio* line. *Int. J. Comp. Psychol.,* **2010**, *23*(1), 43-61.
PMID: 20523756

[110] Mathur, P.; Guo, S. Differences of acute *versus* chronic ethanol exposure on anxiety-like behavioral responses in zebrafish. *Behav. Brain Res.,* **2011**, *219*(2), 234-239.
http://dx.doi.org/10.1016/j.bbr.2011.01.019 PMID: 21255611

[111] Boa-Amponsem, O.; Zhang, C.; Burton, D.; Williams, K.P.; Cole, G.J. Ethanol and cannabinoids regulate zebrafish GABAergic neuron development and behavior in a sonic hedgehog and fibroblast growth factor-dependent mechanism. *Alcohol. Clin. Exp. Res.,* **2020**, *44*(7), 1366-1377.
http://dx.doi.org/10.1111/acer.14383 PMID: 32472575

[112] Baiamonte, M.; Parker, M.O.; Vinson, G.P.; Brennan, C.H. Sustained effects of developmental exposure to ethanol on zebrafish anxiety-like behavior. *PLoS One,* **2016**, *11*(2), e0148425.
http://dx.doi.org/10.1371/journal.pone.0148425 PMID: 26862749

[113] da Silva Chaves, S.N.; Dutra Costa, B.P.; Vidal, G.G.C.; Lima-Maximino, M.; Pacheco, R.E.; Maximino, C. NOS-2 participates in the behavioral effects of ethanol withdrawal in zebrafish. *Neurosci. Lett.,* **2020**, *728*, 134952.
http://dx.doi.org/10.1016/j.neulet.2020.134952 PMID: 32283112

[114] Clayman, C.L.; Malloy, E.J.; Kearns, D.N.; Connaughton, V.P. Differential behavioral effects of ethanol pre-exposure in male and female zebrafish (*Danio rerio*). *Behav. Brain Res.,* **2017**, *335*, 174-184.
http://dx.doi.org/10.1016/j.bbr.2017.08.007 PMID: 28797598

[115] Wong, K.; Elegante, M.; Bartels, B.; Elkhayat, S.; Tien, D.; Roy, S.; Goodspeed, J.; Suciu, C.; Tan, J.; Grimes, C.; Chung, A.; Rosenberg, M.; Gaikwad, S.; Denmark, A.; Jackson, A.; Kadri, F.; Chung, K.M.; Stewart, A.; Gilder, T.; Beeson, E.; Zapolsky, I.; Wu, N.; Cachat, J.; Kalueff, A.V. Analyzing habituation responses to novelty in zebrafish (*Danio rerio*). *Behav. Brain Res.,* **2010**, *208*(2), 450-457.
http://dx.doi.org/10.1016/j.bbr.2009.12.023 PMID: 20035794

[116] Novier, A.; Ornelas, L.C.; Diaz-Granados, J.L.; Matthews, D.B. Differences in behavioral responding in adult and aged rats following chronic ethanol exposure. *Alcohol. Clin. Exp. Res.,* **2016**, *40*(7), 1462-1472.
http://dx.doi.org/10.1111/acer.13098 PMID: 27218698

[117] Younis, R.M.; Wolstenholme, J.T.; Bagdas, D.; Bettinger, J.C.; Miles, M.F.; Damaj, M.I. Adolescent but not adult ethanol binge drinking modulates ethanol behavioral effects in mice later in life. *Pharmacol. Biochem. Behav.,* **2019**, *184*, 172740.
http://dx.doi.org/10.1016/j.pbb.2019.172740 PMID: 31326461

[118] Blaser, R.E.; Chadwick, L.; McGinnis, G.C. Behavioral measures of anxiety in zebrafish (*Danio rerio*). *Behav. Brain Res.,* **2010**, *208*(1), 56-62.
http://dx.doi.org/10.1016/j.bbr.2009.11.009 PMID: 19896505

[119] Maximino, C.; Marques de Brito, T.; Dias, C.A.; Gouveia, A., Jr; Morato, S. Scototaxis as anxiety-like behavior in fish. *Nat. Protoc.,* **2010**, *5*(2), 209-216.
http://dx.doi.org/10.1038/nprot.2009.225 PMID: 20134420

[120] Maximino, C.; da Silva, A.W.; Gouveia, A., Jr; Herculano, A.M. Pharmacological analysis of zebrafish (*Danio rerio*) scototaxis. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **2011**, *35*(2), 624-631.
http://dx.doi.org/10.1016/j.pnpbp.2011.01.006 PMID: 21237231

[121] Holcombe, A.; Howorko, A.; Powell, R.A.; Schalomon, M.; Hamilton, T.J. Reversed scototaxis during withdrawal after daily-moderate, but not weekly-binge, administration of ethanol in zebrafish. *PLoS One,* **2013**, *8*(5), e63319.
http://dx.doi.org/10.1371/journal.pone.0063319 PMID: 23675478

[122] Desikan, A.; Wills, D.N.; Ehlers, C.L. Ontogeny and adolescent alcohol exposure in Wistar rats: open field conflict, light/dark box and forced swim test. *Pharmacol. Biochem. Behav.,* **2014**, *122*, 279-285.
http://dx.doi.org/10.1016/j.pbb.2014.04.011 PMID: 24785000

[123] Fleming, W.; Jones, Q.; Chandra, U.; Saini, A.; Walker, D.; Francis, R.; Ocampo, G.; Kuhn, C. Withdrawal from brief repeated alcohol treatment in adolescent and adult male and female rats. *Alcohol. Clin. Exp. Res.,* **2019**, *43*(2), 204-211.
http://dx.doi.org/10.1111/acer.13936 PMID: 30566247

[124] Echevarria, D.; Jammack, C.; Pratt, D.; Hosemann, J. A novel behavioral test battery to assess global drug effects using the zebrafish. *Int. J. Comp. Psychol.,* **2008**, *21*, 19-34.

[125] Maaswinkel, H.; Le, X.; He, L.; Zhu, L.; Weng, W. Dissociating the effects of habituation, black walls, buspirone and ethanol on anxiety-like behavioral responses in shoaling zebrafish. A 3D approach to social behavior. *Pharmacol. Biochem. Behav.,* **2013**, *108*, 16-27.
http://dx.doi.org/10.1016/j.pbb.2013.04.009 PMID: 23603028

[126] Hughes, R.N.; Hancock, N.J. Strain-dependent effects of acute caffeine on anxiety-related behavior in PVG/c, Long-Evans and Wistar rats. *Pharmacol. Biochem. Behav.,* **2016**, *140*, 51-61.
http://dx.doi.org/10.1016/j.pbb.2015.11.005 PMID: 26577750

[127] Anderson, N.L.; Hughes, R.N. Increased emotional reactivity in rats following exposure to caffeine during adolescence. *Neurotoxicol. Teratol.,* **2008**, *30*(3), 195-201.
http://dx.doi.org/10.1016/j.ntt.2008.02.002 PMID: 18378115

[128] Santucci, A.C.; Cortes, C.; Bettica, A.; Cortes, F. Chronic ethanol consumption in rats produces residual increases in anxiety 4 months after withdrawal. *Behav. Brain Res.,* **2008**, *188*(1), 24-31.
http://dx.doi.org/10.1016/j.bbr.2007.10.009 PMID: 18061285

[129] Saverino, C.; Gerlai, R. The social zebrafish: behavioral responses to conspecific, heterospecific, and computer animated fish. *Behav. Brain Res.,* **2008**, *191*(1), 77-87. http://dx.doi.org/10.1016/j.bbr.2008.03.013 PMID: 18423643

[130] Al-Imari, L.; Gerlai, R. Sight of conspecifics as reward in associative learning in zebrafish (*Danio rerio*). *Behav. Brain Res.,* **2008**, *189*(1), 216-219. http://dx.doi.org/10.1016/j.bbr.2007.12.007 PMID: 18243353

[131] Kurta, A.; Palestis, B.G. Effects of ethanol on the shoaling behavior of zebrafish (*Danio rerio*). *Dose Response,* **2010**, *8*(4), 527-533. http://dx.doi.org/10.2203/dose-response.10-008.Palestis PMID: 21191489

[132] Miller, N.; Greene, K.; Dydinski, A.; Gerlai, R. Effects of nicotine and alcohol on zebrafish (*Danio rerio*) shoaling. *Behav. Brain Res.,* **2013**, *240*, 192-196. http://dx.doi.org/10.1016/j.bbr.2012.11.033 PMID: 23219966

[133] Gerlai, R. Zebra fish: an uncharted behavior genetic model. *Behav. Genet.,* **2003**, *33*(5), 461-468. http://dx.doi.org/10.1023/A:1025762314250 PMID: 14574124

[134] Ariyasiri, K.; Choi, T-I.; Kim, O-H.; Hong, T.I.; Gerlai, R.; Kim, C-H. Pharmacological (ethanol) and mutation (sam2 KO) induced impairment of novelty preference in zebrafish quantified using a new three-chamber social choice task. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **2019**, *88*, 53-65. http://dx.doi.org/10.1016/j.pnpbp.2018.06.009 PMID: 29958859

[135] Gregson, J.; Burt De Perera, T. Shoaling in eyed and blind morphs of the characin Astyanax fasciatus under light and dark conditions. *J. Fish Biol.,* **2007**, *70*, 1615-1619. http://dx.doi.org/10.1111/j.1095-8649.2007.01430.x

[136] Partridge, B.; Pitcher, T. The sensory basis of fish schools: relative roles of lateral line and vision. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.,* **1980**, *135*, 315-325. http://dx.doi.org/10.1007/BF00657647

[137] Gerlai, R. Antipredatory behavior of zebrafish: adaptive function and a tool for translational research. *Evol. Psychol.,* **2013**, *11*(3), 591-605. http://dx.doi.org/10.1177/147470491301100308 PMID: 23864295

[138] Fernandes, Y.; Gerlai, R. Long-term behavioral changes in response to early developmental exposure to ethanol in zebrafish. *Alcohol. Clin. Exp. Res.,* **2009**, *33*(4), 601-609. http://dx.doi.org/10.1111/j.1530-0277.2008.00874.x PMID: 19183139

[139] Buske, C.; Gerlai, R. Early embryonic ethanol exposure impairs shoaling and the dopaminergic and serotoninergic systems in adult zebrafish. *Neurotoxicol. Teratol.,* **2011**, *33*(6), 698-707. http://dx.doi.org/10.1016/j.ntt.2011.05.009 PMID: 21658445

[140] Varlinskaya, E.I.; Hosová, D.; Towner, T.; Werner, D.F.; Spear, L.P. Effects of chronic intermittent ethanol exposure during early and late adolescence on anxiety-like behaviors and behavioral flexibility in adulthood. *Behav. Brain Res.,* **2020**, *378*, 112292. http://dx.doi.org/10.1016/j.bbr.2019.112292 PMID: 31626849

[141] Varlinskaya, E.I.; Spear, L.P. Acute effects of ethanol on social behavior of adolescent and adult rats: role of familiarity of the test situation. *Alcohol. Clin. Exp. Res.,* **2002**, *26*(10), 1502-1511. http://dx.doi.org/10.1111/j.1530-0277.2002.tb02449.x PMID: 12394283

[142] Ferré, S. Mechanisms of the psychostimulant effects of caffeine: implications for substance use disorders. *Psychopharmacology (Berl.),* **2016**, *233*(10), 1963-1979. http://dx.doi.org/10.1007/s00213-016-4212-2 PMID: 26786412

[143] Gutiérrez, H.C.; Vacca, I.; Schoenmacker, G.; Cleal, M.; Tochwin, A.; O'Connor, B.; Young, A.M.J.; Vasquez, A.A.; Winter, M.J.; Parker, M.O.; Norton, W.H.J. Screening for drugs to reduce zebrafish aggression identifies caffeine and sildenafil. *Eur. Neuropsychopharmacol.,* **2020**, *30*, 17-29. http://dx.doi.org/10.1016/j.euroneuro.2019.10.005 PMID: 31679888

[144] Boehmler, W.; Petko, J.; Woll, M.; Frey, C.; Thisse, B.; Thisse, C.; Canfield, V.A.; Levenson, R. Identification of zebrafish A2 adenosine receptors and expression in developing embryos. *Gene Expr. Patterns,* **2009**, *9*(3), 144-151. http://dx.doi.org/10.1016/j.gep.2008.11.006 PMID: 19070682

[145] Nikodijević, O.; Jacobson, K.A.; Daly, J.W. Locomotor activity in mice during chronic treatment with caffeine and withdrawal. *Pharmacol. Biochem. Behav.,* **1993**, *44*(1), 199-216. a http://dx.doi.org/10.1016/0091-3057(93)90299-9 PMID: 7679219

[146] Daly, J.W.; Shi, D.; Wong, V.; Nikodijevic, O. Chronic effects of ethanol on central adenosine function of mice. *Brain Res.,* **1994**, *650*(1), 153-156. http://dx.doi.org/10.1016/0006-8993(94)90219-4 PMID: 7953667

[147] Fredholm, B.B.; Bättig, K.; Holmén, J.; Nehlig, A.; Zvartau, E.E. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol. Rev.,* **1999**, *51*(1), 83-133. PMID: 10049999

[148] Jain, N.S.; Hirani, K.; Chopde, C.T. Reversal of caffeine-induced anxiety by neurosteroid 3-alpha-hydroxy-5-alpha-pregnane-20-one in rats. *Neuropharmacology,* **2005**, *48*(5), 627-638. http://dx.doi.org/10.1016/j.neuropharm.2004.11.016 PMID: 15814098

[149] Gupta, P.; Khobragade, S.; Shingatgeri, V.; Rajaram, S. Assessment of locomotion behavior in adult zebrafish after acute exposure to different pharmacological reference compounds. *Drug Develop Ther.,* **2014**, *5*, 127. http://dx.doi.org/10.4103/2394-2002.139626

[150] Ladu, F.; Mwaffo, V.; Li, J.; Macrì, S.; Porfiri, M. Acute caffeine administration affects zebrafish response to a robotic stimulus. *Behav. Brain Res.,* **2015**, *289*, 48-54. http://dx.doi.org/10.1016/j.bbr.2015.04.020 PMID: 25907748

[151] Richendrfer, H.; Pelkowski, S.D.; Colwill, R.M.; Creton, R. On the edge: pharmacological evidence for anxiety-related behavior in zebrafish larvae. *Behav. Brain Res.,* **2012**, *228*(1), 99-106. http://dx.doi.org/10.1016/j.bbr.2011.11.041 PMID: 22155488

[152] de Carvalho, T.S.; Cardoso, P.B.; Santos-Silva, M.; Lima-Bastos, S.; Luz, W.L.; Assad, N.; Kauffmann, N.; Passos, A.; Brasil, A.; Bahia, C.P.; Moraes, S.; Gouveia, A., Jr; de Jesus Oliveira B.E.; Oliveira, K.R.M.H.; Herculano, A.M. Oxidative stress mediates anxiety-like behavior induced by high caffeine intake in zebrafish: protective effect of alpha-tocopherol. *Oxid. Med. Cell. Longev.,* **2019**, *2019*, 8419810. http://dx.doi.org/10.1155/2019/8419810 PMID: 31772712

[153] Pohanka, M.; Dobes, P. Caffeine inhibits acetylcholinesterase, but not butyrylcholinesterase. *Int. J. Mol. Sci.,* **2013**, *14*(5), 9873-9882. http://dx.doi.org/10.3390/ijms14059873 PMID: 23698772

[154] Ferré, S. An update on the mechanisms of the psychostimulant effects of caffeine. *J. Neurochem.,* **2008**, *105*(4), 1067-1079. http://dx.doi.org/10.1111/j.1471-4159.2007.05196.x PMID: 18088379

[155] Ferré, S.; O'Brien, M.C. Alcohol and caffeine: the perfect storm. *J. Caffeine Res.,* **2011**, *1*(3), 153-162. http://dx.doi.org/10.1089/jcr.2011.0017 PMID: 24761263

[156] Acquas, E.; Tanda, G.; Di Chiara, G. Differential effects of caffeine on dopamine and acetylcholine transmission in brain areas of drug-naive and caffeine-pretreated rats. *Neuropsychopharmacology,* **2002**, *27*(2), 182-193. http://dx.doi.org/10.1016/S0893-133X(02)00290-7 PMID: 12093592

[157] Santos, L.; Ruiz-Oliveira, J.; Silva, P.; Luchiari, A. Caffeine dose-response relationship and behavioral screening in zebrafish. In: *The Question of Caffeine*, **2017**. http://dx.doi.org/10.5772/intechopen.68341

[158] Vada, S.; Goli, D.; Sharma, U.; Bose, A.; Mandal, S. Thorough investigation of epileptic behavioral characterization of caffeine in adult zebrafishes in correlation with drug brain concentration. *Acta Ethol.,* **2017**, *20*, 95-105. http://dx.doi.org/10.1007/s10211-017-0250-y

[159] Ruiz-Oliveira, J.; Silva, P.F.; Luchiari, A.C. Coffee time: Low caffeine dose promotes attention and focus in zebrafish. *Learn. Behav.,* **2019**, *47*(3), 227-233. http://dx.doi.org/10.3758/s13420-018-0369-3 PMID: 30623296

[160] Angelucci, M.E.; Cesário, C.; Hiroi, R.H.; Rosalen, P.L.; Da Cunha, C. Effects of caffeine on learning and memory in rats tested in the Morris water maze. *Braz. J. Med. Biol. Res.,* **2002**, *35*(10), 1201-1208. http://dx.doi.org/10.1590/S0100-879X2002001000013 PMID: 12424493

[161] Baldwin, H.A.; File, S.E. Caffeine-induced anxiogenesis: the role of adenosine, benzodiazepine and noradrenergic receptors. *Pharmacol. Biochem. Behav.,* **1989**, *32*(1), 181-186. http://dx.doi.org/10.1016/0091-3057(89)90230-X PMID: 2543990

[162] Alia, A.O.; Petrunich-Rutherford, M.L. Anxiety-like behavior and whole-body cortisol responses to components of energy drinks in zebrafish (*Danio rerio*). *PeerJ,* **2019**, *7*, e7546. http://dx.doi.org/10.7717/peerj.7546 PMID: 31497403

[163] Cauli, O.; Morelli, M. Caffeine and the dopaminergic system. *Behav. Pharmacol.,* **2005**, *16*(2), 63-77. http://dx.doi.org/10.1097/00008877-200503000-00001 PMID: 15767841

[164] Solinas, M.; Ferré, S.; You, Z.B.; Karcz-Kubicha, M.; Popoli, P.; Goldberg, S.R. Caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens. *J. Neurosci.,* **2002**, *22*(15), 6321-6324. http://dx.doi.org/10.1523/JNEUROSCI.22-15-06321.2002 PMID: 12151508

[165] Hughes, R.N.; Hancock, N.J.; Henwood, G.A.; Rapley, S.A. Evidence for anxiolytic effects of acute caffeine on anxiety-related behavior in male and female rats tested with and without bright light. *Behav. Brain Res.,* **2014**, *271*, 7-15. http://dx.doi.org/10.1016/j.bbr.2014.05.038 PMID: 24875772

[166] Burbano-L, D.A.; Porfiri, M. Data-driven modeling of zebrafish behavioral response to acute caffeine administration. *J. Theor. Biol.,* **2020**, *485*, 110054. http://dx.doi.org/10.1016/j.jtbi.2019.110054 PMID: 31634449

[167] Chen, Y.H.; Huang, Y.H.; Wen, C.C.; Wang, Y.H.; Chen, W.L.; Chen, L.C.; Tsay, H.J. Movement disorder and neuromuscular change in zebrafish embryos after exposure to caffeine. *Neurotoxicol. Teratol.,* **2008**, *30*(5), 440-447. http://dx.doi.org/10.1016/j.ntt.2008.04.003 PMID: 18508234

[168] El Yacoubi, M.; Ledent, C.; Ménard, J-F.; Parmentier, M.; Costentin, J.; Vaugeois, J-M. The stimulant effects of caffeine on locomotor behaviour in mice are mediated through its blockade of adenosine A(2A) receptors. *Br. J. Pharmacol.,* **2000**, *129*(7), 1465-1473. a http://dx.doi.org/10.1038/sj.bjp.0703170 PMID: 10742303

[169] Zhang, Q.; Yu, Y.P.; Ye, Y.L.; Zhang, J.T.; Zhang, W.P.; Wei, E.Q. Spatiotemporal properties of locomotor activity after administration of central nervous stimulants and sedatives in mice. *Pharmacol. Biochem. Behav.,* **2011**, *97*(3), 577-585. http://dx.doi.org/10.1016/j.pbb.2010.09.011 PMID: 20863845

[170] Buckholtz, N.S.; Middaugh, L.D. Effects of caffeine and L-phenylisopropyladenosine on locomotor activity of mice. *Pharmacol. Biochem. Behav.,* **1987**, *28*(2), 179-185. http://dx.doi.org/10.1016/0091-3057(87)90211-5 PMID: 3685054

[171] Hsu, C.W.; Chen, C.Y.; Wang, C.S.; Chiu, T.H. Caffeine and a selective adenosine A2A receptor antagonist induce reward and sensitization behavior associated with increased phospho-Thr75-DARPP-32 in mice. *Psychopharmacology (Berl.),* **2009**, *204*(2), 313-325. http://dx.doi.org/10.1007/s00213-009-1461-3 PMID: 19169672

[172] Kaplan, G.B.; Greenblatt, D.J.; Leduc, B.W.; Thompson, M.L.; Shader, R.I. Relationship of plasma and brain concentrations of caffeine and metabolites to benzodiazepine receptor binding and locomotor activity. *J. Pharmacol. Exp. Ther.,* **1989**, *248*(3), 1078-1083. PMID: 2539455

[173] Marin, M.T.; Zancheta, R.; Paro, A.H.; Possi, A.P.; Cruz, F.C.; Planeta, C.S. Comparison of caffeine-induced locomotor activity between adolescent and adult rats. *Eur. J. Pharmacol.,* **2011**, *660*(2-3), 363-367. http://dx.doi.org/10.1016/j.ejphar.2011.03.052 PMID: 21497160

[174] Hughes, R.N.; Hancock, N.J. Effects of acute caffeine on anxiety-related behavior in rats chronically exposed to the drug, with some evidence of possible withdrawal-reversal. *Behav. Brain Res.,* **2017**, *321*, 87-98. http://dx.doi.org/10.1016/j.bbr.2016.12.019 PMID: 28043898

[175] Raymond, J.; Chanin, S.; Michael Steward, A.; Kyzar, E.; Gaikwad, S.; Roth, A. *Assessing habituation phenotypes in adult zebrafish: intra- and inter-trial habituation in the novel tank task*; Zebrafish Protocols for Neurobehavioral Research, **2012**, pp. 71-84.

[176] Ardais, A.P.; Borges, M.F.; Rocha, A.S.; Sallaberry, C.; Cunha, R.A.; Porciúncula, L.O. Caffeine triggers behavioral and neurochemical alterations in adolescent rats. *Neuroscience,* **2014**, *270*, 27-39. http://dx.doi.org/10.1016/j.neuroscience.2014.04.003 PMID: 24726984

[177] Bhattacharya, S.K.; Satyan, K.S.; Chakrabarti, A. Anxiogenic action of caffeine: an experimental study in rats. *J. Psychopharmacol.,* **1997**, *11*(3), 219-224. http://dx.doi.org/10.1177/026988119701100304 PMID: 9305413

[178] El Yacoubi, M.; Ledent, C.; Parmentier, M.; Costentin, J.; Vaugeois, J-M. The anxiogenic-like effect of caffeine in two experimental procedures measuring anxiety in the mouse is not shared by selective A(2A) adenosine receptor antagonists. *Psychopharmacology (Berl.),* **2000**, *148*(2), 153-163. b http://dx.doi.org/10.1007/s002130050037 PMID: 10663430

[179] Gulick, D.; Gould, T.J. Effects of ethanol and caffeine on behavior in C57BL/6 mice in the plus-maze discriminative avoidance task. *Behav. Neurosci.,* **2009**, *123*(6), 1271-1278. http://dx.doi.org/10.1037/a0017610 PMID: 20001110

[180] Lopez-Cruz, L.; Pardo, M.; Salamone, J.D.; Correa, M. Comparison between high doses of caffeine and theophylline on motor and axiogenic effects in CD1 mice: studies of acute and chronic administration. *Behav. Pharmacol.,* **2011**, *22*, e1-e73.

[181] Maximino, C.; da Silva, A.W.; Araújo, J.; Lima, M.G.; Miranda, V.; Puty, B.; Benzecry, R.; Picanço-Diniz, D.L.; Gouveia, A., Jr; Oliveira, K.R.; Herculano, A.M. Fingerprinting of psychoactive drugs in zebrafish anxiety-like behaviors. *PLoS One,* **2014**, *9*(7), e103943. http://dx.doi.org/10.1371/journal.pone.0103943 PMID: 25079766

[182] Stewart, A.; Wu, N.; Cachat, J.; Hart, P.; Gaikwad, S.; Wong, K.; Utterback, E.; Gilder, T.; Kyzar, E.; Newman, A.; Carlos, D.; Chang, K.; Hook, M.; Rhymes, C.; Caffery, M.; Greenberg, M.; Zadina, J.; Kalueff, A.V. Pharmacological modulation of anxiety-like phenotypes in adult zebrafish behavioral models. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **2011**, *35*(6), 1421-1431. http://dx.doi.org/10.1016/j.pnpbp.2010.11.035 PMID: 21122812

[183] La-Vu, M.; Tobias, B.C.; Schuette, P.J.; Adhikari, A. To approach or to avoid: an introductory overview of teh study of anxiety using rodent assays. *Front. Behav. Neurosci.,* **2020**, *14*, 145. http://dx.doi.org/10.3389/fnbeh.2020.00145 PMID: 33005134

[184] Enríquez-Castillo, A.; Alamilla, J.; Barral, J.; Gourbière, S.; Flores-Serrano, A.G.; Góngora-Alfaro, J.L.; Pineda, J.C. Differential effects of caffeine on the antidepressant-like effect of amitriptyline in female rat subpopulations with low and high immobility in the forced swimming test. *Physiol. Behav.,* **2008**, *94*(3), 501-509. http://dx.doi.org/10.1016/j.physbeh.2008.03.004 PMID: 18436269

[185] Rosa, L.V.; Costa, F.V.; Canzian, J.; Borba, J.V.; Quadros, V.A.; Rosemberg, D.B. Three- and bi-dimensional analyses of the shoaling behavior in zebrafish: Influence of modulators of anxiety-like responses. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **2020**, *102*, 109957. http://dx.doi.org/10.1016/j.pnpbp.2020.109957 PMID: 32360787

[186] Bailey, R.L.; Saldanha, L.G.; Dwyer, J.T.; Dwyer, J. Estimating caffeine intake from energy drinks and dietary supplements in the United States. *Nutr. Rev.,* **2014**, *72*(9-13)(Suppl. 1), 9-13. http://dx.doi.org/10.1111/nure.12138 PMID: 25293539

[187] Seifert, S.M.; Schaechter, J.L.; Hershorin, E.R.; Lipshultz, S.E. Health effects of energy drinks on children, adolescents, and young adults. *Pediatrics,* **2011**, *127*(3), 511-528. http://dx.doi.org/10.1542/peds.2009-3592 PMID: 21321035

[188] Franklin, K.M.; Hauser, S.R.; Bell, R.L.; Engleman, E.A. Caffeinated alcoholic beverages - an emerging trend in alcohol abuse. *J. Addict. Res. Ther.,* **2013**(Suppl. 4), S4-S012. PMID: 25419478

[189] Griffiths, R.; Juliano, L.; Chausmer, A. Caffeine pharmacology and clinical effects. *Principles of Addiction Med.,* **2003**, *3*, 193-224.

[190] McKetin, R.; Coen, A.; Kaye, S. A comprehensive review of the effects of mixing caffeinated energy drinks with alcohol. *Drug Alcohol Depend.,* **2015**, *151*, 15-30. http://dx.doi.org/10.1016/j.drugalcdep.2015.01.047 PMID: 25861944

[191] Peacock, A.; Pennay, A.; Droste, N.; Bruno, R.; Lubman, D.I. 'High' risk? A systematic review of the acute outcomes of mixing alcohol with energy drinks. *Addiction,* **2014**, *109*(10), 1612-1633. http://dx.doi.org/10.1111/add.12622 PMID: 24846217

[192] Arria, A.M.; Caldeira, K.M.; Kasperski, S.J.; Vincent, K.B.; Griffiths, R.R.; O'Grady, K.E. Energy drink consumption and increased risk for alcohol dependence. *Alcohol. Clin. Exp. Res.,* **2011**, *35*(2), 365-375. http://dx.doi.org/10.1111/j.1530-0277.2010.01352.x PMID: 21073486

[193] O'Brien, M.C.; McCoy, T.P.; Rhodes, S.D.; Wagoner, A.; Wolfson, M. Caffeinated cocktails: energy drink consumption, high-risk drinking, and alcohol-related consequences among college students. *Acad. Emerg. Med.,* **2008**, *15*(5), 453-460. http://dx.doi.org/10.1111/j.1553-2712.2008.00085.x PMID: 18439201

[194] Thombs, D.; Rossheim, M.; Barnett, T.E.; Weiler, R.M.; Moorhouse, M.D.; Coleman, B.N. Is there a misplaced focus on AmED? Associations between caffeine mixers and bar patron intoxication. *Drug Alcohol Depend.,* **2011**, *116*(1-3), 31-36. http://dx.doi.org/10.1016/j.drugalcdep.2010.11.014 PMID: 21177047

[195] Jones, S.C.; Barrie, L.; Berry, N. Why (not) alcohol energy drinks? A qualitative study with Australian university students. *Drug Alcohol Rev.,* **2012**, *31*(3), 281-287. http://dx.doi.org/10.1111/j.1465-3362.2011.00319.x PMID: 21605204

[196] Spinetta, M.J.; Woodlee, M.T.; Feinberg, L.M.; Stroud, C.; Schallert, K.; Cormack, L.K.; Schallert, T. Alcohol-induced retrograde memory impairment in rats: prevention by caffeine. *Psychopharmacology (Berl.),* **2008**, *201*(3), 361-371. http://dx.doi.org/10.1007/s00213-008-1294-5 PMID: 18758756

[197] Dash, P.K.; Moore, A.N.; Moody, M.R.; Treadwell, R.; Felix, J.L.; Clifton, G.L. Post-trauma administration of caffeine plus ethanol reduces contusion volume and improves working memory in rats. *J. Neurotrauma,* **2004**, *21*(11), 1573-1583. http://dx.doi.org/10.1089/neu.2004.21.1573 PMID: 15684650

[198] Pires, R.G.; Pereira, S.R.; Oliveira-Silva, I.F.; Franco, G.C.; Ribeiro, A.M. Cholinergic parameters and the retrieval of learned and relearned spatial information: a study using a model of Wernicke-Korsakoff Syndrome. *Behav. Brain Res.,* **2005**, *162*(1), 11-21. http://dx.doi.org/10.1016/j.bbr.2005.02.032 PMID: 15922063

[199] Pereira, S.R.; Menezes, G.A.; Franco, G.C.; Costa, A.E.; Ribeiro, A.M. Chronic ethanol consumption impairs spatial remote memory in rats but does not affect cortical cholinergic parameters. *Pharmacol. Biochem. Behav.,* **1998**, *60*(2), 305-311. http://dx.doi.org/10.1016/S0091-3057(97)00472-3 PMID: 9632211

[200] Stancampiano, R.; Carta, M.; Cocco, S.; Curreli, R.; Rossetti, Z.L.; Fadda, F. Biphasic effects of ethanol on acetylcholine release in the rat prefrontal cortex. *Brain Res.,* **2004**, *997*(1), 128-132. http://dx.doi.org/10.1016/j.brainres.2003.09.078 PMID: 14715158

[201] Santos, L.C.; Ruiz-Oliveira, J.; Oliveira, J.J.; Silva, P.F.; Luchiari, A.C. Irish coffee: Effects of alcohol and caffeine on object discrimination in zebrafish. *Pharmacol. Biochem. Behav.,* **2016**, *143*, 34-43. http://dx.doi.org/10.1016/j.pbb.2016.01.013 PMID: 26850919

[202] Boison, D.; Chen, J-F.; Fredholm, B.B. Adenosine signaling and function in glial cells. *Cell Death Differ.,* **2010**, *17*(7), 1071-1082. http://dx.doi.org/10.1038/cdd.2009.131 PMID: 19763139

[203] Brown, R.M.; Short, J.L. Adenosine A(2A) receptors and their role in drug addiction. *J. Pharm. Pharmacol.,* **2008**, *60*(11), 1409-1430. http://dx.doi.org/10.1211/jpp/60.11.0001 PMID: 18957161

[204] Ruby, C.L.; Adams, C.A.; Knight, E.J.; Nam, H.W.; Choi, D-S. An essential role for adenosine signaling in alcohol abuse. *Curr. Drug Abuse Rev.,* **2010**, *3*(3), 163-174. http://dx.doi.org/10.2174/1874473711003030163 PMID: 21054262

[205] Kaplan, G.B.; Bharmal, N.H.; Leite-Morris, K.A.; Adams, W.R. Role of adenosine A1 and A2A receptors in the alcohol withdrawal syndrome. *Alcohol,* **1999**, *19*(2), 157-162. http://dx.doi.org/10.1016/S0741-8329(99)00033-6 PMID: 10548160

[206] Krauss, S.W.; Ghirnikar, R.B.; Diamond, I.; Gordon, A.S. Inhibition of adenosine uptake by ethanol is specific for one class of nucleoside transporters. *Mol. Pharmacol.,* **1993**, *44*(5), 1021-1026. PMID: 7902530

[207] Nagy, L.E.; Diamond, I.; Casso, D.J.; Franklin, C.; Gordon, A.S. Ethanol increases extracellular adenosine by inhibiting adenosine uptake *via* the nucleoside transporter. *J. Biol. Chem.,* **1990**, *265*(4), 1946-1951. http://dx.doi.org/10.1016/S0021-9258(19)39923-5 PMID: 2298733

[208] Zhang, D.; Xiong, W.; Jackson, M.F.; Parkinson, F.E. Ethanol tolerance affects endogenous adenosine signaling in mouse hippocampus. *J. Pharmacol. Exp. Ther.,* **2016**, *358*(1), 31-38. http://dx.doi.org/10.1124/jpet.116.232231 PMID: 27189965

[209] Lutte, A.H.; Majolo, J.H.; Da Silva, R.S. Inhibition of ecto-5′-nucleotidase and adenosine deaminase is able to reverse long-term behavioural effects of early ethanol exposure in zebrafish (*Danio rerio*). *Sci. Rep.,* **2020**, *10*(1), 17809. http://dx.doi.org/10.1038/s41598-020-74832-0 PMID: 33082435

[210] Rico, E.P.; Rosemberg, D.B.; Berteli, J.F.A.; da Silveira Langoni, A.; Souto, A.A.; Bogo, M.R.; Bonan, C.D.; Souza, D.O. Adenosine deaminase activity and gene expression patterns are altered after chronic ethanol exposure in zebrafish brain. *Neurotoxicol. Teratol.,* **2018**, *65*, 14-18. http://dx.doi.org/10.1016/j.ntt.2017.11.001 PMID: 29122710

[211] Rico, E.P.; Rosemberg, D.B.; Langoni, Ada.S.; Souto, A.A.; Dias, R.D.; Bogo, M.R.; Bonan, C.D.; Souza, D.O. Chronic ethanol treatment alters purine nucleotide hydrolysis and nucleotidase gene expression pattern in zebrafish brain. *Neurotoxicol.,* **2011**, *32*(6), 871-878. http://dx.doi.org/10.1016/j.neuro.2011.05.010 PMID: 21704070

[212] Carmichael, F.J.; Israel, Y.; Crawford, M.; Minhas, K.; Saldivia, V.; Sandrin, S.; Campisi, P.; Orrego, H. Central nervous system effects of acetate: contribution to the central effects of ethanol. *J. Pharmacol. Exp. Ther.,* **1991**, *259*(1), 403-408. PMID: 1920128

[213] Dohrman, D.P.; Diamond, I.; Gordon, A.S. The role of the neuromodulator adenosine in alcohol's actions. *Alcohol Health Res. World,* **1997**, *21*(2), 136-143. PMID: 15704350

[214] Pechlivanova, D.; Tchekalarova, J.; Nikolov, R.; Yakimova, K. Dose-dependent effects of caffeine on behavior and thermoregulation in a chronic unpredictable stress model of depression in rats. *Behav. Brain Res.,* **2010**, *209*(2), 205-211. http://dx.doi.org/10.1016/j.bbr.2010.01.037 PMID: 20122970

[215] Kuzmin, A.; Johansson, B.; Gimenez, L.; Ogren, S.O.; Fredholm, B.B. Combination of adenosine A1 and A2A receptor blocking agents induces caffeine-like locomotor stimulation in mice. *Eur. Neuropsychopharmacol.,* **2006**, *16*(2), 129-136. http://dx.doi.org/10.1016/j.euroneuro.2005.07.001 PMID: 16054807

[216] Jain, N.; Kemp, N.; Adeyemo, O.; Buchanan, P.; Stone, T.W. Anxiolytic activity of adenosine receptor activation in mice. *Br. J. Pharmacol.,* **1995**, *116*(3), 2127-2133. http://dx.doi.org/10.1111/j.1476-5381.1995.tb16421.x PMID: 8640355

[217] Prediger, R.D.; Batista, L.C.; Takahashi, R.N. Adenosine A1 receptors modulate the anxiolytic-like effect of ethanol in the elevated plus-maze in mice. *Eur. J. Pharmacol.,* **2004**, *499*(1-2), 147-154. http://dx.doi.org/10.1016/j.ejphar.2004.07.106 PMID: 15363961

[218] Giménez-Llort, L.; Fernández-Teruel, A.; Escorihuela, R.M.; Fredholm, B.B.; Tobeña, A.; Pekny, M.; Johansson, B. Mice lacking the adenosine A1 receptor are anxious and aggressive, but are normal learners with reduced muscle strength and survival rate. *Eur. J. Neurosci.,* **2002**, *16*(3), 547-550. http://dx.doi.org/10.1046/j.1460-9568.2002.02122.x PMID: 12193199

[219] Johansson, B.; Halldner, L.; Dunwiddie, T.V.; Masino, S.A.; Poelchen, W.; Giménez-Llort, L.; Escorihuela, R.M.; Fernández-Teruel, A.; Wiesenfeld-Hallin, Z.; Xu, X.J.; Hårdemark, A.; Betsholtz, C.; Herlenius, E.; Fredholm, B.B. Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A1 receptor. *Proc. Natl. Acad. Sci. USA,* **2001**, *98*(16), 9407-9412. http://dx.doi.org/10.1073/pnas.161292398 PMID: 11470917

[220] Lang, U.E.; Lang, F.; Richter, K.; Vallon, V.; Lipp, H.P.; Schnermann, J.; Wolfer, D.P. Emotional instability but intact spa-

tial cognition in adenosine receptor 1 knock out mice. *Behav. Brain Res.,* **2003**, *145*(1-2), 179-188. http://dx.doi.org/10.1016/S0166-4328(03)00108-6 PMID: 14529816

[221] Adams, C.L.; Cowen, M.S.; Short, J.L.; Lawrence, A.J. Combined antagonism of glutamate mGlu5 and adenosine A2A receptors interact to regulate alcohol-seeking in rats. *Int. J. Neuropsychopharmacol.,* **2008**, *11*(2), 229-241. http://dx.doi.org/10.1017/S1461145707007845 PMID: 17517168

[222] Arolfo, M.P.; Yao, L.; Gordon, A.S.; Diamond, I.; Janak, P.H. Ethanol operant self-administration in rats is regulated by adenosine A2 receptors. *Alcohol. Clin. Exp. Res.,* **2004**, *28*(9), 1308-1316. http://dx.doi.org/10.1097/01.ALC.0000139821.38167.20 PMID: 15365300

[223] Thorsell, A.; Johnson, J.; Heilig, M. Effect of the adenosine A2a receptor antagonist 3,7-dimethyl-propargylxanthine on anxiety-like and depression-like behavior and alcohol consumption in Wistar Rats. *Alcohol. Clin. Exp. Res.,* **2007**, *31*(8), 1302-1307. http://dx.doi.org/10.1111/j.1530-0277.2007.00425.x PMID: 17550371

[224] Micioni Di Bonaventura, M.V.; Cifani, C.; Lambertucci, C.; Volpini, R.; Cristalli, G.; Froldi, R.; Massi, M. Effects of A2A adenosine receptor blockade or stimulation on alcohol intake in alcohol-preferring rats. *Psychopharmacology (Berl.),* **2012**, *219*(4), 945-957. http://dx.doi.org/10.1007/s00213-011-2430-1 PMID: 21833502

[225] Fredholm, B.B.; IJzerman, A.P.; Jacobson, K.A.; Klotz, K.N.; Linden, J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev.,* **2001**, *53*(4), 527-552. PMID: 11734617

[226] Ferre, S.; Ciruela, F.; Borycz, J.; Solinas, M.; Quarta, D.; Antoniou, K.; Quiroz, C.; Justinova, Z.; Lluis, C.; Franco, R.; Goldberg, S.R. Adenosine A1-A2A receptor heteromers: new targets for caffeine in the brain. *Front. Biosci.,* **2008**, *13*, 2391-2399. http://dx.doi.org/10.2741/2852 PMID: 17981720

[227] Shook, B.C.; Jackson, P.F. Adenosine A2A receptor antagonists and Parkinson's disease. *ACS Chem. Neurosci.,* **2011**, *2*(10), 555-567. http://dx.doi.org/10.1021/cn2000537 PMID: 22860156

[228] Rezvani, A.H.; Sexton, H.G.; Johnson, J.; Wells, C.; Gordon, K.; Levin, E.D. Effects of caffeine on alcohol consumption and nicotine self-administration in rats. *Alcohol. Clin. Exp. Res.,* **2013**, *37*(9), 1609-1617. http://dx.doi.org/10.1111/acer.12127 PMID: 23895206

[229] Collier, A.D.; Min, S.S.; Campbell, S.D.; Roberts, M.Y.; Camidge, K.; Leibowitz, S.F. Maternal ethanol consumption before paternal fertilization: Stimulation of hypocretin neurogenesis and ethanol intake in zebrafish offspring. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **2020**, *96*, 109728. http://dx.doi.org/10.1016/j.pnpbp.2019.109728 PMID: 31394141

[230] Sterling, M.E.; Karatayev, O.; Chang, G.Q.; Algava, D.B.; Leibowitz, S.F. Model of voluntary ethanol intake in zebrafish: effect on behavior and hypothalamic orexigenic peptides. *Brain Behav Res.,* **2015**, *278*, 29-39. http://dx.doi.org/10.1016/j.bbr.2014.09.024 PMID: 25257106

[231] Zheng, X.; Dai, W.; Chen, X.; Wang, K.; Zhang, W.; Liu, L.; Hou, J. Caffeine reduces hepatic lipid accumulation through regulation of lipogenesis and ER stress in zebrafish larvae. *J. Biomed. Sci.,* **2015**, *22*, 105. http://dx.doi.org/10.1186/s12929-015-0206-3 PMID: 26572131

[232] SanMiguel, N.; López-Cruz, L.; Müller, C.E.; Salamone, J.D.; Correa, M. Caffeine modulates voluntary alcohol intake in mice depending on the access conditions: Involvement of adenosine receptors and the role of individual differences. *Pharmacol. Biochem. Behav.,* **2019**, *186*(172789), 172789. http://dx.doi.org/10.1016/j.pbb.2019.172789 PMID: 31499144

[233] Dietze, M.A.; Kulkosky, P.J. Effects of caffeine and bombesin on ethanol and food intake. *Life Sci.,* **1991**, *48*(19), 1837-1844. http://dx.doi.org/10.1016/0024-3205(91)90239-8 PMID: 2041457

[234] Hederra, A.; Aidunate, J.; Segovia-Riquelme, N.; Mardones, J. Effects of caffeine on the voluntary EtOH intake of rats. The effects of centrally active drugs on voluntary EtOH consumption. **1975**, *24*, 9-13.

[235] de Carvalho, C.; da Cruz, J.; Takahashi, R. Prolonged exposure to caffeinated alcoholic solutions prevents the alcohol deprivation effect in rats. *J. Caffeine Res.,* **2012**, *2*, 83-89. http://dx.doi.org/10.1089/jcr.2012.0013

[236] Okhuarobo, A.; Igbe, I.; Yahaya, A.; Sule, Z. Effect of caffeine on alcohol consumption and alcohol-induced conditioned place preference in rodents. *J. Basic Clin. Physiol. Pharmacol.,* **2018**, *30*(1), 19-28. http://dx.doi.org/10.1515/jbcpp-2018-0068 PMID: 30099411

[237] Kunin, D.; Gaskin, S.; Rogan, F.; Smith, B.R.; Amit, Z. Caffeine promotes ethanol drinking in rats. Examination using a limited-access free choice paradigm. *Alcohol,* **2000**, *21*(3), 271-277. http://dx.doi.org/10.1016/S0741-8329(00)00101-4 PMID: 11091031

[238] Fritz, B.M.; Quoilin, C.; Kasten, C.R.; Smoker, M.; Boehm, S.L., II Concomitant caffeine increases binge consumption of ethanol in adolescent and adult mice, but produces additive motor stimulation only in adolescent animals. *Alcohol. Clin. Exp. Res.,* **2016**, *40*(6), 1351-1360. http://dx.doi.org/10.1111/acer.13089 PMID: 27154344

[239] Collier, A. *Anxiety-like behaviors and c-fos expression in adult zebrafish: effects of housing conditions, alcohol and caffeine*; University of Southern Mississippi, **2017**.

[240] El Yacoubi, M.; Ledent, C.; Parmentier, M.; Costentin, J.; Vaugeois, J-M. Caffeine reduces hypnotic effects of alcohol through adenosine A2A receptor blockade. *Neuropharmacology,* **2003**, *45*(7), 977-985. http://dx.doi.org/10.1016/S0028-3908(03)00254-5 PMID: 14573390

[241] Khor, Y.M.; Soga, T.; Parhar, I.S. Caffeine neuroprotects against dexamethasone-induced anxiety-like behaviour in the Zebrafish (*Danio rerio*). *Gen. Comp. Endocrinol.,* **2013**, *181*, 310-315. http://dx.doi.org/10.1016/j.ygcen.2012.09.021 PMID: 23044054

[242] Clayman, C. *The role of ethanol pre-exposure in ethanol-induced behavioral responses and reward pathways in zebrafish (Danio rerio)*; American University: Washington, DC, **2016**.

[243] Hughes, R.N. Adult anxiety-related behavior of rats following consumption during late adolescence of alcohol alone and in combination with caffeine. *Alcohol,* **2011**, *45*(4), 365-372. http://dx.doi.org/10.1016/j.alcohol.2010.10.006 PMID: 21145693

[244] Fuxe, K.; Ferré, S.; Genedani, S.; Franco, R.; Agnati, L.F. Adenosine receptor-dopamine receptor interactions in the basal ganglia and their relevance for brain function. *Physiol. Behav.,* **2007**, *92*(1-2), 210-217. http://dx.doi.org/10.1016/j.physbeh.2007.05.034 PMID: 17572452

[245] Boeck, C.R.; Marques, V.B.; Valvassori, S.S.; Constantino, L.C.; Rosa, D.V.; Lima, F.F.; Romano-Silva, M.A.; Quevedo, J. Early long-term exposure with caffeine induces cross-sensitization to methylphenidate with involvement of DARPP-32 in adulthood of rats. *Neurochem. Int.,* **2009**, *55*(5), 318-322. http://dx.doi.org/10.1016/j.neuint.2009.03.015 PMID: 19576520

[246] Capiotti, K.M.; Menezes, F.P.; Nazario, L.R.; Pohlmann, J.B.; de Oliveira, G.M.; Fazenda, L.; Bogo, M.R.; Bonan, C.D.; Da Silva, R.S. Early exposure to caffeine affects gene expression of adenosine receptors, DARPP-32 and BDNF without affecting sensibility and morphology of developing zebrafish (*Danio rerio*). *Neurotoxicol. Teratol.,* **2011**, *33*(6), 680-685. http://dx.doi.org/10.1016/j.ntt.2011.08.010 PMID: 21914471

[247] López-Cruz, L.; Salamone, J.D.; Correa, M. The impact of caffeine on the behavioral effects of ethanol related to abuse and addiction: a review of animal studies. *J. Caffeine Res.,* **2013**, *3*(1), 9-21. http://dx.doi.org/10.1089/jcr.2013.0003 PMID: 24761272