Behavioural Processes xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc

Sleep deprivation impairs cognitive performance in zebrafish: A matter of fact?

Jaquelinne Pinheiro-da-Silva[a], Steven Tran[b], Ana Carolina Luchiari[a,*]

[a] Departamento de Fisiologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Av Sen Salgado Filho, 3000, Lagoa Nova, Natal, Brazil
[b] Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, United States

ARTICLE INFO

Keywords:
Avoidance learning
Memory
Rest deprivation
Sleep
Danio rerio

ABSTRACT

The zebrafish *(Danio rerio)* has become a valuable model organism for behavioral studies examining learning and memory. Its diurnal circadian rhythm and characterized sleep-like state make it comparable to mammals, features that have contributed to establishing this small vertebrate as a translational model for sleep research. Despite sleep being an evolutionarily conserved behavior, its mechanisms and functions are still debated. Sleep deprivation is commonly associated with decreased attention, reduced responsiveness to external stimuli, altered locomotor activity and impaired performance on cognitive tasks. In the current study, we examined the effects of partial and total sleep deprivation on zebrafish learning performance in an active avoidance conditioning paradigm. In addition, we examined the effects of two drugs known to alter sleep (alcohol and melatonin) on learning performance in sleep deprived animals. Our results suggest that although partial sleep deprivation did not alter learning performance, total sleep deprivation was found to significantly impair behavioral responses to the electric shock as well as avoidance learning. However, when sleep deprived fish were treated with alcohol the night before the learning task, learning performance was similar to the control group. In contrast, melatonin treatment did not alter learning performance in sleep deprived animals. We conclude that the zebrafish is a sensitive tool for investigating the effects of sleep deprivation on cognitive performance and may be a useful model for dissecting the mechanisms underlying learning and memory.

1. Introduction

The zebrafish *(Danio rerio)* has emerged as a vertebrate model in neuroethology, genetics and developmental biology over the past decades, and it has gained popularity in behavioral studies focusing on learning and memory (Luchiari et al., 2015; Spence et al., 2008). This small fish has a well characterized sleep-like state distinguished by circadian regulation, periods of inactivity accompanied by an increased arousal threshold, resting place preference and sleep rebound homeostasis (Yokogawa et al., 2007; Zhdanova, 2006a). These features in combination with the zebrafish diurnal circadian rhythm (del Pozo et al., 2011) have established the species as a relevant and translational model for sleep research.

Sleep is an evolutionarily conserved phenomenon observed in most vertebrates (Schmidt, 2014; Zhdanova, 2011). Although the mechanisms and functions of sleep is still debated, it is a common biological need and clearly important for the learning process and memory consolidation (Graves et al., 2003; Jenkins and Dallenbach, 1924; Leibowitz et al., 2006; Marshall and Born, 2007; Rasch and Born, 2013; Stickgold and Walker, 2013; Watson and Buzsáki, 2015). Deficits associated with altered sleep include reduced attention and health-related problems, which have been demonstrated both clinically and experimentally (Alhola and Polo-Kantola, 2007; Killgore, 2010; Raidy and Scharff, 2005; Van Cauter et al., 2008; Van Dongen et al., 2012). In the United States alone, it is estimated that 50–70 million people suffer from sleep disorders with the majority not being properly diagnosed or treated (Colten and Altevogt, 2006).

Sleep deprivation (SD) negatively impacts the quality of life and normal physiological functions (Leibowitz et al., 2006). Research has shown that sleep deprivation can significantly affect memory consolidation (Andersen et al., 2008; Killgore, 2010; McGaugh, 2000; Rasch and Born, 2013; Stickgold and Walker, 2013; Watson and Buzsáki, 2015), with substantial impairment observed when sleep deprivation takes place during memory acquisition. Memory consolidation is often dependent on sleep-stage. For instance, hippocampus-dependent memories such as declarative and spatial memories are supported by slow-wave sleep and are more severely disrupted by sleep deprivation (Graves et al., 2003; Marshall and Born, 2007). Studies of

* Corresponding author at: Departamento de Fisiologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, PO BOX 1510, 59078-970, Natal, Rio Grande do Norte, Brazil.
E-mail address: analuchiari@yahoo.com.br (A.C. Luchiari).

https://doi.org/10.1016/j.beproc.2018.04.004
Received 5 May 2017; Received in revised form 16 March 2018; Accepted 9 April 2018

Yu et al. (2006), Zhdanova et al. (2008, 2001) and also Pinheiro-da-Silva et al. (2017) demonstrated deficits in learning following sleep deprivation in zebrafish. However, the effects of sleep deprivation on forms of learning that are more critical for survival, such as aversive avoidance learning, has been little studied in zebrafish.

In avoidance learning paradigms, the conditioning process is highly dependent on the nature of the unconditioned stimuli. In classical conditioning protocols, animals learn to associate a neutral conditioned stimulus (CS) with an unconditioned stimulus (US), independent of its appetitive or aversive properties. In a situation where the unconditioned stimulus causes an instinctive avoidance reaction (unconditioned response), the animal responds by actively escaping the aversive stimulus (Moore, 2004, 1973). Learned responses using this type of conditioning are valuable due to its adaptive value. Research has shown that stressful situations such as avoidance conditioning may affect the quality of sleep (Machida et al., 2013), as well as subsequent reactions to the aversive stimuli in zebrafish (Champagne et al., 2010). However, to what extent sleep deprivation interferes on the learning of an aversive stimulus and its subsequent avoidance still needs investigation.

Numerous neurotransmitter systems that are implicated in the regulation of sleep and in the process of learning (Jones, 2000) are also affected by some drugs, such as alcohol (Koob and Roberts, 1999; Vengeliene et al., 2008). Alcohol is one of the most commonly consumed drug in the world and has an enormous societal impact in terms of lost workplace productivity, negative health consequences, and costs associated with the treatment of alcoholism (OMS, 2002; WHO, 2012). Alcohol is a central nervous system depressant and its sedative effects are known to affect sleep as well as behavioral responses during wake state (Gerlai et al., 2000; Stein and Friedmann, 2005). Since ethanol is a small and water-soluble molecule, its effects on the brain are widely diffused. As a result, the mechanisms through which alcohol alters brain function and sleep are poorly understood. However, alcohol has been shown to have both beneficial and adverse effects on sleep and sleep disorders, which are dependent on numerous factors including but not limited to dose, intake frequency, tolerance, and abuse (Roehrs et al., 1999; Roehrs and Roth, 2001).

On the other hand, this vertebrate organism produce a small molecule that exhibits a circadian rhythm and has been suggested to promote sleep, the melatonin (Brzezinski et al., 2005; Zhdanova et al., 2001). Melatonin synthesis by the pineal gland is regulated by the circadian clock, with synthesis occurring primarily at night, and the levels acutely suppressed by light exposure. Due to melatonin's high lipid solubility and ability to cross cell membranes, melatonin can be quickly distributed throughout the organism (Zhdanova, 2006b). Exogenous melatonin administration has been shown to act as sleep-promotor in diurnal vertebrates including humans (Arendt and Skene, 2005; Brzezinski et al., 2005; Mintz et al., 1998; Van Den Heuvel et al., 2005; Zhdanova et al., 2001). Melatonin injections in pigeons and rodents have also been demonstrated to entrain circadian rhythms of activity (Phillips and Berger, 1992; Yamada et al., 1988). As well as for alcohol, melatonin effects on behavior have been reported in both fish and mammals (Brower, 2003; Brzezinski et al., 2005; Fairclough and Graham, 1999; Roehrs and Roth, 2001; Stein and Friedmann, 2005; Zhdanova et al., 2002), while its effects on sleep deprived fish remain unknown.

In order to examine behavioral mechanisms of learning and memory in zebrafish subjected to sleep deprivation, we used an avoidance conditioning paradigm similar to previous studies (Blank et al., 2009; Manuel et al., 2014; Pradel et al., 1996; Xu et al., 2007). However, we approached short-term memory due to its higher vulnerability compared to long-term memory. In addition, to examine the effects of sleep altering drugs, we also observed the behavioral response in sleep-deprived fish exposed to alcohol or melatonin. Therefore, we hypothesized that (1) sleep deprivation, negatively impacts short-term memory formation of an aversive experience, while (2) alcohol and melatonin promotes sleep and ameliorate the effects of sleep deprivation in zebrafish.

2. Material and methods

2.1. Ethical approval

All experiments were performed in accordance with the Ethics Committee for Animal Use of the Federal University of Rio Grande do Norte (application number: CEUA 022/2012).

2.2. Animals and stock conditions

Adult wild-type zebrafish (*Danio rerio*, 3 months old, mixed sexes) were purchased from a local fish farm and transferred to a storage system (50 L tanks) at the Ornamental Fish Vivarium, Department of Physiology - UFRN. The storage system consisted of four 50-L tanks in a closed recirculation system with mechanical, biological, and chemical filtration and UV disinfection. Animals were housed (in a proportion of one fish/L) with aerated and filtered water, with temperature (28 °C), pH (7.1) and oxygen measured regularly. Fish were kept on a 12:12h light-dark cycle, with zeitgeber time (ZT) 0 corresponding to lights on time (07a.m.–07p.m.), and light intensity was measured at 250 lx. Zebrafish were fed twice a day, mornings and afternoons, with brine shrimp and commercial diet (60% protein and 15% fat, Nutricom Pet).

2.3. Sleep conditions and drug treatments

The light -dark cycle is the major zeitgeber for circadian rhythms in most animals. (Krieger, 1979; Panda et al., 2002). The control group was maintained on a 12L:12D light:dark cycle. Constant light conditions can suppress sleep behavior in some species (Berger and Phillips, 1994; Deprés-Brummer et al., 1996) and prior research has demonstrated that light suppresses sleep in zebrafish with no evidence for rebound during the light phase (Yokogawa et al., 2007). Thus, we used two different light treatments to induce sleep deprivation. Sleep deprivation was achieved by both extending the light phase of the cycle and by exposing fish to light-dark cycle during the dark phase. The *partial sleep deprivation* condition consisted of 18 h of light (extended light phase) followed by only 6 h of dark (18L:06D), whereas the *total sleep deprivation* condition consisted of 18 h of light followed by 6 h of light pulses (6 h cycle of 4-min light and 1-min dark). The control, partial and total sleep deprivation protocols were implemented for 3 consecutive days prior to the learning test. The sleep deprivation protocol described above have been previously shown to suppress sleep-like behavior in zebrafish when administered during the dark phase (Pinheiro-da-Silva et al., 2017). In Supplementary Material we provide previous data (with the endorsement of the authors mentioned above), which indicates that partial sleep deprivation and total sleep deprivation lead to differences in zebrafish distribution in the tank and number of sleep episodes.

To pharmacologically alter sleep deprivation, we treated zebrafish with melatonin or ethanol in the total sleep deprivation condition. Melatonin exposure was expected to facilitate sleep entrainment and suppress the effects of sleep deprivation (Berger et al., 2009; Yamada et al., 1988), whereas ethanol was expected to disturb sleep and exacerbate the effects of sleep deprivation (Roehrs and Roth, 2001).

Scheer and Czeisler (2005) discussed the efficiency of single and repeated exogenous melatonin administrations and suggested that repeated doses prior to bedtime were more effective in improving sleep. Therefore, we administered melatonin directly to the housing tank water at a final concentration of 100 nM, once a day, with 30% of the tank water being replaced daily for 10 consecutive days prior to the learning test (adapted from Zhdanova et al. (2008). Note that since the melatonin treatment lasted 10 days, the sleep deprivation protocol was conducted on day 8 (the last 3 days of treatment). In contrast, on the last night of sleep deprivation for the ethanol condition, zebrafish were

exposed to 0.5% ethanol for 1 h prior to the habitual onset of the dark phase in a 4 L tank, and then returned to their housing tank (Gerlai et al., 2000).

Therefore, the 5 experimental conditions used in the current study were: Partial Sleep Deprivation (18L:06D, n = 32), Total Sleep Deprivation (18L:06D + pulses, n = 30), Total Sleep Deprivation + 0.5% Ethanol (Eth 18L:06D + pulses, n = 26), Total Sleep Deprivation + Melatonin (Mel 18L:06D + pulses, n = 28), and Control (12L:12D, n = 24).

2.4. Aversive learning task

An aversive conditioning protocol modified from Blank et al. (2009) and Xu et al. (2007) was administered to each group described above and was performed during the daytime (ZT1 – ZT8). Ethanol and melatonin were not administered during the test day. Zebrafish were individually tested in a 15 L *shuttle box* tank (40 × 25 × 20 cm) divided by an opaque wall with a 2 cm opening at the bottom allowing fish access to both sides of the tank. The walls of the tank were completely covered with opaque plastic self-adhesive white films with different visual cues on the bottom of each side of the tank (white background vs. black and white grid pattern) (Fig. 1).

In the current study, we used an electroshock apparatus which has been successfully utilized for cognitive avoidance tasks (Manuel et al., 2014) to demonstrate learning (Xu et al., 2007) and memory retention (Blank et al., 2009) in zebrafish. The electroshock apparatus consisted of 2 manual shock machines, one for each compartment of the shuttle box, which delivered an electroshock to serve as an aversive stimulus. Each machine had two electrodes positioned through the wall and placed on each side of the tank. The electroshock (6 V) was administered via two schemes: (a) consistently on one side of the tank (either the white or checkered pattern side) or (b) on random sides. Half the number of animals for each sleep deprivation condition was used for each electroshock scheme.

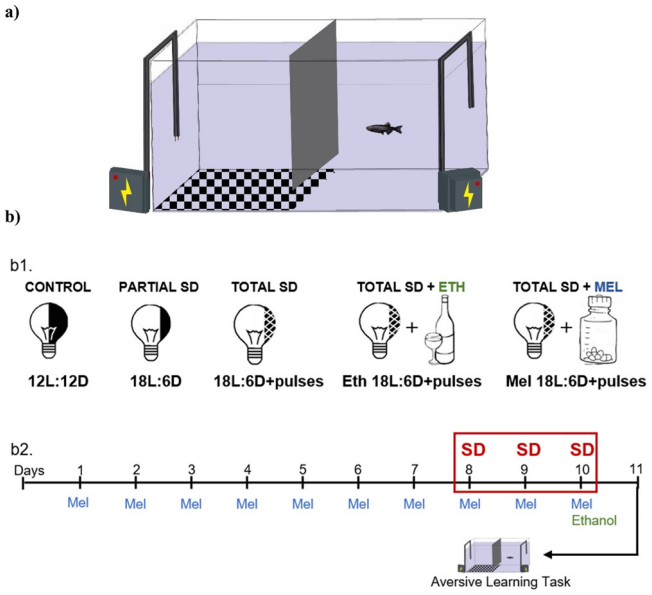

Fig. 1. (a) Schematic overview of the aversive learning apparatus. Tanks were (15 L, 40 × 25 × 20 cm) divided by an opaque wall with a 2 cm opening at the bottom allowing the fish to swim to both sides. All walls were completely covered with opaque plastic self-adhesive white films but different visual cues were present on the bottom of each side of the tank (white background vs. black and white checkered pattern). Wire electrodes extending through each opposing side wall on both sides were attached to a manually operated electroshock machine. (b) Representation of the five different treatments applied, with their respective photoperiods and drugs described. Timeline for sleep deprivation and drug administration is shown: SD took place on day 8, 9 and 10. Melatonin was administered for 10 consecutive days before the test, while Ethanol were delivered only on night before the test.

Animals were tested only once and individually in the shuttle box tank which began with a 2-min habituation period. Following habituation, a 2-s electroshock was applied followed by 60-s interval + 2-s electroshock and so on, during a 20-min trial. Fish were able to swim to the other side of the tank to avoid the adverse stimulus, while the shock was administered on the side corresponding to the respective scheme, regardless of which side the fish was on.

Behavior responses were recorded during the 20 min following habituation and analyzed using an automated video tracking software, ZebTrack. The video tracking software has been previously validated and settings details have been previously described (Pinheiro-da-Silva et al., 2017). In short, Zebtrack is capable of quantifying swimming patterns, including speed, distance travelled, and time spent in predefined areas. To evaluate learning performance in zebrafish, we quantified the time that fish spent on each side of the tank, as well the average speed, maximum speed, freezing and total distance traveled during each trial.

2.5. Statistics

Statistical analysis was performed using SigmaPlot Version 3.5 (Systat Software, San Jose, CA). Paired Student's *t*-test was used to compare the time spent on each side of the tank and One-Way Analysis of Variance (ANOVA) with Tukey's Honest Significant Difference (HSD) tests were used to compare average speed, maximum speed, total distance traveled, and freezing for each sleep deprivation condition. The data was shown to be normally distributed and all groups exhibited equal variance. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Aversive learning task

To quantify learning performance, we quantified the amount of time zebrafish spent on each side of the testing tank. During the test, when fish received shocks consistently on one side of the tank, the Control group, Partial SD and Total SD + Ethanol groups learned to avoid the electroshock by spending more time on the side without the shock (Student t test: Control group: $t(22) = -2.05$ $p = 0.05$; Partial SD group: $t(30) = -3.03$ $p = 0.005$; Total SD + Ethanol: $t(23) = -4.19$ $p = < 0.001$) (Fig. 2). However, there were no significant differences in the time spent on each side of the tank for the Total SD and Total SD + Melatonin groups (Student *t*-test: Total SD: $t(28) = -1.13$ $p = 0.27$; Total SD + Melatonin: $t(26) = -0.62$ $p = 0.54$) (Fig. 2c and e). In contrast, when electroshocks were randomly administered, all groups were unable to learn to avoid the shocks and there were no significant differences in the time spent on each side of the shuttle box (Student t test: Control group: $t(24) = 0.99$ $p = 0.33$; Partial SD group: $t(20) = -0.81$ $p = 0.42$; Total SD group: $t(26) = -1.37$ $p = 0.18$; Total SD + Ethanol: $t(28) = -1.49$ $p = 0.15$; Total SD + Melatonin: $t(20) = 0.71$ $p = 0.48$) (Fig. 3).

3.2. Locomotor parameters

While not quantified, visual observation during the test suggested animals did not exhibit abnormal swimming behavior or were sick. One-Way ANOVA did not detect significant differences in the average speed among the five treatment groups ($F(4,65) = 0.27$ $p = 0.89$) (Fig. 4a), but there was a significant main effect of sleep deprivation on maximum speed ($F(4,70) = 3.18$ $p = 0.019$), with the Control group exhibiting a significantly higher maximum speed compared to all other groups (Tukey's HSD test, $p < 0.05$) (Fig. 4b).

We also observed episodes of freezing behavior, as expected due to

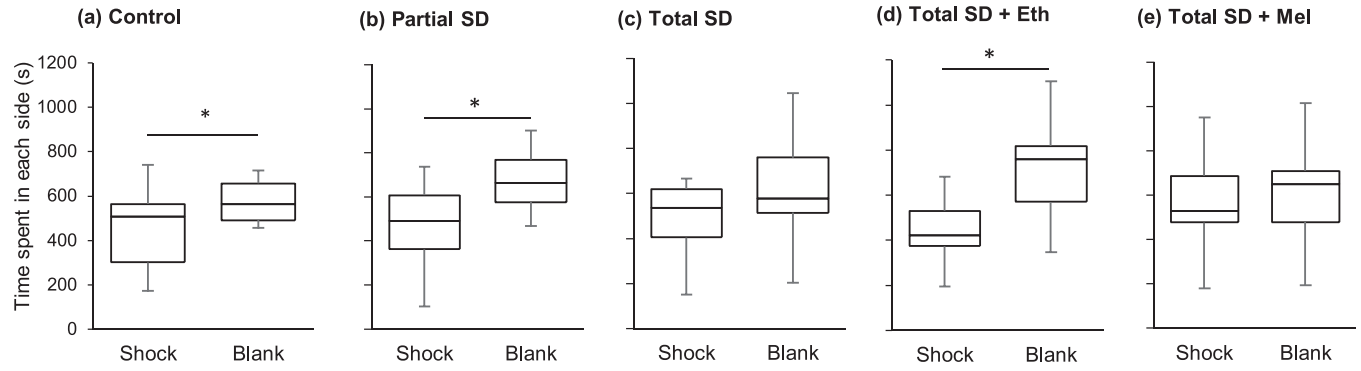

Fig. 2. Adult zebrafish performance in an avoidance conditioning task following 3 nights of Partial sleep deprivation, Total sleep deprivation and Total SD + Ethanol or + Melatonin administration. Electroshock (6 V, 2 s, every 1 min) administered on one side only (white side or black/white side). n = 24–32 fish/group. Boxplots represent the interquartile range divided by a line indicating the median; whisker lines extend to values within 1.5× of the interquartile range. Significant differences between sides during the trials are shown with an asterisk (*), $p < 0.05$.

the aversive nature of the stimulus. There was a significant main effect of sleep deprivation on freezing behavior (One-Way ANOVA, F(4, 66) = 3.34 p = 0.015), and Tukey's HST test revealed that fish from the total SD group froze significant more compared to fish in the control and Total SD + ethanol groups ($p < 0.05$). There was also a significant main effect of sleep deprivation on total distance fish travelled (One-Way ANOVA, F(4, 70) = 14.97 $p < 0.001$), and Tukey's HSD test revealed that the Total SD group travelled a significantly shorter distance compared to all other groups ($p < 0.05$) (Fig. 4d).

4. Discussion

In the current study, we found that sleep deprivation impaired avoidance learning in zebrafish. Although partial sleep deprivation did not affect learning, total sleep deprivation for 72 h prior to the learning task significantly decreased avoidance behavior. Notably, when totally sleep deprived zebrafish were exposed to acute alcohol exposure on the night prior to testing, the behavioral performance in the avoidance task was similar to the control group. In contrast, fish treated with melatonin and totally sleep deprived showed hindered learning performance. Sleep is an important behavioral state associated with energy allocation and conservation, synapses remodeling and memory consolidation (Schmidt, 2014; Siegel, 2005; Tononi and Cirelli, 2006). Sleep deprivation reduces cognitive ability and the prolonged deprivation of the sleep behavior might cause physical and psychological deficits (Alhola and Polo-Kantola, 2007; Killgore, 2010; Prince and Abel, 2013). Our results are in line with these observations, demonstrating that sleep deprivation in zebrafish significantly impairs cognitive performance.

In our study, fish exposed to electroshocks on only one side of the tank learned to avoid the stimulus when they were not sleep deprived (see Fig. 2). These results are in line with previous studies on avoidance learning (Blank et al., 2009; Bueno et al., 1994; Manuel et al., 2014; Xu et al., 2007) and corroborates the ability of zebrafish to learn, form memory and reactivate the memory related to the aversive stimulus. The voltage of the electroshock used in our aversive conditioning paradigm can be considered as a slight stressor. We used 6 V for the electroshock in order to avoid tissue damage, which could affect active response (Cachat et al., 2011; Egan et al., 2009).

Partially sleep deprived fish were able to respond and learn the association between the conditioned and unconditioned stimulus similarly to the control group. The performance of partially sleep deprived fish may be attributed to some sleep rebound during the shortened dark phase (Schmidt, 2014; Sigurgeirsson et al., 2013). Sleep rebound is related to a homeostatic sleep response, which is a process through which partially sleep deprived animals can recover from sleep debt (Sigurgeirsson et al., 2013; Yokogawa et al., 2007; Zhdanova et al., 2001). Thus, although partial sleep deprivation reduced maximum speed and there was a trend towards decreased freezing behavior (Fig. 4), 6 h of darkness may have been enough to allow zebrafish to recover from sleep deprivation, which leads to increased performance as compared to animals in the Total SD condition.

Similar to most animals, zebrafish exhibit a natural tendency to explore new environments in search of food, mates, and shelter (Avdesh et al., 2012; Oliveira et al., 2015; von Krogh et al., 2010). Associating an aversive unconditioned stimulus such as an electric shock with a conditioned stimulus such as a distinct environment is an important cognitive ability that can increase an animal's chances for survival

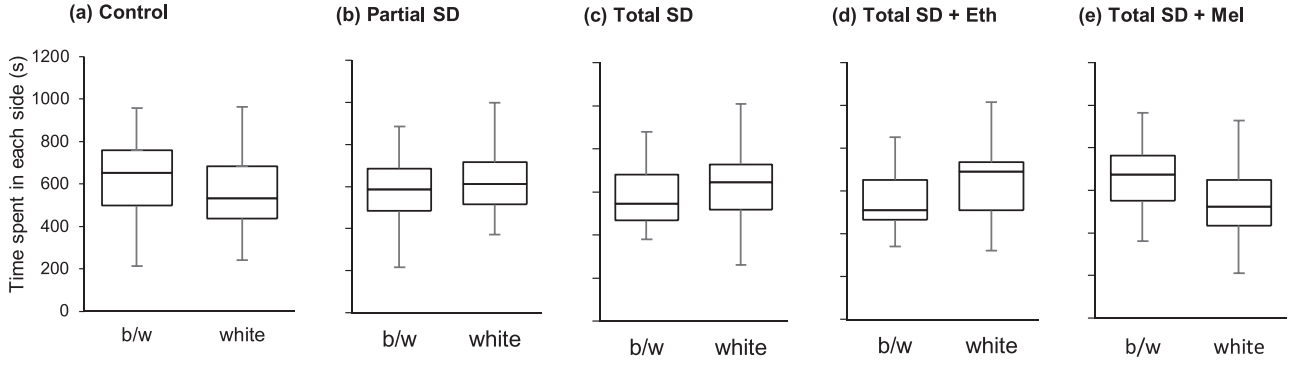

Fig. 3. Adult zebrafish performance in an avoidance conditioning task following 3 nights of partial sleep deprivation, total sleep deprivation and total SD + Ethanol or + Melatonin administration. Electroshock (6 V, 0.2 s, every 1 min) administered on random sides. n = 24–32 fish/group. Boxplots represent the interquartile range divided by a line indicating the median; whisker lines extend to values within 1.5× of the interquartile range. Analysis indicated non-significant effects between groups ($p > 0.05$). (1.5 column).

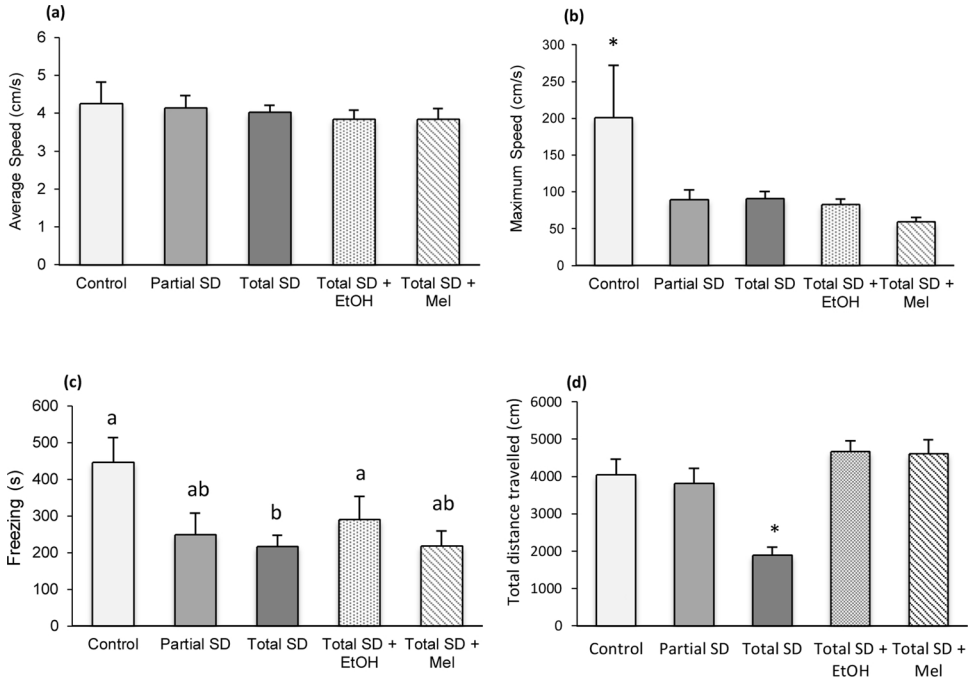

Fig. 4. One-Way ANOVA applied to compare (a) average speed, (b) maximum speed, (c) freezing behavior and (d) total distance the animal traveled during the avoidance conditioning test, among the five groups: Control, Partial SD, Total SD, Total SD + Ethanol, Total SD + Melatonin. Bars (mean ± SEM) represent data corresponding to 20 min behavioral trial during the test and analyzed using video-tracking software (ZebTrack), $n = 11–16$ fish/group. For this data, we consider the results of all presentation schemes (shock on the white side or checkered pattern side). (a) One-Way ANOVA showed non-significant effects of groups; (b) One-Way ANOVA revealed significant effects of groups and Tukey HSD test showed that the control group had significantly higher values of maximum speed ($p < 0.05$) compared to all other groups, result indicated by asterisk, (c) One-Way ANOVA presented significant effects of groups and Tukey HSD test showed that the control and total SD + Eth groups had significantly higher values of freezing ($p < 0.05$) compared to Total SD group, result indicated by the presence of at least one different letter indicates statistical difference between groups, (d) One-Way ANOVA revealed significant effects of groups and Tukey HSD test showed that the Total SD group had significantly lower values of total distance traveled ($p < 0.05$) compared to all other groups, result indicated by asterisk.

(Serra et al., 1999; Zhdanova et al., 2008). We expected zebrafish to avoid the compartment associated with the electroshock even after three nights of sleep deprivation. However, totally sleep deprived fish receiving electroshocks consistently on one side of the tank did not learn to avoid the compartment associated with the shock and spent an equal amount of time on both sides of the shuttle box (see Fig. 2c). While aversive stimuli have been shown to be more reinforcing than positive stimuli for associative learning tasks (Walker and Stickgold, 2006), the intensity of the shock (only 6 V) may have been too little to form an US-CS association in sleep deprived animals. In contrast, the stimulus was strong enough to allow the US-CS associations to form in control fish and partially sleep deprived fish. Our results indicate that sleep deprivation affects both the perception of the aversive stimulus as well as the formation of US-CS association. Our findings are in accordance with previous studies examining the deleterious effects of sleep deprivation on cognition (Alhola and Polo-Kantola, 2007; Killgore, 2010; Prince and Abel, 2013). Studies examining rodent and human behavior following sleep deprivation have highlighted the individuals' resistance to act in response to an earlier emotional memory (e.g. fear elicited by electric shocks in our study) to the effects of sleep deprivation (Phelps, 2004; Ruskin et al., 2004). Zebrafish and rodents brains show some structural differences, such as the hemispheres size and layout of the telencephalon (Mueller and Wullimann, 2003; Wullimann and Mueller, 2004). However, several homologous areas were already described, including the lateral pallium in zebrafish that appears to correspond to the hippocampus in mammals (Broglio et al., 2005; Salas et al., 2006; Tropepe and Sive, 2003), a region mainly related to learning and memory processes. Moreover, a number of neurotransmitter system as highly conserved in zebrafish, such as the cholinergic, adenosinergic, and dopaminergic pathways (Wullimann and Mueller, 2004).

The impaired learning observed in the total SD group may be attributed to place preference and decreased locomotion. For instance, fish may have exhibited a place preference and simply avoided the non-paired compartment. To test these alternative possibilities, fish from each treatment group also received electroshocks on random sides (Fig. 3). As expected, fish receiving electroshock on random sides of the tank spent an equal amount of time on both sides of the tank, demonstrating that zebrafish did not exhibit a place preference during this task. Behavioral parameters including average and maximum swimming speed, freezing behavior and total distance traveled were also analyzed (Fig. 4). Although average speed did not differ between groups, totally sleep deprived fish exhibited the lowest total distance traveled. Zhdanova et al. (2001) reported a similar finding in that sleep deprivation decreases daytime locomotor activity in zebrafish. Examination of freezing behavior revealed that the control group froze for a longer period compared to all sleep deprivation groups which may be associated with the aversive nature of the electric shock. Previous studies have shown that sleep deprivation reduces response time and impairs sustained attention (Binks et al., 1999; Killgore, 2010). Our group has also previously observed that sleep deprivation impairs discrimination of novel object (Pinheiro-da-Silva et al., 2017). Thus, sleep deprivation in this study may have affected the fish's ability to properly respond to the stimulus. Freezing behavior is a complete suspension of movement resulting from increased stress/anxiety and may reflect submissive behavior (Kalueff et al., 2013). We expected to observe freezing behavior elicited by the avoidance conditioning paradigm. Our results demonstrating impaired behavioral responses to the electric shock as well as impaired learning following 3 nights of total sleep deprivation is in accordance with previous study examining the effects of sleep deprivation on learning and memory (Alhola and Polo-Kantola, 2007; Inostroza et al., 2013; Killgore, 2010; Raidy and Scharff, 2005; Ruskin et al., 2004).

Regarding the exogenous molecules used in the study, alcohol is one of the most commonly examined pharmacological agent in zebrafish research and has been shown to alter behavioral responses as well as impair learning (Luchiari et al., 2015; Obernier et al., 2002; Tran and Gerlai, 2013). This substance when added to the water can easily be absorbed by the fish with blood alcohol levels quickly reaching equilibrium with the external alcohol concentration (Ryback et al., 1969). Here, fish were exposed to an acute alcohol dose (0.5%) on the last night of sleep deprivation for 1 h and subsequently tested for avoidance learning on the following day. The sleep deprivation + alcohol group was able to acquire the association between the US and CS similar to the control group, suggesting normal learning performance.

Alcohol consumption is commonly associated with sleep disruptions

(Roehrs and Roth, 2001). However, alcohol has dose-dependent effects with stimulant and sedative properties (Earleywine and Martin, 1993; Martin et al., 1993; Stein and Friedmann, 2005; Tran and Gerlai, 2013). Based on our results, alcohol exposure was able to rescue the learning impairment observed in totally sleep deprived fish. Alcohol's sedative effects may have promoted sleep onset similar to a previous study (Roehrs and Roth, 2001). In fact, alcohol was shown to interfere with the adenosine transmission (Dohrman et al., 1997), which may be related to the sedation effects of alcohol. More than that, Sharma et al. (2014) showed that acute alcohol exposure provokes adenosine A1 receptor-mediated inhibition of orexin neurons, leading to sleep promotion. Alcohol is known to reduce core body temperature, contribute to the entrainment of sleep (Kleitman, 1939), and low doses may beneficial for treating insomnia (Roehrs et al., 1999). However, the development of alcohol tolerance should be taken into consideration. For instance, tolerance to alcohol's sedative effects can be established after only a few days of consumption (Turner, 2000), as the neurotransmitter systems decrease the response to the presence of alcohol over time. Moreover, the use of alcohol to improve sleep may lead to excessive intake. Thus, the relationship between alcohol and sleep requires greater attention, with a focus on identifying the physiological mechanisms underlying alcohol's sleep-promoting properties.

In contrast to alcohol's effect on avoidance learning following sleep deprivation, exposure to melatonin did not affect learning in sleep deprived fish. Melatonin is an important sleep-regulating hormone in zebrafish (Gandhi et al., 2015) and is produced in the pineal gland (Lima-Cabello et al., 2014). Administration of exogenous melatonin has been shown to increase sleep under light:dark conditions (Zhdanova et al., 2001) and has dose dependent effects on locomotor behavior (Wang et al., 2014) which can be observed within 20 min of drug exposure (Zhdanova et al., 2001). In the current study, melatonin administration did not rescue the impaired stimulus perception and learning performance of sleep deprived fish, and this could be related to the drug exposure regimen and/or the sleep deprivation protocol used. We exposed fish to melatonin for 1 h prior to the habitual onset of the dark phase. However, melatonin has been reported to promote sleep within 20 min of exposure and thus, the effects of this hormone may have been limited during the dark phase which occurred 6 h later.

Previous studies have demonstrated that melatonin injections can induced sleep, even in constant light following sleep deprivation (Phillips and Berger, 1992; Yamada et al., 1988). However, our total sleep deprivation protocol consisted of light pulses, which may have inhibited melatonin's action in the brain. For example, melatonin is ineffective in sleep disorder patients when combined with improper sleep conditions (Buscemi et al., 2004; Rawashdeh et al., 2007). Additionally, Rawashdeh et al. (2007) previously reported that melatonin's action in zebrafish is inhibited under constant light in an active-avoidance conditioning paradigm. These authors also showed that a pinealectomy or blocking melatonin signaling improved memory consolidation, suggesting the melatonin role in memory formation.

Finally, our results reinforce the zebrafish as a valuable model organism for high throughput screening of sleep-related drugs. Sleep is an essential behavioral phenomenon and our data support the negative effects of sleep deprivation on simple (perception) and more complex (learning and memory) cognition. We found a significant learning impairment in zebrafish following total sleep deprivation. Although melatonin did not alter learning behavior, acute alcohol exposure improved learning performance in sleep deprived fish. In future studies, approaching other alcohol and melatonin doses and exposure regimes, the stressful effects of sleep deprivation in terms of hormonal levels, and the effects of sleep deprivation in long–term memory formation deserve to be investigated. In the present study, the simplicity of the protocol due to the lack of a time-consuming training phase will make it a useful tool for future behavioral and brain tissue analyses. It will also allow investigators to examine how sleep deprivation affects cognitive function and whether different drugs can be used for the treatment of sleep disorders.

Acknowledgments

The authors are grateful to Ms. Adrielly Nascimento and Mr. Rômulo Almeida for their assistance with data collection, and Mrs. Priscila Fernandes for her valuable suggestions and insights. This work was supported by the FAPERN [grants number: PPP2012].

References

Alhola, P., Polo-Kantola, P., 2007. Sleep deprivation: impact on cognitive performance. Neuropsychiatr. Dis. Treat. 3, 553–567.

Andersen, M.L., Antunes, I.B., Silva, a., Alvarenga, T., a, F., Baracat, E.C., Tufik, S., 2008. Effects of sleep loss on sleep architecture in Wistar rats: gender-specific rebound sleep. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 32, 975–983. http://dx.doi.org/10.1016/j.pnpbp.2008.01.007.

Arendt, J., Skene, D.J., 2005. Melatonin as a chronobiotic. Sleep Med. Rev. http://dx.doi.org/10.1016/j.smrv.2004.05.002.

Avdesh, A., Martin-Iverson, M.T., Mondal, A., Chen, M., Askraba, S., Morgan, N., Lardelli, M., Groth, D.M., Verdile, G., Martins, R.N., 2012. Evaluation of color preference in zebrafish for learning and memory. J. Alzheimer's Dis. 28, 459.

Berger, R.J., Phillips, N.H., 1994. Constant light suppresses sleep and circadian rhythms in pigeons without consequent sleep rebound in darkness. Am. J. Physiol. Integr. Comp. Physiol. 267, R945–R952.

Berger, S.L., Kouzarides, T., Shiekhattar, R., Shilatifard, A., 2009. An operational definition of epigenetics. Genes Dev. 23, 781–783.

Binks, P.G., Waters, W.F., Hurry, M., 1999. Short-term total sleep deprivations does not selectively impair higher cortical functioning. Sleep 22, 328–334.

Blank, M., Guerim, L.D., Cordeiro, R.F., Vianna, M.R.M., 2009. A one-trial inhibitory avoidance task to zebrafish: rapid acquisition of an NMDA-dependent long-term memory. Neurobiol. Learn. Mem. 92, 529–534. http://dx.doi.org/10.1016/j.nlm.2009.07.001.

Broglio, C., Gomez, A., Duran, E., Ocana, F.M., Jiménez-Moya, F., Rodriguez, F., Salas, C., 2005. Hallmarks of a common forebrain vertebrate plan: specialized pallial areas for spatial, temporal and emotional memory in actinopterygian fish. Brain Res. Bull. 66, 277–281.

Brower, K.J., 2003. Insomnia, alcoholism and relapse. Sleep Med. Rev. 7, 523–539. http://dx.doi.org/10.1016/S1087-0792(03)90005-0.

Brzezinski, A., Vangel, M.G., Wurtman, R.J., Norrie, G., Zhdanova, I., Ben-Shushan, A., Ford, I., 2005. Effects of exogenous melatonin on sleep: a meta-analysis. Sleep Med. Rev. 9, 41–50.

Bueno, O.F., Lobo, L.L., Oliveira, M.G., Gugliano, E.B., Pomarico, a C., Tufik, S., 1994. Dissociated paradoxical sleep deprivation effects on inhibitory avoidance and conditioned fear. Physiol. Behav. 56, 775–779. http://dx.doi.org/10.1016/0031-9384(94)90241-0.

Buscemi, N., Vandermeer, B., Pandya, R., Hooton, N., Tjosvold, L., Hartling, L., Baker, G., Vohra, S., Klassen, T., 2004. Melatonin for treatment of sleep disorders. Evidence Report/Technology Assessment.

Cachat, J.M., Canavello, P.R., Elegante, M.F., Bartels, B.K., Elkhayat, S.I., Hart, P.C., Tien, A.K., Tien, D.H., Beeson, E., Mohnot, S., Laffoon, A.L., Stewart, A.M., Gaikwad, S., Wong, K., Haymore, W., Kalueff, A.V., 2011. Modeling stress and anxiety in Zebrafish. Neuromethods. Springer, pp. 73–88. http://dx.doi.org/10.1007/978-1-60761-922-2_3.

Champagne, D.L., Hoefnagels, C.C.M., de Kloet, R.E., Richardson, M.K., 2010. Translating rodent behavioral repertoire to zebrafish (Danio rerio): relevance for stress research. Behav. Brain Res. 214, 332–342. http://dx.doi.org/10.1016/j.bbr.2010.06.001.

Colten, H.R., Altevogt, B.M., 2006. Sleep Disorders and Sleep Deprivation: an Unmet Public Health Problem. Institute of Medicine.

del Pozo, A., Sánchez-Férez, J.A., Sánchez-Vázquez, F.J., 2011. Circadian rhythms of self-feeding and locomotor activity in zebrafish (Danio rerio). Chronobiol. Int. 28, 39–47.

Deprés-Brummer, P., Metzger, G., Morin, D., Urien, S., Touitou, Y., Tillement, J.-P., Claustrat, B., Lévi, F., 1996. Pharmacokinetically guided melatonin scheduling in rats with circadian system suppression. Eur. J. Pharmacol. 312, 171–178.

Dohrman, D.P., Diamond, I., Gordon, A.S., 1997. The role of the neuromodulator adenosine in alcohol's actions. Alcohol Health Res. W. 21, 136–143.

Earleywine, M., Martin, C.S., 1993. Anticipated stimulant and sedative effects of alcohol vary with dosage and limb of the blood alcohol curve. Alcohol. Clin. Exp. Res. 17, 135–139. http://dx.doi.org/10.1111/j.1530-0277.1993.tb00738.x.

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., 2009. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behav. Brain Res. 205, 38–44. http://dx.doi.org/10.1016/j.bbr.2009.06.022.

Fairclough, S.H., Graham, R., 1999. Impairment of driving performance caused by sleep deprivation or alcohol: a comparative study. Hum. Factors 41, 118–128. http://dx.doi.org/10.1518/001872099779577336.

Gandhi, A.V., Mosser, Ea., Oikonomou, G., Prober, D.A., 2015. Melatonin is required for the circadian regulation of sleep. Neuron 1–7. http://dx.doi.org/10.1016/j.neuron.2015.02.016.

Gerlai, R., Lahav, M., Guo, S., Rosenthal, a, 2000. Drinks like a fish: zebra fish (Danio rerio) as a behavior genetic model to study alcohol effects. Pharmacol. Biochem.

Behav. 67, 773–782.
Graves, L.A., Heller, E.A., Pack, A.I., Abel, T., 2003. Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. Learn. Mem. 10, 168–176. http://dx.doi.org/10.1101/lm.48803.
Inostroza, M., Binder, S., Born, J., 2013. Sleep-dependency of episodic-like memory consolidation in rats. Behav. Brain Res. 237, 15–22. http://dx.doi.org/10.1016/j.bbr.2012.09.011.
Jenkins, J.G., Dallenbach, K.M., 1924. Obliviscence during sleep and waking. Am. J. Psychol. 605–612.
Jones, B.E., 2000. Basic mechanisms of sleep-wake states. Princ. Pract. Sleep. Med. 4, 136–153.
Kalueff, A.V., Gebhardt, M., Stewart, A.M., Cachat, J.M., Brimmer, M., Chawla, J.S., Craddock, C., Kyzar, E.J., Roth, A., Landsman, S., Gaikwad, S., Robinson, K., Baatrup, E., Tierney, K., Shamchuk, A., Norton, W., Miller, N., Nicolson, T., Braubach, O., Gilman, C.P., Pittman, J., Rosemberg, D.B., Gerlai, R., Echevarria, D., Lamb, E., Neuhauss, S.C.F., Weng, W., Bally-Cuif, L., Schneider, H., 2013. Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. Zebrafish 10, 70–86. http://dx.doi.org/10.1089/zeb.2012.0861.
Killgore, W.D.S., 2010. Effects of sleep deprivation on cognition. Prog. Brain Res. 105–129. http://dx.doi.org/10.1016/B978-0-444-53702-7.00007-5.
Kleitman, N., 1939. Sleep and Wakefulness as Alternating Phases in the Cycle of Existence.
Koob, G.F., Roberts, A.J., 1999. Brain reward circuits in alcoholism. CNS Spectr. 4, 23–37.
Krieger, D.T., 1979. Endocrine Rhythms. Raven Press.
Leibowitz, S.M., Lopes, M.-C.C., Andersen, M.L., Kushida, C.A., 2006. Sleep deprivation and sleepiness caused by sleep loss. Sleep Med. Clin. 1, 31–45. http://dx.doi.org/10.1016/j.jsmc.2005.11.010.
Lima-Cabello, E., Díaz-Casado, M.E., Guerrero, J.A., Otalora, B.B., Escames, G., López, L.C., Reiter, R.J., Acuña-Castroviejo, D., 2014. A review of the melatonin functions in zebrafish physiology. J. Pineal Res. 1–9. http://dx.doi.org/10.1111/jpi.12149.
Luchiari, A.C., Salajan, D.C., Gerlai, R., 2015. Acute and chronic alcohol administration: effects on performance of zebrafish in a latent learning task. Behav. Brain Res. 282, 76–83. http://dx.doi.org/10.1016/j.bbr.2014.12.013.
Machida, M., Yang, L., Wellman, L.L., Sanford, L.D., 2013. Effects of stressor predictability on escape learning and sleep in mice. Sleep 36, 421–430. http://dx.doi.org/10.5665/sleep.2464.
Manuel, R., Gorissen, M., Piza Roca, C., Zethof, J., van de Vis, H., Flik, G., van den Bos, R., 2014. Inhibitory avoidance learning in brafish (Danio rerio): effects of shock intensity and unraveling differences in task performance. Zebrafish 11, 341–352. http://dx.doi.org/10.1089/zeb.2013.0970.
Marshall, L., Born, J., 2007. The contribution of sleep to hippocampus-dependent memory consolidation. Trends Cogn. Sci. 11, 442–450. http://dx.doi.org/10.1016/j.tics.2007.09.001.
Martin, C.S., Earleywine, M., Musty, R.E., Perrine, M.W., Swift, R.M., 1993. Development and validation of the biphasic alcohol effects scale. Alcohol. Clin. Exp. Res. 17, 140–146. http://dx.doi.org/10.1111/j.1530-0277.1993.tb00739.x.
McGaugh, J.L., 2000. Memory–a century of consolidation. Science 287 (80), 248–251. http://dx.doi.org/10.1126/science.287.5451.248.
Mintz, E.M., Phillips, N.H., Berger, R.J., 1998. Daytime melatonin infusions induce sleep in pigeons without altering subsequent amounts of nocturnal sleep. Neurosci. Lett. 258, 61–64. http://dx.doi.org/10.1016/S0304-3940(98)00849-0.
Moore, B.R., 2004. The evolution of learning. Biol. Rev. Camb. Philos. Soc. 79, 301–335. http://dx.doi.org/10.1017/S1464793103006225.
Moore, B.R., 1973. The Role of Directed Pavlovian Reactions in Simple Instrumental Learning in the Pigeon.
Mueller, T., Wullimann, M.F., 2003. Anatomy of neurogenesis in the early zebrafish brain. Dev. Brain Res. 140, 137–155. http://dx.doi.org/10.1016/S0165-3806(02)00583-7.
Obernier, J.A., White, A.M., Swartzwelder, H.S., Crews, F.T., 2002. Cognitive deficits and CNS damage after a 4-day binge ethanol exposure in rats. Pharmacol. Biochem. Behav. 72, 521–532. http://dx.doi.org/10.1016/S0091-3057(02)00715-3.
Oliveira, J., Silveira, M., Chacon, D., Luchiari, A., 2015. The zebrafish world of colors and shapes: preference and discrimination. Zebrafish 12, 166–173. http://dx.doi.org/10.1089/zeb.2014.1019.
OMS, 2002. The World Health Report 2002: Reducing Risks, Promoting Healthy Life. World Health Organization.
Panda, S., Hogenesch, J.B., Kay, S.A., 2002. Circadian rhythms from flies to human. Nature 417, 329–335.
Phelps, E.A., 2004. Human emotion and memory: interactions of the amygdala and hippocampal complex. Curr. Opin. Neurobiol. 14, 198–202.
Phillips, N.H., Berger, R.J., 1992. Melatonin infusions restore sleep suppressed by continuous bright light in pigeons. Neurosci. Lett. 145, 217–220. http://dx.doi.org/10.1016/0304-3940(92)90026-4.
Pinheiro-da-Silva, J., Silva, P.F., Nogueira, M.B., Luchiari, A.C., 2017. Sleep deprivation effects on object discrimination task in zebrafish (Danio rerio). Anim. Cogn. 20, 159–169. http://dx.doi.org/10.1007/s10071-016-1034-x.
Pradel, G., Schmidt, R., Schachner, M., 1996. Inhibition of memory consolidation by antibodies against the HNK-1 carbohydrate after active avoidance conditioning in zebrafish. J. Neurochem. 66http://dx.doi.org/10.1002/(SICI)1097-4695(199905)39.S19–S19.
Prince, T.-M., Abel, T., 2013. The impact of sleep loss on hippocampal function. Learn. Mem. 20, 558–569. http://dx.doi.org/10.1101/lm.031674.113.
Raidy, D.J., Scharff, L.F.V., 2005. Effects of sleep deprivation on auditory and visual memory tasks. Percept. Mot. Skills 101, 451–467.
Rasch, B., Born, J., 2013. About sleep's role in memory. Physiol. Rev. 93, 681–766. http://dx.doi.org/10.1152/physrev.00032.2012.
Rawashdeh, O., de Borsetti, N.H., Roman, G., Cahill, G.M., 2007. Melatonin suppresses nighttime memory formation in zebrafish. Science 318, 1144–1146. http://dx.doi.org/10.1126/science.1148564.
Roehrs, T., Papineau, K., Rosenthal, L., Roth, T., 1999. Ethanol as a hypnotic in insomniacs: self administration and effects on sleep and mood. Neuropsychopharmacology 20, 279–286.
Roehrs, T., Roth, T., 2001. Sleep, sleepiness, sleep disorders and alcohol use and abuse. Sleep Med. Rev. 5, 287–297. http://dx.doi.org/10.1053/smrv.2001.0162.
Ruskin, D.N., Liu, C., Dunn, K.E., Bazan, N.G., LaHoste, G.J., 2004. Sleep deprivation impairs hippocampus-mediated contextual learning but not amygdala-mediated cued learning in rats. Eur. J. Neurosci. 19, 3121–3124.
Ryback, R., Percarpio, B., Vitale, J., 1969. Equilibration and metabolism of ethanol in the goldfish. Nature 222, 1068–1070. http://dx.doi.org/10.1038/2221068a0.
Salas, C., Broglio, C., Durán, E., Gómez, A., Ocaña, F.M., Jiménez-Moya, F., Rodríguez, F., 2006. Neuropsychology of learning and memory in teleost fish. Zebrafish 3, 157–171.
Scheer, F.A., Czeisler, C.A., 2005. Melatonin, sleep, and circadian rhythms. Sleep Med. Rev. 9, 5–9. http://dx.doi.org/10.1016/j.smrv.2004.11.004.
Schmidt, M.H., 2014. The energy allocation function of sleep: a unifying theory of sleep, torpor, and continuous wakefulness. Neurosci. Biobehav. Rev. 47, 122–153. http://dx.doi.org/10.1016/j.neubiorev.2014.08.001.
Serra, E.L., Medalha, C.C., Mattioli, R., 1999. Natural preference of zebrafish (Danio rerio) for a dark environment. Braz. J. Med. Biol. Res. 32, 1551–1553.
Sharma, A., Vandenberg, B., Hollingsworth, B., 2014. Minimum pricing of alcohol versus volumetric taxation: which policy will reduce heavy consumption without adversely affecting light and moderate consumers? PLoS One 9, e80936.
Siegel, J.M., 2005. Clues to the functions of mammalian sleep. Nature 437, 1264–1271. http://dx.doi.org/10.1038/nature04285.
Sigurgeirsson, B., Thornorsteinsson, H., Sigmundsdóttir, S., Lieder, R., Sveinsdóttir, H.S., Sigurjónsson, Ó.E., Halldórsson, B., Karlsson, K., 2013. Sleep-wake dynamics under extended light and extended dark conditions in adult zebrafish. Behav. Brain Res. 256, 377–390. http://dx.doi.org/10.1016/j.bbr.2013.08.032.
Spence, R., Gerlach, G., Lawrence, C., Smith, C., 2008. The behaviour and ecology of the zebrafish, Danio rerio. Biol. Rev. Camb. Philos. Soc. 83, 13–34. http://dx.doi.org/10.1111/j.1469-185X.2007.00030.x.
Stein, M.D., Friedmann, P.D., 2005. Disturbed sleep and its relationship to alcohol use. Subst. Abus. 26, 1–13. http://dx.doi.org/10.1300/J465v26n01_01.
Stickgold, R., Walker, M.P., 2013. Sleep-dependent memory triage: evolving generalization through selective processing. Nat. Neurosci. 16, 139–145.
Tononi, G., Cirelli, C., 2006. Sleep function and synaptic homeostasis. Sleep Med. Rev. http://dx.doi.org/10.1016/j.smrv.2005.05.002.
Tran, S., Gerlai, R., 2013. Time-course of behavioural changes induced by ethanol in zebrafish (Danio rerio). Behav. Brain Res. 252, 204–213. http://dx.doi.org/10.1016/j.bbr.2013.05.065.
Tropepe, V., Sive, H.L., 2003. Can zebrafish be used as a model to study the neurodevelopmental causes of autism? Genes Brain Behav. 2, 268–281.
Turner, R.T., 2000. Skeletal response to alcohol. Alcohol. Clin. Exp. Res. 24, 1693–1701.
Van Cauter, E., Spiegel, K., Tasali, E., Leproult, R., 2008. Metabolic consequences of sleep and sleep loss. Sleep Med. 9, S23–S28.
Van Den Heuvel, C.J., Ferguson, S.A., Mila MacChi, M., Dawson, D., 2005. Melatonin as a hypnotic: con. Sleep Med. Rev. 9, 71–80. http://dx.doi.org/10.1016/j.smrv.2004.07.001.
Van Dongen, H.P.A., Bender, A.M., Dinges, D.F., 2012. Systematic individual differences in sleep homeostatic and circadian rhythm contributions to neurobehavioral impairment during sleep deprivation. Accid. Anal. Prev. 45 (Suppl), 11–16. http://dx.doi.org/10.1016/j.aap.2011.09.018.
Vengeliene, V., Bilbao, A., Molander, A., Spanagel, R., 2008. Neuropharmacology of alcohol addiction. Br. J. Pharmacol. 154, 299–315. http://dx.doi.org/10.1038/bjp.2008.30.
von Krogh, K., Sørensen, C., Nilsson, G.E., Øverli, Ø., 2010. Forebrain cell proliferation, behavior, and physiology of zebrafish, Danio rerio, kept in enriched or barren environments. Physiol. Behav. 101, 32–39.
Walker, M.P., Stickgold, R., 2006. Sleep, memory, and plasticity. Annu. Rev. Psychol. 57, 139–166. http://dx.doi.org/10.1146/annurev.psych.56.091103.070307.
Wang, J., Liu, C., Ma, F., Chen, W., Liu, J., Hu, B., Zheng, L., 2014. Circadian clock mediates light/dark preference in zebrafish (Danio Rerio). Zebrafish 11, 1–7. http://dx.doi.org/10.1089/zeb.2013.0929.
Watson, B.O., Buzsáki, G., 2015. Sleep, Memory & Brain Rhythms. Daedalus 144, 67–82. http://dx.doi.org/10.1162/DAED_a_00318.
WHO, 2012. Alcohol in the European Union: Consumption, Harm and Policy Approaches: Final Report. Copenhagen 27 March 2012. .
Wullimann, M.F., Mueller, T., 2004. Identification and morphogenesis of the eminentia thalami in the zebrafish. J. Comp. Neurol. 471, 37–48.
Xu, X., Scott-Scheiern, T., Kempker, L., Simons, K., 2007. Active avoidance conditioning in zebrafish (Danio rerio). Neurobiol. Learn. Mem. 87, 72–77. http://dx.doi.org/10.1016/j.nlm.2006.06.002.
Yamada, H., Oshima, I., Sato, K., Ebihara, S., 1988. Loss of the circadian rhythms of locomotor activity, food intake, and plasma melatonin concentration induced by constant bright light in the pigeon (Columba livia). J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol. 163, 459–463.
Yokogawa, T., Marin, W., Faraco, J., Pézeron, G., Appelbaum, L., Zhang, J., Rosa, F., Mourrain, P., Mignot, E., 2007. Characterization of sleep in zebrafish and insomnia in hypocretin receptor mutants. PLoS Biol. 5, e277. http://dx.doi.org/10.1371/journal.pbio.0050277.
Yu, L., Tucci, V., Kishi, S., Zhdanova, I.V., 2006. Cognitive aging in zebrafish. PLoS One 1, e14. http://dx.doi.org/10.1371/journal.pone.0000014.
Zhdanova, I.V., Yu, L., Lopez-Patino, M., Shang, E., Kishi, S., Guelin, E., 2008. Aging of the circadian system in zebrafish and the effects of melatonin on sleep and cognitive

performance. Brain Res. Bull 275, 433–441. http://dx.doi.org/10.1016/j.biotechadv.2011.08.021.Secreted.
Zhdanova, I.V., 2011. Sleep and its regulation in zebrafish. Rev. Neurosci. 22, 27–36. http://dx.doi.org/10.1515/RNS.2011.005.
Zhdanova, I.V., 2006a. Sleep in zebrafish. Zebrafish 3, 215–226. http://dx.doi.org/10.1089/zeb.2006.3.215.
Zhdanova, I.V., 2006b. Sleep and melatonin in diurnal species. Melatonin Biol. Basis 162.
Zhdanova, I.V., Geiger, D.A., Schwagerl, A.L., Leclair, O.U., Killiany, R., Taylor, J.A., Rosene, D.L., Moss, M.B., Madras, B.K., 2002. Melatonin promotes sleep in three species of diurnal nonhuman primates. Physiol. Behav. 75, 523–529. http://dx.doi.org/10.1016/S0031-9384(02)00654-6.
Zhdanova, I.V., Wang, S.Y., Leclair, O.U., Danilova, N.P., 2001. Melatonin promotes sleep-like state in zebrafish. Brain Res. 903, 263–268. http://dx.doi.org/10.1016/S0006-8993(01)02444-1.