Anim Cogn (2017) 20:159–169
DOI 10.1007/s10071-016-1034-x

ORIGINAL PAPER

Sleep deprivation effects on object discrimination task in zebrafish (*Danio rerio*)

Jaquelinne Pinheiro-da-Silva[1] · Priscila Fernandes Silva[1] · Marcelo Borges Nogueira[2] · Ana Carolina Luchiari[1]

Received: 10 December 2015 / Revised: 31 August 2016 / Accepted: 3 September 2016 / Published online: 19 September 2016

Abstract The zebrafish is an ideal vertebrate model for neurobehavioral studies with translational relevance to humans. Many aspects of sleep have been studied, but we still do not understand how and why sleep deprivation alters behavioral and physiological processes. A number of hypotheses suggest its role in memory consolidation. In this respect, the aim of this study was to analyze the effects of sleep deprivation on memory in zebrafish (*Danio rerio*), using an object discrimination paradigm. Four treatments were tested: control, partial sleep deprivation, total sleep deprivation by light pulses, and total sleep deprivation by extended light. The control group explored the new object more than the known object, indicating clear discrimination. The partially sleep-deprived group explored the new object more than the other object in the discrimination phase, suggesting a certain degree of discriminative performance. By contrast, both total sleep deprivation groups equally explored all objects, regardless of their novelty. It seems that only one night of sleep deprivation is enough to affect discriminative response in zebrafish, indicating its negative impact on cognitive processes. We suggest that this study could be a useful screening tool for cognitive dysfunction and a better understanding of the effect of sleep-wake cycles on cognition.

Keywords Sleep · Fish · Memory · Discrimination · Bayesian analysis

Introduction

Sleep is a naturally recurring condition characterized by rest, altered state of consciousness, and suspension of sensory, perceptual and voluntary activities (Schmidt 2014). While it is a universal behavioral and physiological phenomenon present in most vertebrates, not all animals exhibit the same set of sleep state characteristics (Lyamin et al. 2007). The presence, quality, intensity and functions of sleep vary between species and across the lifespan (Siegel 2008). However, it remains unknown exactly why animals sleep. Hypotheses range from energy allocation and conservation to synapse remodeling and memory consolidation, with a myriad of possible functions throughout their evolutionary history (Siegel 2005; Tononi and Cirelli 2006; Schmidt 2014; Herculano-Houzel 2015).

With respect to the purpose of sleeping, some studies suggest that the REM (rapid eye movement) stage of sleep favors learning, acting to consolidate important information and eliminate irrelevant information to avoid unnecessary energy expenditure (Poe et al. 2000; Louie and Wilson 2001; Stickgold and Walker 2005; Stickgold 2005). It is also proposed that brain activity during REM sleep may facilitate memory development and maintenance by strengthening previously formed circuits and promoting new synapse connections (Roffwarg et al. 1966; Rasch et al. 2009; Blumberg 2010; Hobson and Steriade 2011; Schmidt 2014). Other studies also indicate that the non-REM stage of sleep

Electronic supplementary material The online version of this article (doi:10.1007/s10071-016-1034-x) contains supplementary material, which is available to authorized users.

✉ Ana Carolina Luchiari
analuchiari@yahoo.com.br

[1] Departamento de Fisiologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, PO BOX 1511, Natal, Rio Grande do Norte 59078-970, Brazil

[2] Escola de Ciências e Tecnologia, CCET, Universidade Federal do Rio Grande do Norte, Natal, Rio Grande do Norte 59078-970, Brazil

plays a significant role in incorporating memories to cerebral cortex areas (Euston et al. 2007; Prince and Abel 2013). Learning and memory are critical processes that provide numerous advantages for the species, such as recognizing conspecifics and mates, remembering routes and identifying feeding times/places, which are important features for animal fitness (Johnston 1982; Sison and Gerlai 2010).

While it is suggested that sleep plays a vital role in an animal's life, sleep deprivation (SD) has a significant impact on neurological and physiological processes. Indeed, several studies indicate that SD is harmful to neurogenesis, attention, learning and memory retention (Spiegel 2004; Van Cauter 2005; Guzman-Marin et al. 2005; Leibowitz et al. 2006; Yu et al. 2006). Prolonged sleep deprivation causes attention and memory problems, resulting in a state of unreality similar to sleep, loss of autonomic and endocrine control, and even exhaustion and death (Andersen et al. 2008). However, the extent to which SD could affect learning and memory processes needs to be clarified for future genetic or drug screening.

A novel memory paradigm based on the principles of one-trial learning was recently developed specifically for the zebrafish (Oliveira et al. 2015). In this paradigm, the fish explored a dyad of objects with no reinforcement and is then tested for its recognition of a new object. Object discrimination protocols were previously tested in several animal models, such as rats (Bevins and Besheer 2006), pigeons (Koban and Cook 2009), and fish (Siebeck et al. 2009; Schluessel et al. 2012, 2014; Lucon-Xiccato and Dadda 2014). The paradigm is simple and requires only a brief experimental period, resulting in a potentially high throughput. However, this paradigm has not been used for behavioral brain research. In the current study, we investigate the effect of sleep deprivation on the behavioral performance of zebrafish in the object discrimination paradigm. Thus, we tested zebrafish (*Danio rerio*), a valuable model for sleep research (Zhdanova et al. 2001; Zhdanova 2006), in order to address the following questions: Is one night of SD enough to alter performance in a one-trial learning task? Does partial SD affect memory the same way as total SD?

Materials and methods

Stock conditions

Adult zebrafish (*Danio rerio*, wild-type, 3 months of age) obtained from a local fish farm were transferred to a storage system (50 L tanks) at the Ornamental Fish Vivarium, Department of Physiology—Federal University of Rio Grande do Norte. Each set of four 50-L tanks formed a recirculating system with multistage filtration, including a mechanical filter, biological filter, activated carbon filter, and UV light sterilizing unit. The animals were kept in the tanks at a density of one fish/l, with aerated and filtered water, at a temperature of ±26.5 °C, and pH and oxygen measured regularly. The photoperiod used was a 12:12 light/dark cycle, with zeitgeber time (ZT) 0 corresponding to lights-on at 7 a.m., and light intensity of 250 lx. Twice-a-day ad libitum feeding, which always occurred at the same time, consisted of a brine shrimp and flake food diet (60 % protein and 15 % fat). The Animal Ethics Committee of the Federal University of Rio Grande do Norte authorized all animal procedures (CEUA 022/2012).

Sleep deprivation (SD)

According to Yokogawa et al. (2007), light has a powerful suppressive effect on sleep in zebrafish, with no evidence of sleep rebound. Sigurgeirsson et al. (2013) compared an extended period of light and electroshock as promoters of sleep deprivation and confirmed that both light and shock are effective in disrupting sleep, functioning as sleep and wakefulness modulators, but light-induced deprivation causes less deviation from normal sleep–wake bouts. Therefore, we used night light instead of the electroshock protocol to cause one night of sleep deprivation in zebrafish and tested its effects on memory.

In the present study, sleep deprivation was achieved by (1) extending the light phase or (2) exposing fish to brief light pulses during the dark phase. In this case, the stimulus used to induce deprivation could act as an interference or stress element, impairing the animal's performance during the learning task. Therefore, in order to evaluate the effects of light pulses on fish cognition, two groups of 12 fish were used to validate the SD protocol: Control + pulses, in which fish were exposed to a 12L:12D cycle and 6 h of 2-min light pulses (2 min lights on + 2 min lights off), were applied during the waking period (from ZT0 to ZT6), and Control SD + pulses, in which fish were exposed to 18L:06D cycle and 6 h of 2-min light pulses (2 min lights on +2 min lights off), were applied during the entire dark period. These two groups were submitted to the object discrimination task (described below), in which the discrimination phase (memory evaluation) was applied after the light pulse treatment, and data collection started at ZT7.

Another group of fish not used for the cognition test was kept individually in 15-L tanks and their behavior recorded over a 24 h-period. Eight fish were maintained under a 12L:12D cycle (ZT0 at 7 a.m.), another eight were exposed to an extended period of light (Partial SD; 18L:06D), with lights turned on at 7 a.m. and off at 1 a.m. (ZT18), and another group of eight fish to a 24L:00D cycle (Total SD). The behavioral records were analyzed to determine average swimming speed and the fish were visually followed to identify sleeping episodes (Fig. S1 and S2).

In order to compare the effects of sleep deprivation on zebrafish performance on a memory test, 44 fish were divided into four distinct light–dark conditions: control group (12L:12D; $n = 10$), partial sleep deprivation (18L:06D; $n = 11$), total sleep deprivation by light pulses (18L:06D + pulses; $n = 11$), and total sleep deprivation by extended light (24L:00D; $n = 12$). For light deprivation pulses, a 4-min light pulse was administered every 5 min (4 min light + 1 min dark), during the entire 6-h dark phase, preventing the fish from resting in the dark for more than 1 min. The above light–dark conditions were imposed only during the night, after the task memorization phase (see below).

Object discrimination task

For the memory test, we used a one-trial object discrimination procedure in which fish were given only one training trial before being tested. This procedure was adapted from Siebeck et al. (2009); Schluessel et al. (2014); Lucon-Xiccato and Dadda (2014) and previously tested and validated for zebrafish by Oliveira et al. (2015), who showed that zebrafish are able to discriminate objects based on color and shape, but not on size. In the present study, only color was used to facilitate object perception and discrimination processing.

The object discrimination test took place in three phases: (1) tank acclimation, (2) memorization phase, and (3) discrimination phase. During the period between the memorization (2) and discrimination phases (3), the fish were exposed to the light–dark conditions described above. All phases occurred in 15-L tanks (40 × 25 × 20 cm) with all walls covered in white to avoid external interferences. The objects used were LEGO® plastic blocks (4 × 4 × 4 cm; Fig. 1a), and to avoid preferences, the colors of the objects were totally randomized between animals and treatments (Table S1).

The acclimation phase (1) lasted 5 days. Fish were allowed to explore the test tank for 15 min per day, without objects, to acclimatize to the new environment and reduce novelty stress. To reduce isolation stress, since zebrafish are highly social animals, 11 fish explored the test tank together on the first day, half the group on the second day, and so forth, so that by the 5th day each fish had explored the tank alone for 15 min. After the 15-min period in the test tank, the fish were transferred to their home tank.

The memorization phase (2) occurred on the 6th day. Two 3D blocks (A_1 and A_2) of the same color, size, and shape were introduced into the tank, each one positioned next to one of the narrower walls, approximately 30 cm apart (Fig. 1b). Fish were individually allowed to explore the tank containing the two objects for 15 min. Behavior was recorded from above using a Sony DCR-SX45 handycam. The fish were then returned to their home tank. In both phases, we considered the animal's residence time in a 3-cm area around the objects to define exploration behavior (Lucon-Xiccato and Dadda 2014).

On the night following the memorization phase, each group was exposed to one of the light–dark conditions: 12L:12D (control group), 18L:06D (partial sleep deprivation), 18L:06D + pulses (light pulse deprivation), and 24L:00D (extended light deprivation).

The discrimination phase (3) occurred 24 h after the memorization phase. To that end, we presented the same object from the previous phase (now denominated object A_3) in one area and the other object was replaced by a new one (object B), with the same size and shape but different color (Fig. 1c). Fish were able to explore the objects in the tank for 15 min, and behavior was recorded.

Behavioral analysis

Video frames from each trial were analyzed using a new custom-made multi-target tracking software (denominated ZebTrack/UFRN) developed in MATLAB (R2014a; MathWorks, Natick, MA). This software, designed by our

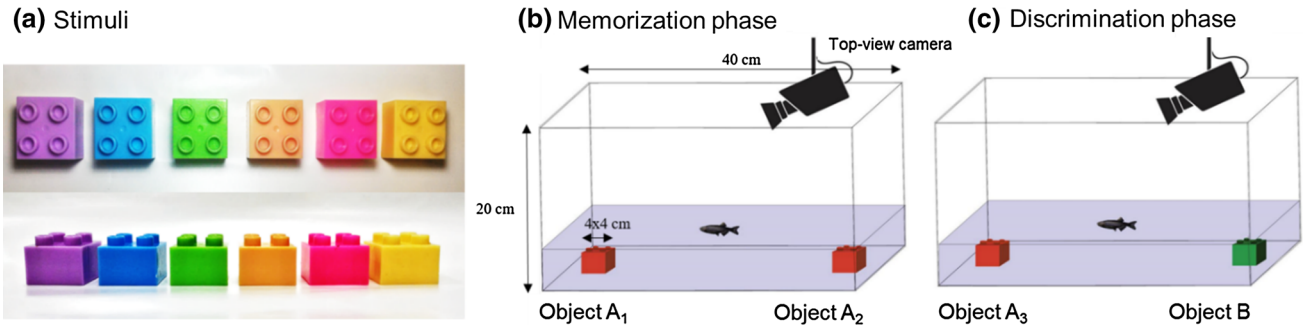

Fig. 1 **a** LEGO® blocks used as stimuli in the one-trial objects discrimination paradigm. **b** Schematic overview of the one-trial object discrimination paradigm (40 × 25 × 20 cm^3) at the memorization phase (with objects A_1 and A_2 in *same colors*) and **c** discrimination phase (with objects A_3 and B in *different colors*). The tank was all covered in *white* self-adhesive plastic film. The objects colors were randomized among all animals and treatments. For both phases, fish were able to explore the objects for 15 min and behavior was registered with a *top-view* camera (color figure online)

laboratory as an alternative to other costly tracking systems, is able to access and quantify several swimming path patterns, including speed, distance traveled, and residence time in specific areas of the tank. It is more appropriate than a manual recording method because after the area of interest is established, the software tracks the fish without any human error. Details of the tracking software are available in the Online Resource.

Statistical analysis

To apply inferential statistics, data were assessed using exploratory analysis due to potential problems with outliers, homoscedasticity, normality, zero trouble, collinearity and independence of variables, as suggested by Zuur et al. (2010).

The time fish spent around the objects (up to 3 cm from each side of the objects) was used to estimate exploration and compared in the memorization and discrimination phases. The difference in exploration time between the objects in each phase was determined using Bayesian estimation, which provides complete posterior distributions for models, yielding richer inference than traditional hypothesis tests (Kruschke 2013). Specifically, this analysis is equivalent to a traditional ANOVA, but robust against outliers. Accounting for the heteroscedasticity of the data and outliers, we used t distribution instead of normal distributions and provided every group with its own standard-deviation (SD) parameter. Furthermore, we set a hierarchical prior on the sd parameters, so that each object mutually informed the sd of the other groups via higher-level distribution (Kruschke 2014). Bayesian analysis uses the highest density interval (HDI) instead of the confidence interval employed for frequentist analysis. The HDI reduces uncertainty, indicating the most credible values and covering 95 % of data distribution. The comparison value was set at around 0, and the region of practical equivalence (ROPE) was defined as ±5.

An index of how much a fish explored each object during the test, previously applied for object recognition tests by Akkerman et al. (2012) and May et al. (2016) was applied to examine differences across conditions. The exploration index for the memorization phase (exploration of memorization = $E_m = A_1 + A_2$) and discrimination index for the discrimination phase (discrimination index = $D_i = B - A_3$) were calculated for each group to establish whether there were differences in object exploration time. Linear regression analysis was conducted to determine whether E_m was predictive of D_i, that is, to assess whether time spent exploring the objects in the memorization phase relates to new object exploration in the discrimination phase. The discrimination indices were then compared by Bayesian analysis for each group, considering the theoretical mean as 0.

Total distance traveled and maximum swimming speed were analyzed by one-way ANOVA followed by post hoc comparisons using the Student–Newman–Keuls test. Statistical hypothesis tests and p values were calculated using Sigma Stat 3.5, and the alpha level was set at 0.05.

Results

Validation of sleep deprivation protocol

Figure 2 depicts exploration time of objects A_1, A_2, A_3, and B for the two control groups. Figure 2a, b shows group data and variability outside the upper and lower quartiles, while Fig. 2c, d presents the posterior distribution of the Bayesian analysis between exploration time of objects A_1 versus A_2 and A_3 versus B. The Control + pulses group, in which fish were exposed to light pulses during the waking period, exhibited no significant difference between exploration time of objects A_1 and A_2 in the memorization phase (posterior mean = 2.35; 95 % HDI = [−40.74, 48.83]), but a significant difference between exploration time of objects A_3 and B in the discrimination phase, suggesting increased exploration of the novel object (posterior mean = 57.86; 95 % HDI = [26.34, 88.85]). On the other hand, the Control SD + pulses group showed no significant difference between object exploration in the memorization phase (A_1 vs. A_2: posterior mean = −20.71; 95 % HDI = [−63.86, 11.26]) and discrimination phase (A_3 vs. B: posterior mean = −2.00; 95 % HDI = [−31.29, 27.37]).

Figure 3 illustrates the discrimination index (D_i) for the Control + pulses and Control SD + pulses groups. Bayesian analysis compared the D_i with the theoretical mean, exhibiting a significant difference for the group that received light pulses during the waking period (Control + pulses: posterior mean = 61.41; 95 % HDI = [32.74, 89.55]), but not for the sleep-deprived group (Control SD + pulses: posterior mean = −4.82; 95 % HDI = [−46.88, 33.46]).

Object discrimination task

Figure 4 shows object exploration time for the control (12L:12D), partial sleep deprivation (18L:06D), total sleep deprivation by light pulses (18L:06D + pulses) and Total sleep deprivation by extended light (24L:00D) groups. The posterior distribution of Bayesian analysis between the exploration time of objects A_1 vs. A_2 and A_3 vs. B for these groups is presented in Fig. 5. Bayesian inferences of these data sets revealed no significant differences in object exploration time in the memorization phase (control group: posterior mean = 9.19; 95 % HDI = [−27.55, 45.32]; partial SD: posterior mean = 1.79; 95 % HDI = [−34.02, 33.84]).

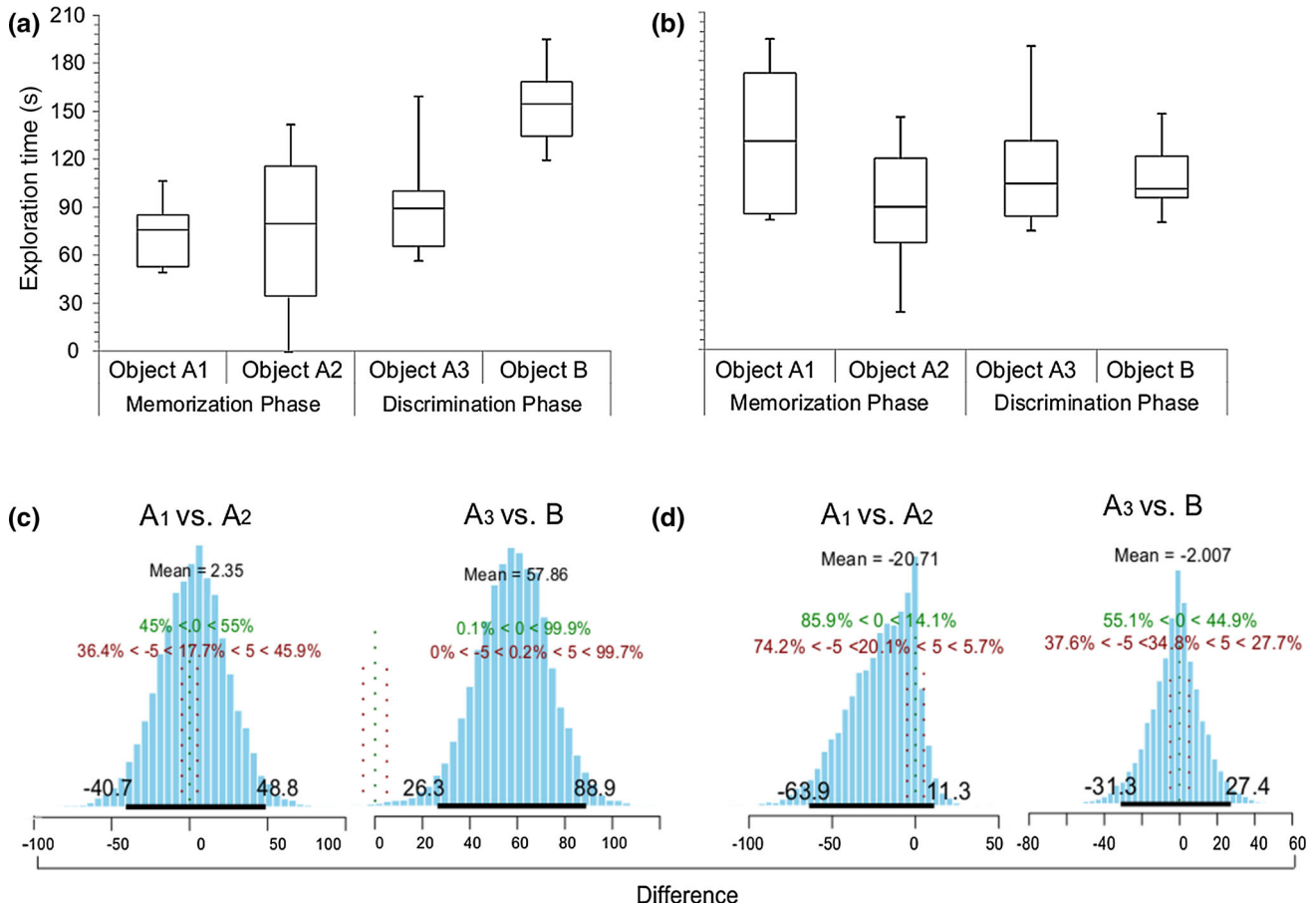

Fig. 2 Exploration time of objects A_1, A_2, A_3, and B for control groups (validation of the sleep deprivation protocol). *Box* plots represent the relative median values of exploration time for the **a** Control + pulses and **b** Control SD + pulses groups. Posterior distribution of the Bayesian analysis shows comparison between the exploration time of objects A_1 versus A_2 and A_3 versus B for **c** Control + pulses and **d** Control SD + pulses groups. *Green dashed lines* (central dashed line) indicate the comparison value = 0 and *red dashed lines* (lateral dashed lines) indicate the ROPE ± 5. 95 %. HDI interval is marked by the *black bar* on the floor of the distribution. For results of statistical analysis, see "Results" section (color figure online)

Although Fig. 5a (right side) showed that the posterior 95 % HDI overlaps the ROPE, it is clear that the effect is quite large, with 97.7 % credibility in favor of the difference between exploration time of objects A_3 and B for the control group (posterior mean = 41.32; 95 % HDI = [−1.39, 78.5]). However, we found no significant difference for the partial SD group (posterior mean = 29.67; 95 % HDI = [−14.69, 85.25]), the total SD + light pulses group (posterior mean = 1.68; 95 % HDI = [−27.87, 29.77]), or the total SD with extended light group (posterior mean = 3.99; 95 % HDI = [−29.88, 41.58]) (Fig. 5b–d.).

Figure 6 illustrates the linear correlation of exploration in the memorization phase (E_m) and discrimination index (D_i) for the tested groups. Comparisons with the theoretical mean showed significant differences for the control group (posterior mean = 53.26; 95 % HDI = [18.51, 86.66]), but not for the other groups (Partial SD: posterior mean = 66.44; 95 % HDI = [−15.39, 153.92]; Total SD + light pulses: posterior mean = 1.99; 95 % HDI = [−39.60, 41.17]; Total SD +extended light: posterior mean = −28.91; 95 % HDI = [−33.45, 94.84] (Fig. 7).

The maximum swimming speed was similar among the four groups in the memorization phase [ANOVA, $F(11, 44) = 0.44$ $p = 0.72$], but fish from the partial and totally sleep-deprived groups showed higher maximum speed than the control group in the discrimination phase [ANOVA, $F(11, 44) = 4.73$ $p = 0.008$]. Comparison between the phases (6th vs. 7th day) for each group showed that the control and totally SD with extended light groups exhibited similar maximum speed between the 2 days (Student's t test: control: $t = 0.39$ $p = 0.69$; SD with extended light: $t = 0.90$ $p = 0.37$), while the other groups increased speed on the 7th day (Student's t test: partially SD: $t = -2.44$ $p = 0.02$; SD with light pulses: $t = -3.42$ $p = 0.003$ $t = -3.38$ $p = 0.007$) (Fig. 8a).

The total distance traveled did not differ among the groups in the memorization phase [ANOVA, $F(11,$

44) = 1.46 $p = 0.24$] or discrimination phase [ANOVA, $F(11, 44) = 1.44$ $p = 0.26$]. Likewise, comparison between the phases showed none of the groups differed in terms of distance traveled on the 6th and 7th days (Student's t test: control: $t = 0.13$ $p = 0.90$; partially SD: $t = -0.33$ $p = 0.74$; SD with light pulses: $t = -0.3$ $p = 0.77$; SD with extended light: $t = 0.99$ $p = 0.33$) (Fig. 8b).

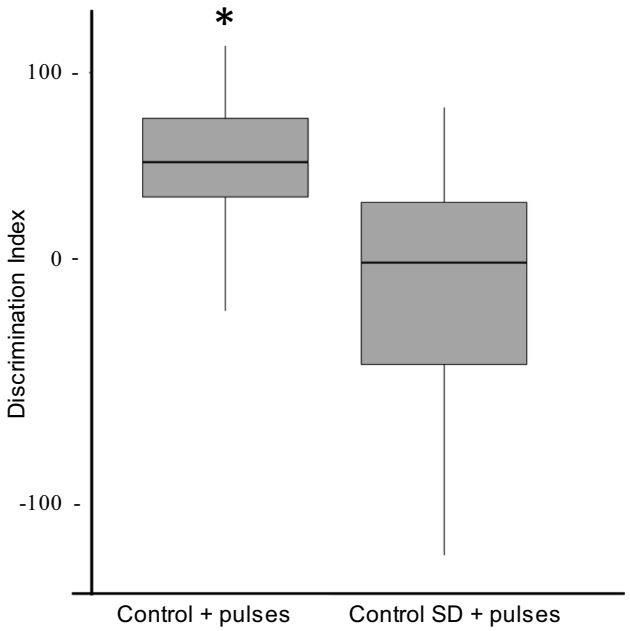

Fig. 3 Discrimination index for control groups (validation of the sleep deprivation protocol). *Index box* plots represent the relative median values of discrimination index ($D_i = B-A3$) for both groups (Control + pulses and Control SD + pulses) in comparison with a theoretical mean of 0. For results of statistical analysis, see "Results" section

Discussion

In this study, we observed that total sleep deprivation prevents memory of a single event in zebrafish, while restricted sleep still allows memory formation. Adding to other studies on the role of sleep in memory and learning tasks, the current study used a recently validated protocol on object discrimination (Oliveira et al. 2015) and showed its relevance for sleep investigations. Our results confirm that zebrafish are able to discriminate visual stimuli based on colors, corroborating other authors' findings (Fetsko 2002; Colwill et al. 2005; Oliveira et al. 2015). Moreover, we show that light is a powerful stimulus in promoting sleep deprivation, confirming studies by Yokogawa et al. (2007) and Sigurgeirsson et al. (2013). Light can be properly used both as an uninterrupted stimulus (24L:00D) and of 2 × 2 min (light × dark) or 4 × 1 min pulses (light × dark) to disrupt sleep in fish. In this study, only one night of sleep deprivation was sufficient to abolish discriminative response, while animals partially sleep deprived exhibited less evident object discrimination, as observed in Fig. 5b.

Exploration is an important behavioral response to environmental changes and its novelties (Kalueff and Zimbardo 2007) and is part of the zebrafish's behavioral repertoire. The control group (12 h dark phase) explored both objects equally on the first day of the test, but on the following day when a new object was introduced, the fish

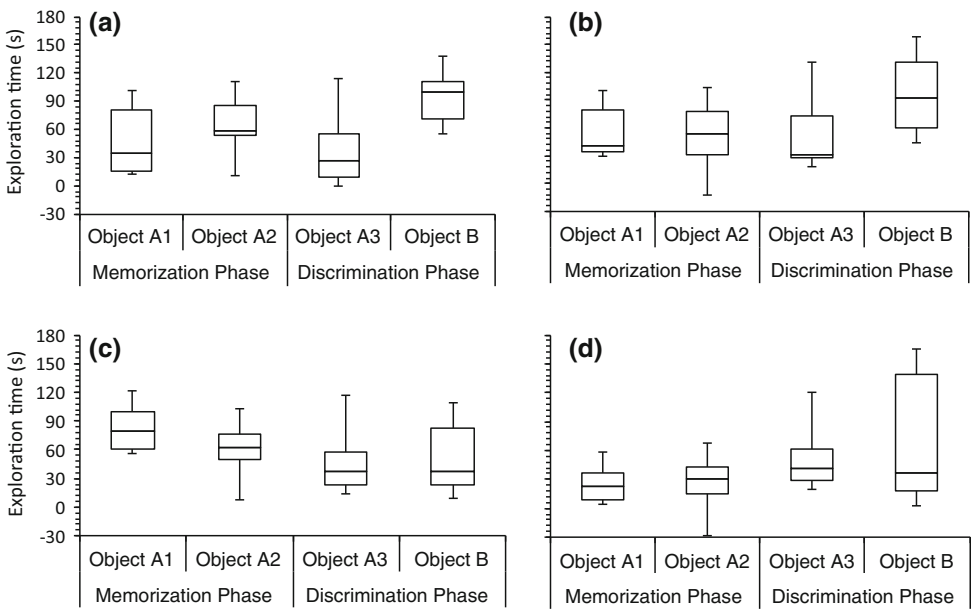

Fig. 4 Zebrafish exploration of objects A_1 versus A_2 and A_3 versus B: **a** Control (12L:12D), **b** partial SD (18L:06D), **c** total SD with light pulses (18L:06D + pulses) and **d** total SD with extended light (24L:00D). *Box* plots represent median values of exploration time in each object, in memorization and discrimination phases. Fish were observed for 15 min. For results of statistical analysis, see "Results" section (color figure online)

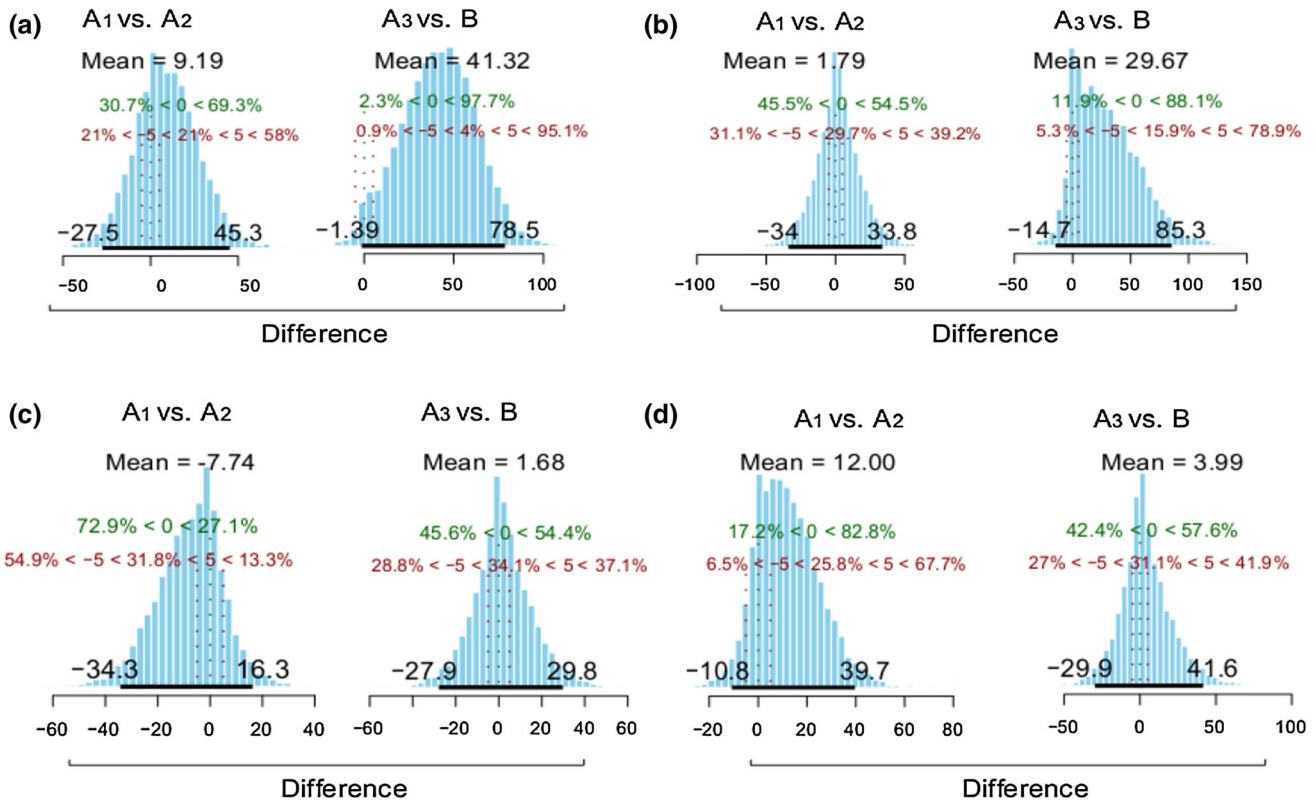

Fig. 5 Posterior distribution of the Bayesian analysis between the exploration time of objects A_1 versus A_2 and A_3 versus B for the **a** Control, **b** Partial SD, **c** Total SD with light pulses and **d** Total SD with extended light. *Green dashed lines* (central dashed line) indicate the comparison value = 0, and *red dashed lines* (lateral dashed lines) indicate the ROPE ± 5. 95 %. HDI interval is marked by the *black bar* on the floor of the distribution. For results of statistical analysis, see "Results" section (color figure online)

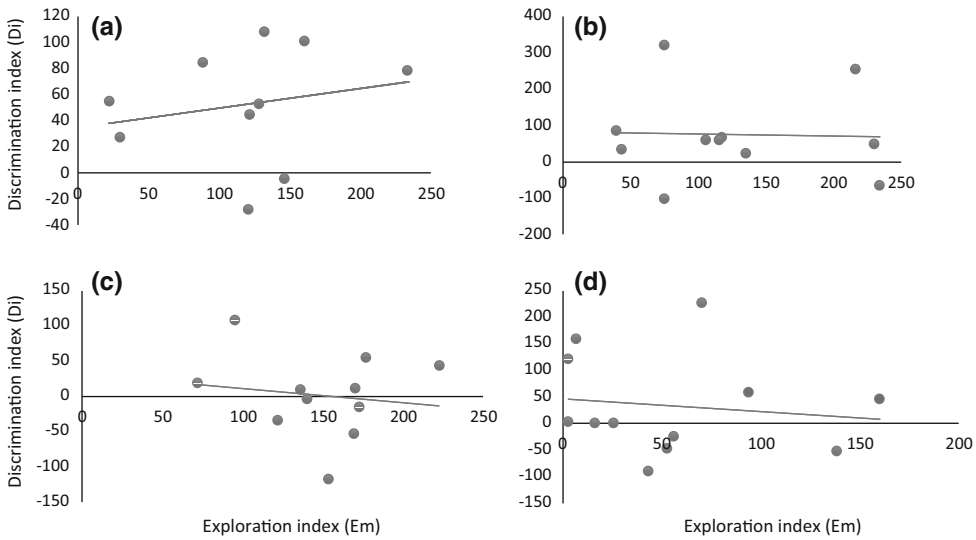

Fig. 6 Linear regression between discrimination index (D_i) and exploration in the memorization phase (E_m). **a** Control group, **b** partial SD, **c** total SD with extended light and **d** total SD with light pulses groups. Reduced exploration in the sleep-deprived groups may explain decreased objects discrimination. For results of statistical analysis, see "Results" section

explored the novelty (object B) more than the known object (object A_3) and more than the former objects (objects A_1 and A_2 on day 6) (Figs. 4a and 5a).

The increased exploration of object B in the discrimination phase compared to both objects A_1 and A_2 in the memorization phase is intriguing because zebrafish seem to be less interested in objects when they are initially presented. In fact, in the memorization phase, individuals divided their exploratory interest between the two identical objects, and the time spent moving from one object to the other is not counted as exploration of any object. In this respect, on the second day, once the familiar object was

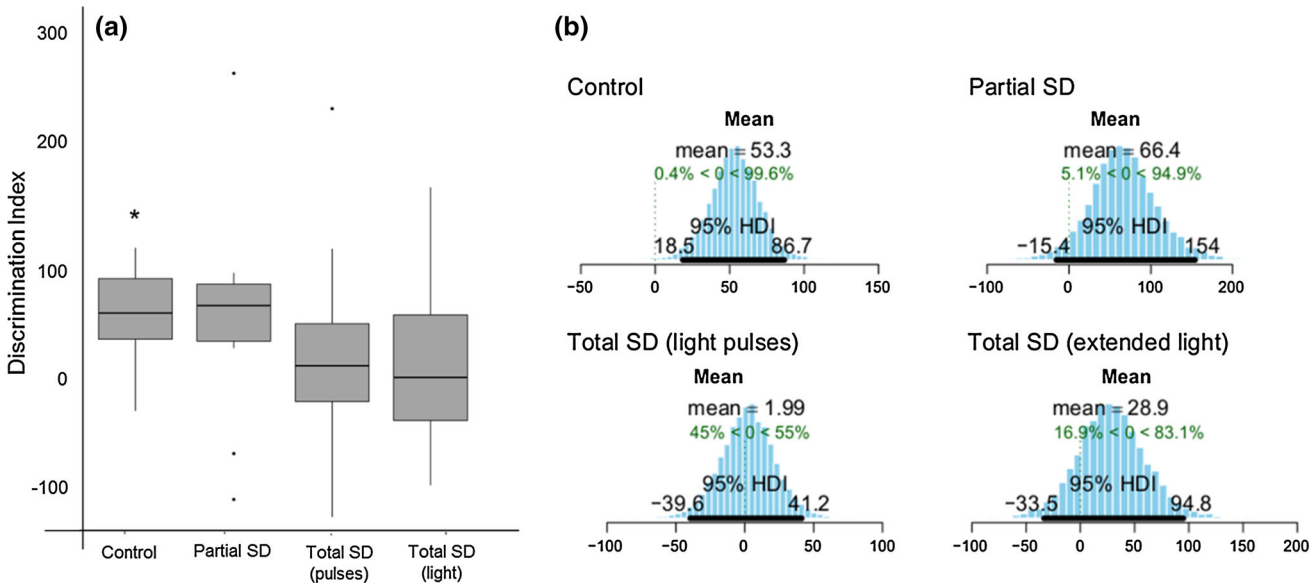

Fig. 7 **a** Discrimination index (D_i) of the control, partial SD, total SD with light pulses, and total SD with extended light. *Error bars* represent standard deviation. (*) indicates a statistically relevant difference between each treatment and the theoretical mean of 0. **b** Posterior distribution of the Bayesian analysis of the discrimination indices. *Green dashed lines* indicate the comparison value = 0. HDI interval is marked by the *black bar* on the floor of the distribution. For results of statistical analysis, see "Results" section (color figure online)

recognized, the fish spent more time around the new object, instead of moving between them. This behavior pattern was also observed in the studies by Oliveira et al. (2015) and Santos et al. (2016). Another element that could draw the fish's attention is the color of the object. According to Spence and Smith (2008), Avdesh et al. (2010) and Oliveira et al. (2015), zebrafish show an innate preference for blue/green. Hence, to avoid increased exploration due to color preference, blue and green objects were used only in the memorization phase and as known objects in the discrimination phase (Table S1). Moreover, it is known that exploratory behavior in zebrafish increases in enriched environments (Manuel et al. 2015), and the environment in the discrimination phase could be considered richer than that of the memorization phase, triggering greater exploration.

However, exploration seems to be affected by attention and discrimination, as animals decrease exploration over time and when the environment does not change (Kim et al. 2005; Kliethermes and Crabbe 2006; Kalueff and Zimbardo 2007). For the control group, the discrimination index (D_i) reinforces the increased exploration of the new object and suggests that more exploration during the memorization phase seems to allow better performance in discriminating the new object (Figs. 6a and 7).Considering that the partially sleep-deprived group explored the new object more and was able to form a memory from the previous day, it was also observed that this group did not show the same exploration pattern exhibited in the control group (Fig. 4a, b). For the partially deprived group, the Bayesian estimation of the time spent exploring objects suggests a tendency toward greater exploration of object B vs. A_3 (Fig. 5b). However, we cannot consider that the partially SD group properly discriminated objects, which is even more evident in D_i analysis (Figs. 6 and 7). Six hours in the dark, after the SD period, may have allowed some sleep recovery. While it did not prevent fish from object discrimination, 12 h in the dark (control) led to better performance in the discrimination task. Thus, a longer period of restricted sleeping nights (for instance more than 3 days) may promote a cumulative effect and produce higher losses in cognitive function, a hypothesis that remains to be tested.

Even though SD causes a range of effects, which vary widely among species, a common argument put forth by authors is its significant impairment of memory consolidation (McGaugh 2000; Andersen et al. 2008; Killgore 2010; Rasch and Born 2013; Watson and Buzsáki 2015). In the present study, all groups had the opportunity to interact with the objects on the first testing day, but only the totally sleep-deprived groups were unable to discriminate the objects on the following day. These fish did not recognize object A_3 from the previous experience (day 6) and responded to both objects as novelties, indicating impaired memory formation (Fig. 4). Other authors have also shown that sleep loss prevents the consolidation of acquired memory (Leconte et al. 1974; Linden et al. 1975; Prince and Abel 2013). According to Marshall and Born (2007),

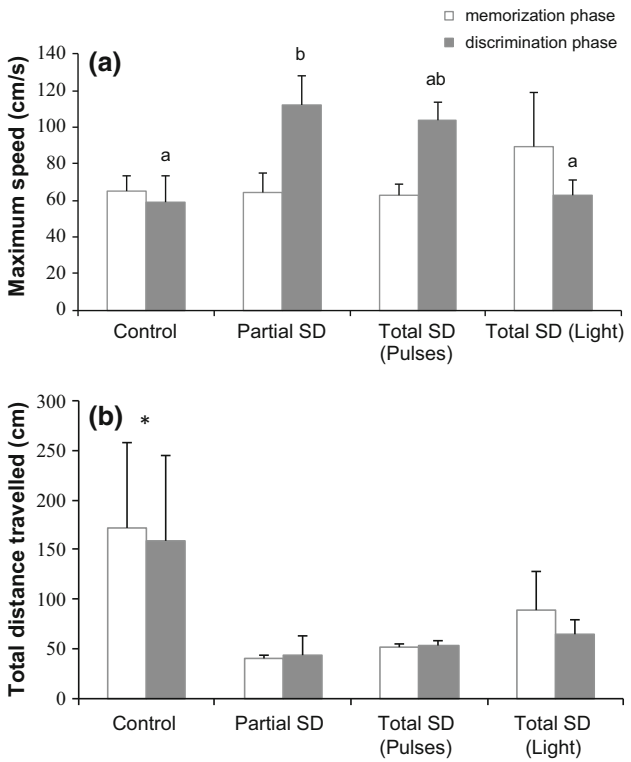

Fig. 8 Behavioral analysis during memorization phase and discrimination phase of a one-trial learning paradigm. One-way ANOVA applied to compare **a** maximum speed swimming + SD and **b** total distance traveled by the fish + SD, between the four groups: Control, Partial SD, Total SD with light pulses, and Total SD with extended light. *Error bars* represent standard error. Data correspond to 15 min of behavioral observation during the test, both in memorization phase as discrimination phase, analyzed using video-tracking software (ZebTrack). (*), and different letters indicate statistically differences ($p < 0.05$)

memory consolidation seems to occur mostly during periods of sleep or inactivity. Moreover, zebrafish have directly light-responsive cells (Weger et al. 2011) and the use of light to avoid sleep probably reduces melatonin levels. In this regard, the impaired discrimination observed in the present study is consistent with investigations showing that abnormalities in human circadian melatonin rhythms lead to changes in cognition and behavior (Melke et al. 2008).

Although the partially SD group cannot be considered to have completely impaired discrimination, both the partially and totally sleep-deprived animals showed higher maximum speed during the second testing day (Fig. 8). Pilcher and Huffcutt (1996) argued that partial SD has a greater effect on body function than even long- or short-term sleep deprivation. In addition, it is well known that SD is a stressful condition that causes significant agitation and anxiety behavior (Meerlo et al. 2002; Andersen et al. 2004; Mueller et al. 2008; Mashoodh et al. 2008). The hyperactive behavior observed in the present study supports this idea. Furthermore, agitation and impairment in working memory and attention were related to prolonged wakefulness (Harrison et al. 2000; Thomas et al. 2000), due to the effect of cognitive vulnerability on the brain after sleep deprivation (Alhola and Polo-Kantola 2007).

In this respect, total sleep deprivation seems to induce a stressful condition for memory consolidation. One could argue that light pulses act as a stressor *per se* and sleep deprivation using light pulses impairs memory due to stress and not sleep deprivation. This possibility, however, can probably be discarded in the present study because light pulses applied during the waking hours did not cause memory degradation, or decrease motivation for exploration (Figs. 2 and 3). On the other hand, it was found that sleep deprivation using 2 × 2 min light pulses (light × -dark) produced the same results as SD applying 4 × 1 min light pulses (light × dark), suggesting that the latter are effective sleep deprivation stimuli in fish.

Along with sleep deprivation, our test required remembering a single neutral episode, which is much weaker than memory based on repetition or a single aversive reinforcer that involves emotional response (Ennaceur and Delacour 1988; McGaugh 2004; Blank et al. 2009). The object discrimination task was chosen due to its vulnerability to the sleep-deprived brain. In addition, visual tasks would be especially susceptible to lack of sleep because iconic memory has a short duration and limited capacity (Raidy and Scharff 2005). Thus, it would be important to examine how sleep deprivation affects memory formation in tasks comprising multiple exposures to a stimulus, which would imply disrupting a stronger memory. Moreover, despite the fact that zebrafish exhibit similar cognitive performance to mammals and allowing translational interpretation, future studies are needed to address a number of limitations. For instance, the impact of chronic sleep deprivation should be investigated in order to understand the cumulative effects of sleeplessness. Binks et al. (1999) report that the effects of sleep loss do not become apparent until after 36–40 h.

Finally, the present study has some practical implications. Zebrafish have become an appropriate model for understanding the relationship between sleep deprivation and memory consolidation, with reliable translational relevance. Zebrafish show a number of advantages over the most widely used model in sleep research (rodents), namely the diurnal nature (Yokogawa et al. 2007; Zhdanova 2011) and secretion of important rhythm regulators such as cortisol (Azpeleta et al. 2010; Sigurgeirsson et al. 2013) and melatonin (Zhdanova et al. 2001; Lima-Cabello et al. 2014; Gandhi et al. 2015) as promoters of a sleep-like state, which are comparable to those observed in humans. Thus, our cognitive protocol can be used in future sleep deprivation studies, focusing on techniques that reveal changes in the brain (neurotransmitters, proteins, neuroplasticity), given that clinical sleep deprivation trials are

expensive, difficult to carry out, ethically questionable, and consequently limited (Alhola and Polo-Kantola 2007). While partial sleep deprivation did not cause immediate memory decline, total sleep deprivation for a single night was highly damaging. Further studies focusing on sleep and sleep deprivation are needed, and the zebrafish can pave the way for a better understanding of sleep disorders and their cognitive relationship.

Acknowledgments The authors would like to thank Mr. Freire and Mr. Fonseca for statistical support, Mr. and Mrs. Haghverdian for English revision, and Ms. Nascimento and Ms. Jordão for technical assistance. We are thankful to the anonymous reviewers, who provided helpful comments and constructive criticism. The authors declare no competing interests.

References

Akkerman S, Prickaerts J, Steinbusch HWM, Blokland A (2012) Object recognition testing: statistical considerations. Behav Brain Res 232:317–322

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

Andersen ML, Bignotto M, Tufik S (2004) Hormone treatment facilitates penile erection in castrated rats after sleep deprivation and cocaine. J Neuroendocrinol 16:154–159. doi:10.1111/j.0953-8194.2004.01145.x

Andersen ML, Antunes IB, Silva a A et al (2008) Effects of sleep loss on sleep architecture in Wistar rats: gender-specific rebound sleep. Prog Neuropsychopharmacol Biol Psychiatry 32:975–983. doi:10.1016/j.pnpbp.2008.01.007

Avdesh A, Chen M, Martin-iverson MT et al (2010) Natural colour preference in the zebrafish (*Danio rerio*). Proc Meas Behav 2010:155–157

Azpeleta C, Martinez-Alvarez RM, Delgado MJ et al (2010) Melatonin reduces locomotor activity and circulating cortisol in goldfish. Horm Behav 57:323–329

Bevins RA, Besheer J (2006) Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study "recognition memory". Nat Protoc 1:1306–1311

Binks PG, Waters WF, Hurry M (1999) Short-term total sleep deprivations does not selectively impair higher cortical functioning. Sleep 22:328–334

Blank M, Guerim LD, Cordeiro RF, Vianna MRM (2009) A one-trial inhibitory avoidance task to zebrafish: rapid acquisition of an NMDA-dependent long-term memory. Neurobiol Learn Mem 92:529–534. doi:10.1016/j.nlm.2009.07.001

Blumberg M (2010) Beyond dreams: do sleep-related movements contribute to brain development? Front Neurol 1:140. doi:10.3389/fneur.2010.00140

Colwill RM, Raymond MP, Ferreira L, Escudero H (2005) Visual discrimination learning in zebrafish (*Danio rerio*). Behav Processes 70:19–31. doi:10.1016/j.beproc.2005.03.001

Ennaceur A, Delacour J (1988) A new one-trial test for neurobiological studies of memory in rats. 1: behavioral data. Behav Brain Res 31:47–59

Euston DR, Tatsuno M, McNaughton BL (2007) Fast-forward playback of recent memory sequences in prefrontal cortex during sleep. Science 318:1147–1150. doi:10.1126/science.1148979

Fetsko LA (2002) What can be learned from a fish: an analysis of visual discrimination in the zebrafish, *Danio Rerio*. Temple University, Philadelphia

Gandhi AV, Mosser EA, Oikonomou G, Prober DA (2015) Melatonin is required for the circadian regulation of sleep. Neuron 85:1193–1199

Guzman-Marin R, Suntsova N, Methippara M et al (2005) Sleep deprivation suppresses neurogenesis in the adult hippocampus of rats. Eur J Neurosci 22:2111–2116. doi:10.1111/j.1460-9568.2005.04376.x

Harrison Y, Horne JA, Rothwell A (2000) Prefrontal neuropsychological effects of sleep deprivation in young adults–a model for healthy aging? Sleep 23:1067–1073

Herculano-Houzel S (2015) Decreasing sleep requirement with increasing numbers of neurons as a driver for bigger brains and bodies in mammalian evolution. Proc R Soc B Biol Sci 282:20151853. doi:10.1098/rspb.2015.1853

Hobson JA, Steriade M (2011) Neuronal basis of behavioral state control. Comprehensive physiology. Wiley, Hoboken, pp 701–823

Johnston TD (1982) Selective costs and benefits in the evolution of learning. Adv Study Behav 12:65–106. doi:10.1016/S0065-3454(08)60046-7

Kalueff AV, Zimbardo PG (2007) Behavioral neuroscience, exploration, and K.C. Montgomery's legacy. Brain Res Rev 53:328–331. doi:10.1016/j.brainresrev.2006.09.003

Killgore WDS (2010) Effects of sleep deprivation on cognition. Prog Brain Res pp 105–129

Kim D, Chae S, Lee J et al (2005) Variations in the behaviors to novel objects among five inbred strains of mice. Genes, Brain Behav 4:302–306

Kliethermes CL, Crabbe JC (2006) Pharmacological and genetic influences on hole-board behaviors in mice. Pharmacol Biochem Behav 85:57–65

Koban A, Cook R (2009) Rotational object discrimination by pigeons. J Exp Psychol Anim Behav Process 35:250

Kruschke JK (2013) Bayesian estimation supersedes the t test. J Exp Psychol Gen 142(2):573–603. doi:10.1037/a0029146

Kruschke J (2014) Doing Bayesian data analysis: a tutorial with R, JAGS, and Stan. Academic Press, Cambridge

Leconte P, Hennevin E, Bloch V (1974) Duration of paradoxical sleep necessary for the acquisition of conditioned avoidance in the rat. Physiol Behav 13:675–681. doi:10.1016/0031-9384(74)90239-X

Leibowitz SM, Lopes M-CC, Andersen ML, Kushida CA (2006) Sleep deprivation and sleepiness caused by sleep loss. Sleep Med Clin 1:31–45. doi:10.1016/j.jsmc.2005.11.010

Lima-Cabello E, Díaz-Casado ME, Guerrero JA et al (2014) A review of the melatonin functions in zebrafish physiology. J Pineal Res 57:1–9

Linden ER, Bern D, Fishbein W (1975) Retrograde amnesia: prolonging the fixation phase of memory consolidation by paradoxical sleep deprivation. Physiol Behav 14:409–412. doi:10.1016/0031-9384(75)90004-9

Louie K, Wilson MA (2001) Temporally structured replay of awake hippocampal ensemble activity during rapid eye movement sleep. Neuron 29:145–156. doi:10.1016/S0896-6273(01)00186-6

Lucon-Xiccato T, Dadda M (2014) Assessing memory in zebrafish using the one-trial test. Behav Process 106:1–4. doi:10.1016/j.beproc.2014.03.010

Lyamin O, Pryaslova J, Kosenko P, Siegel J (2007) Behavioral aspects of sleep in bottlenose dolphin mothers and their calves. Physiol Behav 92:725–733. doi:10.1016/j.physbeh.2007.05.064

Manuel R, Gorissen M, Stokkermans M et al (2015) The effects of environmental enrichment and age-related differences on inhibitory avoidance in zebrafish (*Danio rerio* Hamilton). Zebrafish 12:152–165

Marshall L, Born J (2007) The contribution of sleep to hippocampus-dependent memory consolidation. Trends Cogn Sci 11:442–450. doi:10.1016/j.tics.2007.09.001

Mashoodh R, Stamp JA, Wilkinson M et al (2008) Lack of estradiol modulation of sleep deprivation-induced c-Fos in the rat brain. Physiol Behav 95:562–569. doi:10.1016/j.physbeh.2008.08.001

May Z, Morrill A, Holcombe A et al (2016) Object recognition memory in zebrafish. Behav Brain Res 296:199–210. doi:10.1016/j.bbr.2015.09.016

McGaugh JL (2000) Memory–a century of consolidation. Science 287:248–251. doi:10.1126/science.287.5451.248

McGaugh JL (2004) The amygdala modulates the consolidation of memories of emotionally arousing experiences. Annu Rev Neurosci 27:1–28

Meerlo P, Koehl M, Van Der Borght K, Turek FW (2002) Sleep restriction alters the hypothalamic-pituitary-adrenal response to stress. J Neuroendocrinol 14:397–402. doi:10.1046/j.0007-1331.2002.00790.x

Melke J, Botros HG, Chaste P et al (2008) Abnormal melatonin synthesis in autism spectrum disorders. Mol Psychiatry 13:90–98

Mueller AD, Pollock MS, Lieblich SE et al (2008) Sleep deprivation can inhibit adult hippocampal neurogenesis independent of adrenal stress hormones. Am J Physiol Regul Integr Comp Physiol 294:R1693–R1703. doi:10.1152/ajpregu.00858.2007

Oliveira J, Silveira M, Chacon D, Luchiari A (2015) The zebrafish world of colors and shapes: preference and discrimination. Zebrafish 12:166–173. doi:10.1089/zeb.2014.1019

Pilcher JJ, Huffcutt AJ (1996) Effects of sleep deprivation on performance: a meta-analysis. Sleep 19:318–326

Poe GR, Nitz DA, McNaughton BL, Barnes CA (2000) Experience-dependent phase-reversal of hippocampal neuron firing during REM sleep. Brain Res 855:176–180

Prince T-M, Abel T (2013) The impact of sleep loss on hippocampal function. Learn Mem 20:558–569. doi:10.1101/lm.031674.113

Raidy DJ, Scharff LFV (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. doi:10.1152/physrev.00032.2012

Rasch B, Pommer J, Diekelmann S, Born J (2009) Pharmacological REM sleep suppression paradoxically improves rather than impairs skill memory. Nat Neurosci 12:396–397

Roffwarg HP, Muzio JN, Dement WC (1966) Ontogenetic development of the human sleep-dream cycle. Science 152:604–619. doi:10.1126/science.152.3722.604

Santos LC, Oliveira JR, Oliveira JJ et al (2016) Irish coffee: effects of alcohol and caffeine on object discrimination in zebrafish. Pharmacol Biochem Behav. doi:10.1016/j.pbb.2016.01.013

Schluessel V, Fricke G, Bleckmann H (2012) Visual discrimination and object categorization in the cichlid Pseudotropheus sp. Anim Cogn 15:525–537. doi:10.1007/s10071-012-0480-3

Schluessel V, Kraniotakes H, Bleckmann H (2014) Visual discrimination of rotated 3D objects in Malawi cichlids (Pseudotropheus sp.): a first indication for form constancy in fishes. Anim Cogn 17:359–371. doi:10.1007/s10071-013-0667-2

Schmidt MH (2014) The energy allocation function of sleep: a unifying theory of sleep, torpor, and continuous wakefulness. Neurosci Biobehav Rev 47:122–153. doi:10.1016/j.neubiorev.2014.08.001

Siebeck UE, Litherland L, Wallis GM (2009) Shape learning and discrimination in reef fish. J Exp Biol 212:2113–2119. doi:10.1242/jeb.028936

Siegel JM (2005) Clues to the functions of mammalian sleep. Nature 437:1264–1271. doi:10.1038/nature04285

Siegel JM (2008) Do all animals sleep? Trends Neurosci 31:208–213. doi:10.1016/j.tins.2008.02.001

Sigurgeirsson B, Thornorsteinsson H, Sigmundsdóttir S et al (2013) Sleep-wake dynamics under extended light and extended dark conditions in adult zebrafish. Behav Brain Res 256:377–390. doi:10.1016/j.bbr.2013.08.032

Sison M, Gerlai R (2010) Associative learning in zebrafish (*Danio rerio*) in the plus maze. Behav Brain Res 207:99–104. doi:10.1016/j.bbr.2009.09.043

Spence R, Smith C (2008) Innate and learned colour preference in the zebrafish, *Danio rerio*. Ethology 114:582–588

Spiegel K (2004) Brief communication: sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite. Ann Intern Med 141:846. doi:10.7326/0003-4819-141-11-200412070-00008

Stickgold R (2005) Sleep-dependent memory consolidation. Nature 437:1272–1278. doi:10.1038/nature04286

Stickgold R, Walker MP (2005) Memory consolidation and reconsolidation: what is the role of sleep? Trends Neurosci 28:408–415

Thomas M, Sing H, Belenky G et al (2000) Neural basis of alertness and cognitive performance impairments during sleepiness. I. Effects of 24 h of sleep deprivation on waking human regional brain activity. J Sleep Res 9:335–352. doi:10.1046/j.1365-2869.2000.00225.x

Tononi G, Cirelli C (2006) Sleep function and synaptic homeostasis. Sleep Med Rev 10:49–62

Van Cauter E (2005) Endocrine physiology. In: Principles and practice of sleep medicine. Elsevier, p 266–282

Watson BO, Buzsáki G (2015) Sleep, memory & brain rhythms. Daedalus 144:67–82. doi:10.1162/DAED_a_00318

Weger BD, Sahinbas M, Otto GW et al (2011) The light responsive transcriptome of the zebrafish: function and regulation. PLoS ONE 6:e17080. doi:10.1371/journal.pone.0017080

Yokogawa T, Marin W, Faraco J et al (2007) Characterization of sleep in zebrafish and insomnia in hypocretin receptor mutants. PLoS Biol 5:e277. doi:10.1371/journal.pbio.0050277

Yu L, Tucci V, Kishi S, Zhdanova IV (2006) Cognitive aging in zebrafish. PLoS One 1:e14. doi:10.1371/journal.pone.0000014

Zhdanova IV (2006) Sleep in zebrafish. Zebrafish 3:215–226. doi:10.1089/zeb.2006.3.215

Zhdanova IV (2011) Sleep and its regulation in zebrafish. Rev Neurosci 22:27–36

Zhdanova IV, Wang SY, Leclair OU, Danilova NP (2001) Melatonin promotes sleep-like state in zebrafish. Brain Res 903:263–268. doi:10.1016/S0006-8993(01)02444-1

Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol 1:3–14