Effects of sleep deprivation on learning and memory in Zebrafish (Danio rerio)

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HÁSKÓLI ÍSLANDS

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Final project for the degree of Bachelor of Science in Medicine

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Abstract

Introduction

Sleep is a behavioral state exhibited by nearly all species and widely considered to be instrumental in health and cognitive function. Yet, the function of sleep remains obscure and its particular effects on various physiological functions, behavior, neurogenesis and development remains to be determined. The aim of this study is to investigate how sleep deprivation affects learning and memory in the zebrafish *(Danio rerio)* but zebrafish has in recent years been shown to be an excellent animal model for behavioral neuroscience.

Material and methods

24 Zebrafish of wild type (AB) were exposed to a conditioned avoidance task in which they were trained to avoid a mild electric stimulus associated with either red or green colored and equally large halves of the conditioning chamber. The fish were then divided in two groups recorded in either sleep-promoting environment with lights-off from 22:00 to 8:00 (naive group) or in wake-promoting environment with no lights-off period (light group). The morning after naive and light groups were re-introduced into the conditioning chamber for a retention test.

Results

Time spent in safe half of the conditioning chamber was significantly longer after training compared to before training for both groups ($p<0.005$) but no significant difference was found in time spent in safe half for neither group in pre-training and post-sleep ($p>0.05$) nor post-training and post-sleep ($p>0.05$). No significant differences ($p>0.05$) were found between groups in any of the measurements for time spent in safe half of the conditioning chamber. Significant differences were neither found in average swimming velocity overnight nor sleep percentage ($p>0.05$) between the two groups.

Discussion

These results suggest that this particular conditioning avoidance task does not contribute to long-term memory in the zebrafish and therefore the effects of sleep deprivation cannot be evaluated. The data also shows that sleep deprivation with light does not contribute to the same loss of sleep in the zebrafish as other research suggests. Therefore both the conditioning avoidance task and sleep deprivation should be revised and restructured.

Abbreviations

Pre-train = the first fifteen minutes in the forty-five minute training period

Post-train = the last fifteen minutes in the forty-five minute training period

Post-sleep = the fifteen minutes in the memory retention test.

EEG = electroencephalogram

AD = Alzheimer's disease

PD = Parkinson's disease

Hctr/Orx = hypocretin/orexin

Introduction

1. Aim of project

Sleep is widely considered an important physiological and behavioral state that has been preserved throughout evolution of all species closely studied to this day (1). Although the importance of sleep is accepted in modern medicine, physiology and psychology scientists have yet to answer why exactly we need to sleep and what is happening in the nervous system during this seemingly essential behavioral state. A common assumption is that sleep plays a role in learning and memory formation and cognition. The aim of this project was to measure associative learning and memory in the zebrafish by connecting two previously unknown stimuli, color and mild electric shock and then evaluate the effects of different sleep deprivation paradigms on memory formation. The zebrafish or *Danio rerio* has in recent years become an important model organism for behavioral science. Results of previous studies suggest that the processes that underlie certain behaviors in mammals such as learning and memory, sleep and anxiety are comparable to underlying processes of behavior in the zebrafish (2).

2. Zebrafish (*Danio rerio*) as an animal model

2.1 General properties

The zebrafish is a small freshwater fish and a vertebrate unlike other common model organisms of small size, like the *Drosophila melanogaster* and *Caenorhabditis elegans*. It was Dr. George Streisinger who began to study the zebrafish as a promising animal model in the 1980's. He was interested in finding a vertebrate model organism that was more simple and easier to manipulate genetically than the mouse or rat and easier to study in large quantities. Zebrafish are small, inexpensive to maintain in a laboratory facility, their genome has been fully sequenced, their development and growth is rapid and drugs can be applied in their tank water among other desirable qualities of an animal model. Today, they are used as an animal model all over the world in many different scientific fields such as pharmacology, developmental biology, cancer research, and in the field of behavioral neuroscience among others (3-6).

2.2 Behavior

Why are zebrafish a suitable animal model for behavioral neuroscience? Zebrafish behavior has been well described. Zebrafish are diurnal and thus have decreased activity during the night compared to the daytime similar to the circadian pattern in mammals (7). They are social animals that prefer staying in groups also known as shoaling (8), they show similar response to chemicals like caffeine and ethanol as humans (2) and their behavioral and physiological responses to stress indicate that they are an adequate model for preclinical studies of stress (9). Research also show that the neurotransmitter systems in the zebrafish brain are highly similar to the neurotransmitter systems in the human brain (10). These factors indicate that the zebrafish is a useful animal model in the field of behavioral neuroscience.

3. Learning and memory

3.1 In general

Learning and memory are cognitive changes necessary for all living beings to adapt to and survive in their environment. Learning and memory consist of acquisition, consolidation and retention that together create the information process of learning and memory. Acquisition being the state during which information is perceived, consolidation the state where the perceived information is consolidated or stored into memory and retention the recollection of the former perceived information. If there is a misconnection between any one of these three steps in memory formation and for some reason acquisition does not lead to consolidation or retention, long-term memory will most likely not form (11, 12).

The hippocampus has been described as a critical organ for memory formation. This can be seen by the presentation of severe memory loss in patients where surgical removal of the hippocampus and adjacent structures has been performed (13, 14). The amygdala also seems to play an important role in learning and memory, especially emotional memory as in fear conditioning (15). NMDA glutamate receptors are considered to play a large role in the synaptic plasticity that leads to learning and memory (16). Fear conditioning, where a neutral stimulus like tone or color is associated with an unconditioned stimulus like electric shock, is one of the best understood conditioning method for learning and memory at the molecular and behavioral level (17).

3.2 Learning and memory in the zebrafish

The zebrafish is a convenient model for behavioral neuroscience and has good potential to be an important model for understanding behavioral neuroscience and neurodegenerative diseases (18). To understand what influence behavior and external variables like drugs, sleep and diseases have on memory it is necessary to have a well-described experimental setup for learning and memory that is both efficient and effective. Many different training methods have been used to evaluate learning and memory performance of the zebrafish in general and to see if various stimuli affect that performance. Zebrafish behavior and memory has been shown to be sensitive to an NMDA antagonist (19, 20). Zebrafish have shown ability for visual discrimination in a T-maze setup and can associate between a visual stimulus and a reward stimulus (21, 22). Color preferences for zebrafish have been studied for appropriate use of color in associative conditioning with colors that indicate the fish have similar preferences for green and red (23). Inhibitory avoidance in zebrafish has been studied in a setup with quick and effective learning protocols that showed both acquisition and retention as well as being NMDA sensitive (24). Non-associative learning has also been described in zebrafish (25).

4. Sleep

4.1 Characteristic features of sleep

Sleep is an important behavioral and physiological state. Sleep is essential for all species and without it, health and cognitive function and even lives are at jeopardy (1, 26). Historical evidence shows it has long been known that diseases that present with severe sleep loss, for example Familial Fatal Insomnia eventually, lead to death (27). Nevertheless, the reason for why sleep has been preserved throughout evolution among almost all animals studied to this day, is still not fully understood (1). Most scientists would probably agree that there must be a good reason behind the fact that this state has been as well preserved throughout evolution as observed. Especially when looking at the fact that sleep is a state that makes the animal vulnerable to predators and unable to collect food. Recognizing the mechanisms behind sleep could not only help us understand the nature of sleep but could also give us instruments to understand and treat sleep disorders.

Sleep consists of higher sensitivity thresholds for external stimulus, sleep-pressure and sleep rebound (28, 29). Sleep-pressure consists of the increased need for sleep following a long time period without sleep and sleep-rebound the powerful tendency to sleep more after a period of little sleep . Sleep can be divided into two stages, slow wave sleep or non-REM sleep and REM sleep that is characterized with desynchronized EEG activity, muscular paralysis and rapid eye but to this day no studies have been made on these brain wave activities in the zebrafish (30).

4.2 Hypocretin/Orexin system

The hypocretin/orexin (hctr/orx) system is a physiological and well-established system involved in sleep, wakefulness and energy metabolism. It is one of the best characterized sleep/wake homeostasis regulators in mammals and has been shown to support wakefulness (31). Loss of hctr/orx neurons results in the disease narcolepsy that presents with symptoms of recurrent and uncontrollable episodes of sleep (32). Research indicate that the hctr/orx system and the sleep wake cycle play a role in the pathogenesis of well known neurodegenerative diseases like Alzheimer's disease (AD) and Parkinson's disease (PD) (33, 34).

Hctr/Orx is produced by neurons in the hypothalamus that connect to many different areas in the brain connected to the sleep-waking cycle (35). Deficiency in the signaling of these neurons can be caused by mutations in the peptide, it's receptors or loss of neurons themselves (36).

The hctr/orx system has been studied in the zebrafish animal model and results show that this system is similar in zebrafish and mammals (10, 37). Other study shows that the correspondence between the human and zebrafish hctr/orx systems might not be that straight forward and that hypocretin receptor mutants show insomnia instead of narcolepsy symptoms(38). Although not explained fully, the hctr/orx system seems to play an important role in sleep-wake promoting behavior. Zebrafish larvae that overexpress hctr/orx have reduced sleep and show sleep behavior similar to humans that suffer from insomnia (39). Wakefulness promoter drugs that are used to treat narcolepsy also have sleep-reducing effects in larval zebrafish (40).

4.3 Sleep and synaptic plasticity

It's a common assumption that lack of sleep affects cognitive performance and memory but the subject of whether sleep is important for memory consolidation or not has been a debate among scientists (41, 42). Sleep deprivation in young adult humans shows increased reaction time for working memory (43). Chronic sleep deprivation also seems to impact mental and physical health (44). The exact mechanisms of how sleep effects synaptic plasticity are still unknown but the synaptic homeostasis hypothesis indicates that sleep might indeed have a homeostatic role for synaptic plasticity (45-47).

Both the synaptic homeostasis theory and the evidence that the hctr/orexin system might play a role in the pathology of neurodegenerative disease like AD(34) and PD(33) suggests that studying the correlation between sleep and behavior dependent on synaptic plasticity, like learning and memory, is a worthwhile subject. Recent findings also suggest that the zebrafish is not only a useful model to study behavior as stated earlier but also a useful model to study neurodegenerative diseases (18, 48).

Therefore, this study aims to study the effects of sleep deprivation on learning in memory in the zebrafish using a simple model of conditioning avoidance task and sleep deprivation with light.

Material and methods

5. Materials

5.1 Animals

Zebrafish were provided by the University of Reykjavík Neurolab but the wild type stock of AB strain was originally from the University of Oregon Zebrafish International Resource center (ZIRC, Oregon, USA). All fish were bred between 27 to 32 weeks prior to the experiment. Fish were fed twice a day with a Zeigler Adult Zebrafish Diet (Zeigler, Pennsylvania, USA) and kept in a 14:10 light:dark cycle (lights turned off at 22:00 in the evening and on at 8:00 in the morning). Water temperature was held at a constant 28,5°C in the housing tanks and 28°C +/- 1°C during the training sessions and sleep conditions. All procedures were in compliance with the regulations of the National Bioethics Committee of Iceland.

5.2 Apparatus setup

Zebrafish behavior was tracked using a Sony XC-E150 (Sony, Japan) infrared cameras with a 50 mm Pentax lens (Pentax, Japan) and the video tracking software Ethovision (Noldus Information Technology, Netherlands). Two types of tanks were used in this study, a conditioning tank and a sleeping tank.

The conditioning tank consisted of a 26x10x10cm rectangular water area where temperature was held at a constant 28°C +/- 1°C. A stainless steel grid was put in the conditioning tank along the inner area of both longitudinal sides and connected to electrical stimulus device (Grass, West Warwick, USA). One half of the longitudinal sides of the tank was characterized by red plexiglass and the other half by green plexiglass, both halves were equally large (13x10x10cm). Both ends of the conditioning chamber (10 cm) were black and the bottom of the tank was white. **Figure 1** and **figure 2** show the conditioning tank. Fish were recorded in the conditioning tank during the conditioning avoidance task, one fish at a time.

The sleep tank contained four 10x15x6,5 sized rectangular chambers defined as arenas. Four fish were recorded at a time in the sleeping tank, one in each chamber. Light in the sleeping tanks was measured with a lux meter (VICTOR, Guangdong, China) to make sure that no external light entered the sleeping tank during recordings. 0,01-0,02 lux were measured during the periods when lights were turned off.

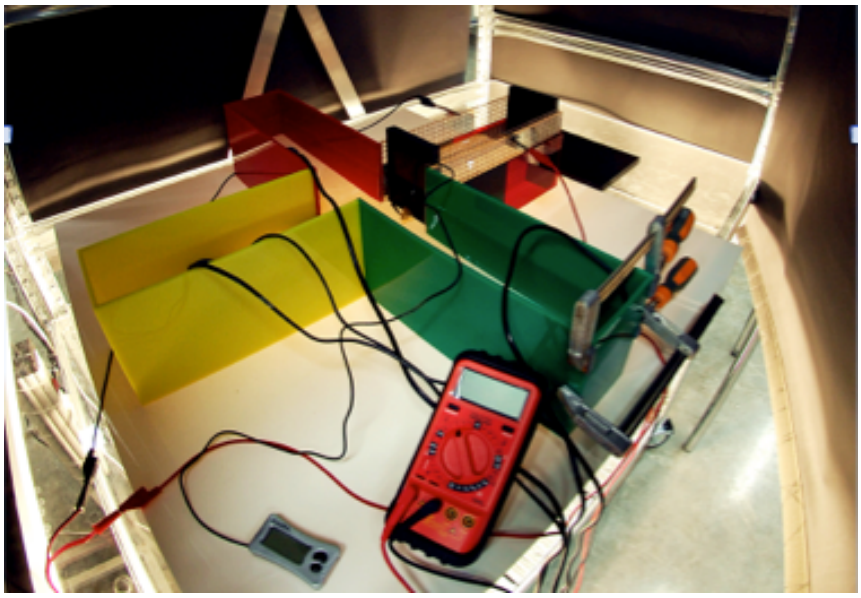

Figure 1: This picture shows the training tank setup. The two-colored arm of this plus maze is the conditioning chamber with black dividing walls on both ends.

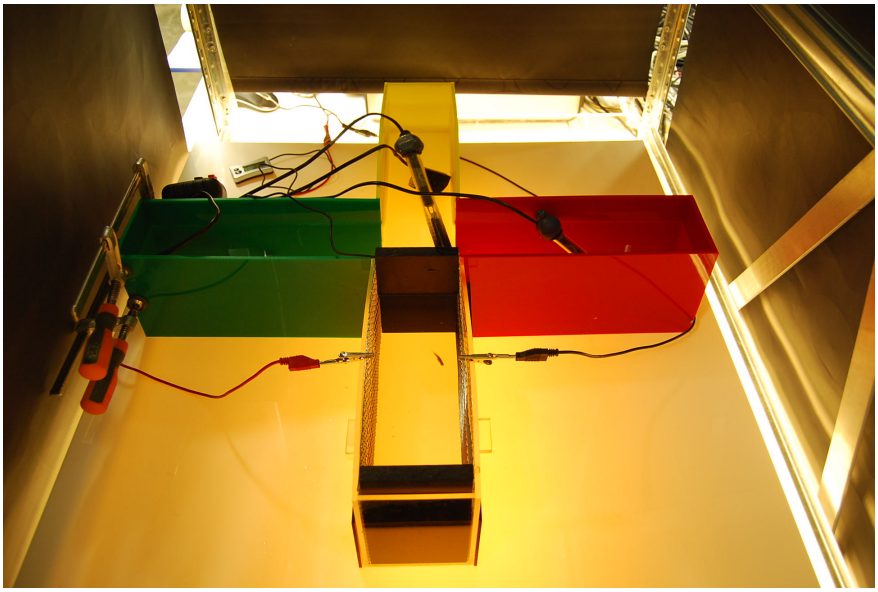

Figure 2: Here the conditioning chamber is shown during a training session. The electric stimulator is connected to grids on both sides

Electrical stimuli for the training sessions were delivered via programmed commands in Ethovision XT7. No electrical stimulus was used in the sleep conditions. The electrical tension between the grids in the water was measured before the training procedure with a multimeter and adjusted to 1 V/cm and duration of 0,2 seconds with a 1 second interval between pulses. These adjustments were made to control for variability in stimulus strength in the water.

6. Methods

6.1 Training

Twenty-four zebrafish were trained on a conditioned avoidance task. Each fish spent a forty-five minute training period in the conditioning tank further described in chapter 5.2 (see **figure 1** and **figure 2**). Before each training session the green and red zones in the conditioning tank were identified either as the stimulus zone or the safe zone and that identification was changed in every other trial run. Each trial run contained four zebrafish. The training period was divided into three fifteen-minute (900 seconds) session. At the beginning of each training session the fish was put into the training tank at the line of demarcation between the green zone and the red zone. In the first session (Pre-training) fish were allowed a free exploration of the conditioning tank without any conditioning stimulus. During the second session mild electrical stimulus was associated with either the green or red half of the tank. S48 Square Pulse Stimulator (Grass Technology, U.S.A.) was used to give the electrical stimulus when the fish was situated in either green or red half of the tank during the training. The electrical stimulus (1 V/cm for 0,2 seconds with a 1,0 second interval) was given if the fish had a position in the half of the conditioning tank defined as the shock zone but no electrical stimulus was given when the fish was in the safe zone. Finally place preference was recorded in the last session (Post-training) of the forty-five minute training period but the conditioning stimulus was removed during that last session. Fish movement was tracked at 5Hz from the end of the training session, and overnight, until memory retention test was carried out the following morning.

6.2 Sleep conditions

After the learning sessions each zebrafish was randomly put into one of four compartments in the sleep tank and its movement velocity and behavior recorded over night. The twenty-four zebrafish were divided in groups recorded either in a sleep-promoting environment with lights-off from 22:00 to 8:00 (naive group) or in a wake-promoting environment with no lights-off period (light group) but light suppresses sleep in zebrafish (38).

6.3 Memory testing

The morning after the sleep recordings each fish was put into the learning tank again in a random order for fifteen minutes (Post-sleep). Time in the half of the tank defined as the safe zone during the training period the day before was measured to evaluate long-term memory.

6.4 Euthanasia procedure and tissue collection

Fish were killed after the memory test by putting them in ice water of temperature below 4°C. This procedure was used because as it leads to quicker death and shows fewer indicators of stress than using high doses of the anesthetic tricane methanosulfate (MS222) (49). Fish were then dissected according to previously established protocols (50) and brain tissue collected and put into a formalin solution for future analysis that were not included in this study.

6.5 Data analysis

Behavioral data was exported from Ethovision XT7 as an Excel file. All statistical calculations were performed in IMB SPSS statistics (SPSS inc., Chicago, USA). Repeated measures ANOVA was used for analysis of duration in safe zone pre-training, post-training and post-sleep. Alpha level was set at p=0.05, Bonferroni post hoc tests were used for pairwise comparisons. Independent T-test was used to compare swimming velocity and average sleep percentage over night between groups. Charts were made in Excel. Sleep analysis was made with the free statistic software R.

Results

7. Measurements and statistics

7.1 Learning and memory measurements

A repeated measures ANOVA determined that there was a significant difference between time points during the training sessions ($F(2, 44) = 0{,}034$, $p < 0.0005$). Mauchly's Test of Sphericity was not significant ($p>0{,}05$). Pairwise comparisons showed that there was a significant increase in time spent in safe zone during post-training compared to pre-training for both groups ($p<0{,}005$), there was significantly less time spent in the safe zone in post-sleep compared to post-training ($p<0{,}005$) and there was no significant difference between time in safe zone during pre-train and post-sleep ($p>0{,}05$). There was no significant differenc between groups in any one of the three time measurements, pre-training, post-training and post-sleep. **Figure 3** shows time spent in safe zone of the conditioning chamber in both groups with standard deviation.

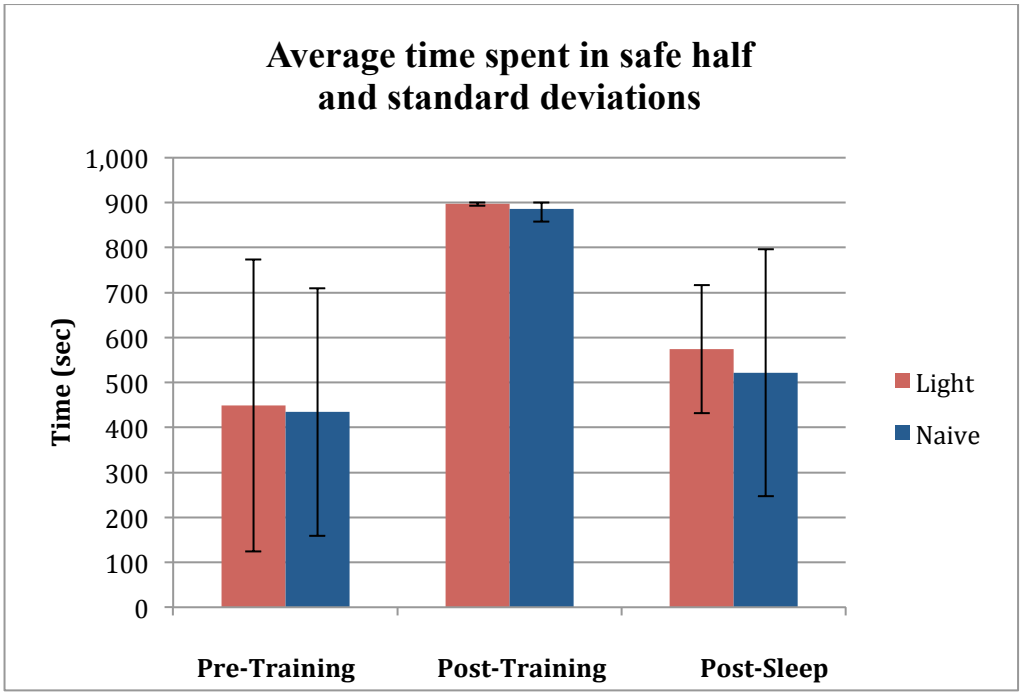

Figure 3: Average time in safe zone during the fifteen minutes before training (pre-training), fifteen minutes after training (post-training) and in the fifteen minute memory test (post-sleep) 24hours later. Standard deviation is also shown.

7.2 Sleep measurements

Independent t-test determined that there was no significant difference in average swimming velocity overnight between the two groups ($t(19)$= - 1,747 and $p>0,05$). Mean overnight (22:00-08:00) velocity for the light group shown in **table 1** was 1.3468 cm/sec with a 95% confidence interval of 0,9630-1,8151. Standard error for the average overnight velocity was 0,2069, standard deviation 0,6863 and median 1,2506. Average velocity from 19:20 to 09:00 o'clock for the light group is shown in **figure 4** with the standard error for every 10 minute period. Mean overnight velocity for the naïve group also shown in **table 1** was 0,8812 cm/sec with a 95% confidence interval 0,5103-1,1815. Standard error for the average overnight velocity was 0,1619, standard deviation 0,5119 and median 0,6813. Average velocity from 19:20 to 09:00 o'clock for the naïve group is shown in **figure 5** with the standard error for every 10 minute period. The two groups compared together with the standard error is shown **figure 6**.

	Light group	Naïve group
Average velocity (cm/se	1,3468	0,8812
Median	1,2506	0,6813
Standard deviation	0,6863	0,5119
Standard error mean	0,2069	0,1619

Table 1: Average velocity, median and standard deviation and standard error mean for both groups overnight (the time from 22:00 to 08:00)

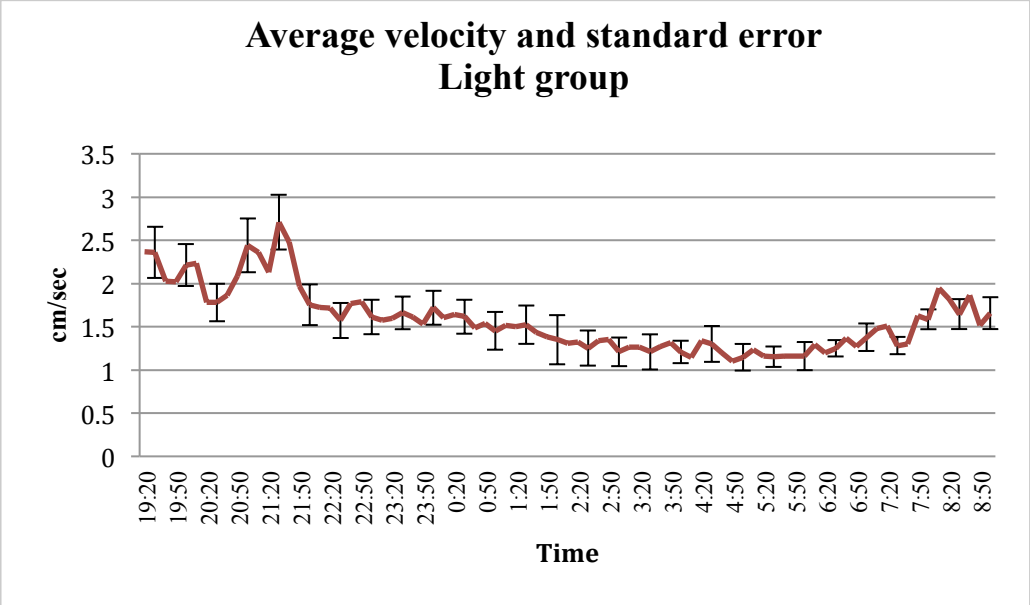

Figure 4: Linear graph of the average swim velocity in the evening and overnight and standard error for the light group. Lights were turned on during the night for this group.

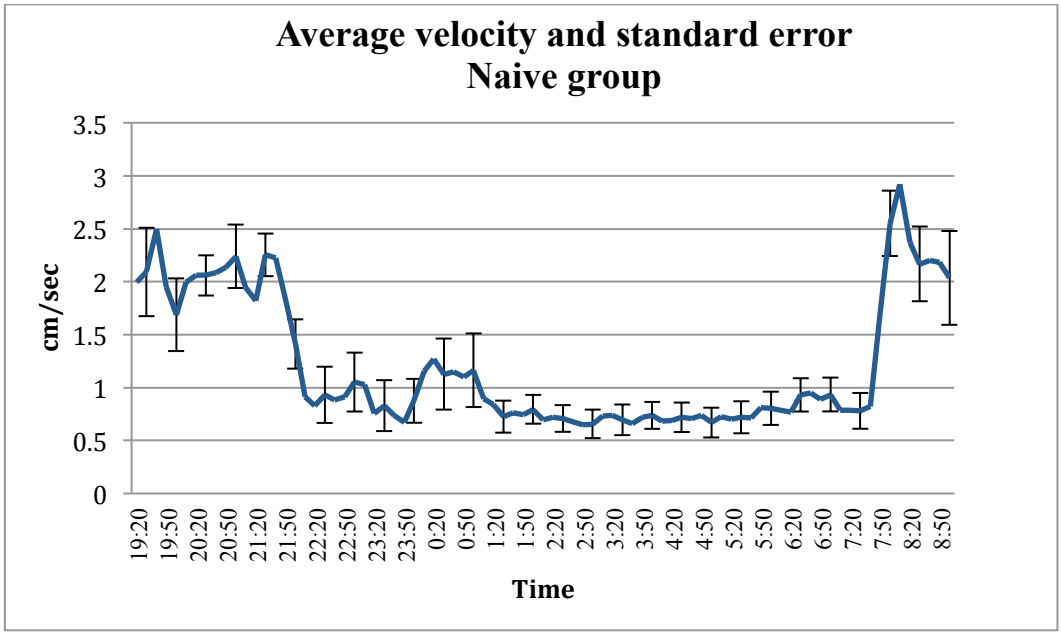

Figure 5: Linear graph of the average swim velocity overnight and standard error for the naïve group. Lights were turned off at 22:00 and on again 8:00 in the morning.

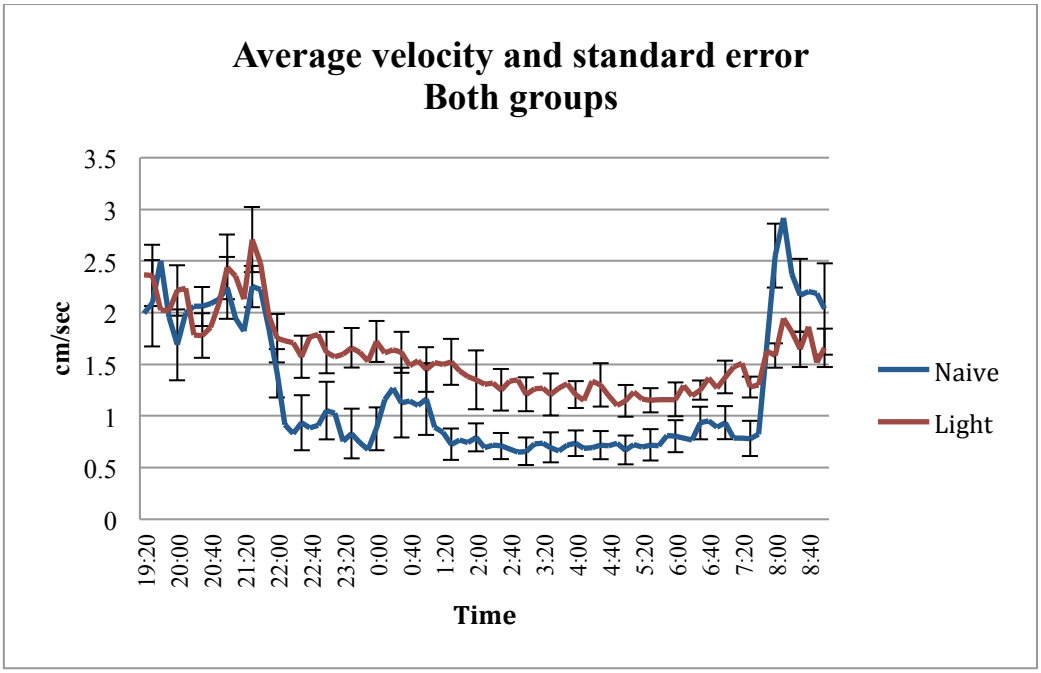

Figure 6: Average swim velocity overnight and standard error for both groups shown with standard error.

A sleep analysis was done in R. The sleep analysis shows the portion of immobility during the night that can be interpreted as sleep. Sleep for the zebrafish is defined as the accumulated period of immobility (mobility threshold 1.5 cm/sec) after an initial immobility period of 6 seconds. Average sleeping percentage over the night from 22:00 to 08:00 is shown in **figure 7**. The average sleep percentage for the Light group was 22.38% with a standard deviation of 19,23 but the average sleeping percentage for the Naïve group was 44.77% with a standard deviation of 27.67, **figure 8**. Independent t-test determined that there was no significant difference in average sleep percentage overnight between the two groups ($t(17)= 1,758$ and $p>0,05$).

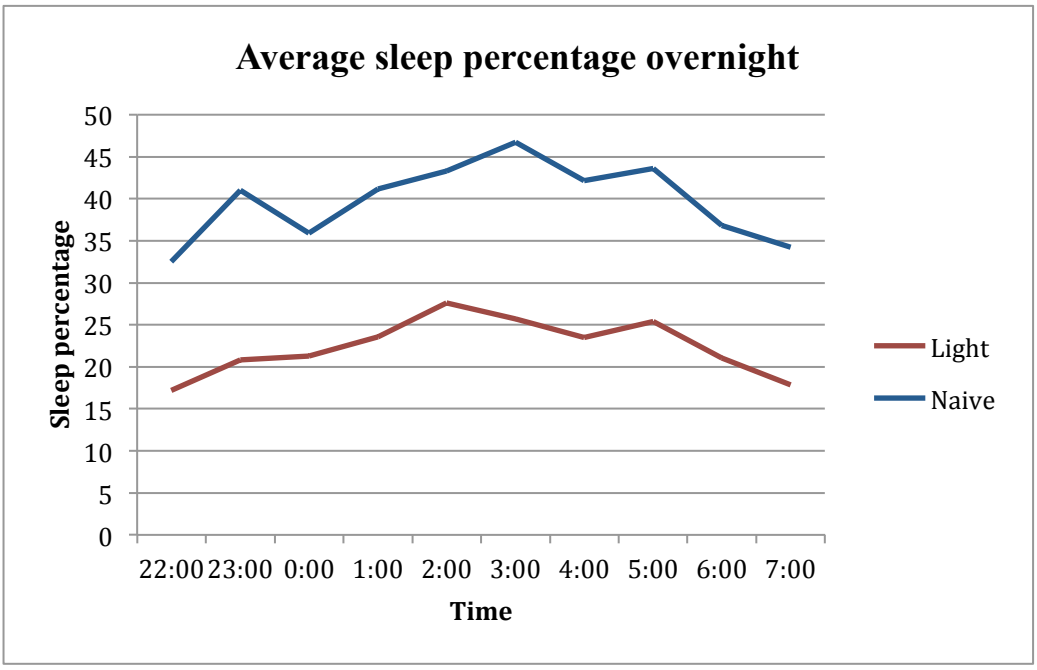

Figure 6: This graph shows the average sleep percentage for both groups during the night.

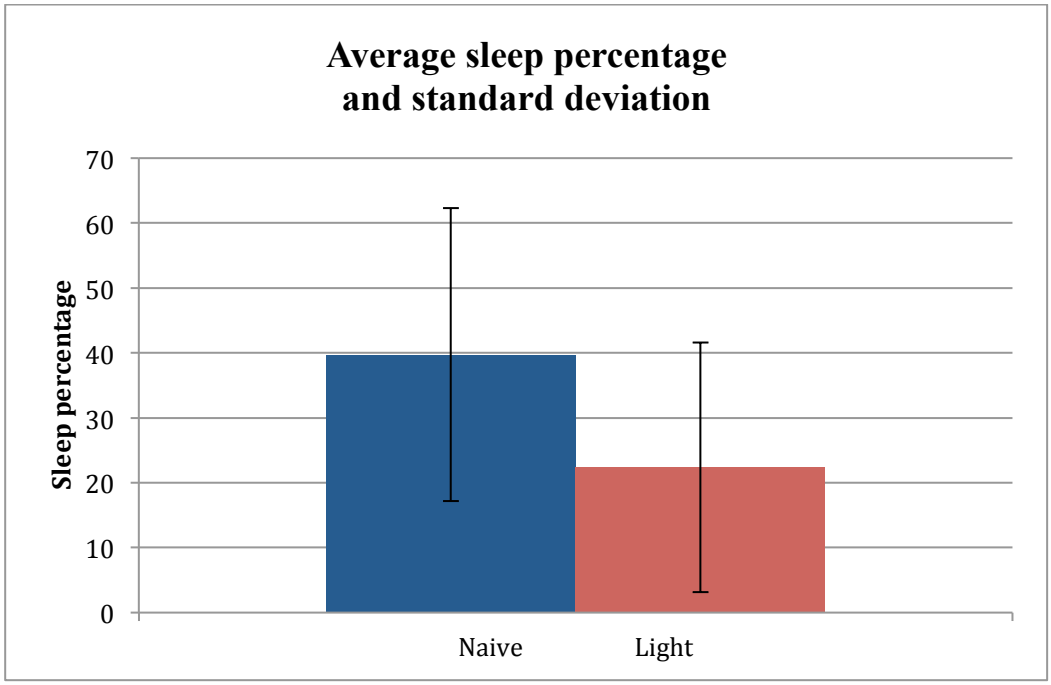

Figure 7: This graph shows the average sleep percentage overnight for the Light group and standard deviation.

Discussion

Zebrafish are a useful model to study behavioral neurophysiology like sleep, learning and memory. Studying behavior in a small vertebrate animal model that has well defined behavior, can be studied in large quantities and over a relatively short period of time offers significant research advantages compared to other animal models. To be able to study the effects of sleep or sleep deprivation on learning and memory and evaluate the effects of one variable on the other it is necessary that the protocols for both conditions are well defined and effective.

In this study a simple conditioning training tank consisting of two differently colored halves was used and an electric shock associated to either one of the halves and changed for each trial group that contained four fishes to control for place preference. Fish were then moved to a sleeping tank where half of the fish was in lights-on condition overnight (n=12) and the other half a naïve group that had normal housing lights-off condition during the night (n=12). Time in the safe-zone, the zone that no electric shock was associated with, was measured in the conditioning training tank and the movement and behavior of the fish measured in the sleep conditions. Performance on the conditioning training task and memory retention test was then calculated and the two sleep conditions groups compared together.

The results show that fish spent significantly more time in the safe zone of the conditioning tank post-training compared to pre-training. This suggests that the zebrafish perceived the electric stimulus, associated it with the part of the conditioning tank it received the electric stimulus in and avoided it for the last fifteen minutes of the training session.

In the memory test, on the other hand, there was no significant increase in the amount of time spent in the safe half of the conditioning chamber between pre-training and post-sleep. This suggests that there was no long-term memory formation overnight. There was also a significant decrease in the time spent in safe zone in post-sleep compared to post-training **(see figure 3)**. This suggests that either zebrafish can't learn to associate two differently colored areas with an avoidance stimulus or this specific training did not cause significant memory retention. Other studies show that zebrafish can learn to associate visual stimulus with a stimulus like electric shock and during this study the zebrafish showed short time response to the training (20), (51). The explanation that zebrafish can't learn to associate visual stimulus

to an avoidance stimulus is therefore highly unlikely. A more likely explanation is that this specific training task did not cause efficient memory retention.

The reasons for this may be one or more of the following. The stimulus was not strong enough and a higher voltage might be needed for proper learning and association, stimulus was not frequent enough to make the stimulus association with either the green or red areas in the conditioning tank strong enough for memory retention. It is likely that this conditioning tank did not have all the qualities to make the color association clear. As mentioned earlier, a color preference study has been made in the zebrafish which shows similar preference for green and red and stronger reference to both those colors than the colors blue and yellow so the color choice was suitable (23). On the other hand, both ends of the conditioning chamber were black instead of presenting the same colors as were on either side of the ends, green or red. Zebrafish have behavioral tendencies (52) to stay in darker areas rather than bright so the fact that both ends were dark and of same color might effect the conditioning.

Another study has shown that sleep can be decreased in the zebrafish using only light as a stimulus (38). The results of this current study show, on the other hand, that the light stimulus did not significantly decrease sleeping percentage in the Light group compared to the Naive group. The standard deviation shows that the sleep percentage varies markedly within both groups. It might therefore be interesting to measure sleep in the fish before applying sleep deprivation method with light and see if there would be significant individual difference in sleep percentage. Another approach would be to study larger groups to see more reliable results. It would also be necessary to evaluate the effects of other sleep deprivation techniques on learning and memory to fully describe its effects.

Temperature was measured and held at an appropriate level during all procedures as well as measurements of the electrical tension between the grids. Water from the housing system in the laboratory was used in the training tank and the sleeping tank. Future procedures should include checking the nitrite, ammonia and pH levels in the water before all measurements as well as keeping the temperature constant.

In this study the same personnel did all the measurements and procedures. This can be an advantage and a flaw. The advantages of one person performing all procedures of the study for example is that it makes it more likely that procedures are done exactly the same each time. The flaw is that the researcher can always make mistakes in protocols that could affect the study and might be avoided with the participation of more than one well trained personnel. An observer drift is a possible bias although measuring behavior with a software like Ethovision XT7 helps minimizing observer drift.

Zebrafish are very sensitive to stress factors such as handling and being moved from one tank to the other so it could well be that the stress from handling could have effected the results. Ideally measures should be made to make the handling a minimal stress factor when changing between tanks.

To improve this study technique the colors in the conditioning tank should be applied to both ends of the tank and the bottom to see if that improves the color association with the stimulus and memory retention. Different methods of sleep deprivation should also be applied to study if there is truly a difference in sleep deprivation with light and other stimuli like electric shock and if both, neither or only one of them affects memory retention. Protocols for handling should be revised and improved to control for the stress of handling. Protocols for water quality should be inspected and improved if found necessary to make sure the fish is in as healthy environment as possible.

Comparing the effects of wake-promoting and sleep-promoting environments after training on the *c-fos* expression in the brains of the zebrafish would also be an interesting addition to the future research of the effects of sleep deprivation on learning and memory in zebrafish. *C-Fos* expression in the zebrafish brain reflects polysynaptic activated neurons and can be useful to study patterns of neuronal activity (53, 54). *C-Fos* expression has been used in zebrafish to identify neuronal pathways (55). Another interesting addition would be to look at and compare hctr/orx expression in the same groups and see if there is a difference in hctr/orx expression between fish in sleep-conducting environment after training and those put in a wake-conducting environment after training. Study that consist of both behavioral and immunohistochemical methods could give further clues to if and how synaptic plasticity in learning and memory is consistent to sleep/wake homeostasis.

As described earlier zebrafish are a convenient animal model to study the effects of sleep deprivation on learning and memory. It is nevertheless always important to state that a mouse is not a man and neither is the zebrafish. Even though it goes without saying, behavior will not be easily studied in a cell culture and it is important to study the organism as a whole where external influence can be carefully monitored. It's therefore important to develop simple and efficient procedures to study behavior and its underlying mechanisms in an appropriate animal model. It is also important that the procedures to study the effects of sleep deprivation on learning and memory show long-term memory in the animals so that the effects of sleep deprivation can be evaluated.

Conclusions and perspectives

From the results of this research it can be concluded that this conditioning avoidance task did cause response in the animals short-term behavior but no long-term memory formation. Therefore the effects of sleep deprivation with light on learning and memory in zebrafish cannot be evaluated at this point and further adjustments are needed to develop a good memory training procedure. These results also indicate that sleep deprivation with light does not result in significant decrease in sleep percentage and might therefore not be a strong sleep deprivation technique to evaluate the effects of sleep deprivation on learning and memory in the future, at least not on its own. A better approach might be to study the effects of different sleep deprivation methods, for example both light and electric stimulus, on learning and memory in a better-adjusted version of the conditioning avoidance training.

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