SCREENING OF COMPOUNDS THAT ALTER SLEEP-WAKE STATE IN ZEBRAFISH

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INTRODUCTION

Zebrafish have emerged as an exceptional animal model for many research and applications in several fields, such as developmental biology, oncology, toxicology, neurobiology, as well as for sleep and wake behaviors.

The **advantages of the zebrafish** are mainly their low cost and ease of maintaining and breeding. Moreover, many of the research conducted in zebrafish has been carried out in larvae to take advantage of zebrafish fecundity, larvae small size, ease of handling and transparency. In addition, the use of zebrafish larvae is in accordance with the 3R principle.

Sleep is an integrative process that has to be studied in whole organisms. The main advantage of zebrafish for sleep studies is that zebrafish is diurnal. Sleep in zebrafish (adults and larval stages) is associated with reduced locomotor activity, specific postures and increased arousal threshold and it is regulated by homeostatic and circadian mechanisms (Zhdanova, 2011).

OBJECTIVE To evaluate zebrafish as an animal model for screening of compounds that can alter sleep-wake state

MATERIAL AND METHODS

Material: 5 days post fertilization (dpf) zebrafish embryos from wild type AB strain.

Compounds: psychoactive drugs (stimulants and depressants) see Table 1

Treatment: 5dpf embryos were placed in a 96-well plate (one embryo per well). Each well contained the appropiate concentration of compound in 600ul of total volume. In case DMSO was needed as vehicle its concentration was 1%.

Tracking: Immediately after embryos treatment, the plate was introduced in the tracking system. Zebrafish locomotor activity was recorded over 24 hours under their habitual photoperiod (lights off at 22:00, lights on at 8:00) using DanioVision system powered by Ethovision (Noldus).

Analysis: the total distance moved, as well as sleep duration (defined as more than 6 second in immobility state - based on Sigurgeirsson et al., 2011) were measured. For statistical analysis these parameters were measured every one hour.

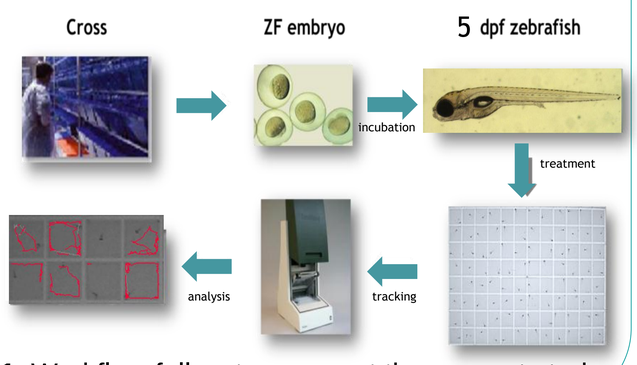

Fig 1: Workflow follow to carry out the present study.

RESULTS

Compound	Human effect
Melatonin	Sleep promoting
Doxylamine	Sedative
Promethazine	Sedative
Caffeine	CNS Stimulant
Modafinil	Wakefulness promoting

Table 1: stimulant and sedative compounds used in the present work and the effect produced in humans.

Sleep Promoting - Sedative compounds

In the case of Sleep Promoting or Sedative agents, a decrease in the distance moved at day was seen, usually clearer in the second day period (Fig. 2). Two posible explanations for this could be: the selected compounds need more time to penetrate and therefore to affect embryo and/or in the first hours embryos were not habitutated to the system so that the variability was greater.

The sedative agents were also detectable by sleep duration parameter (Fig. 3).

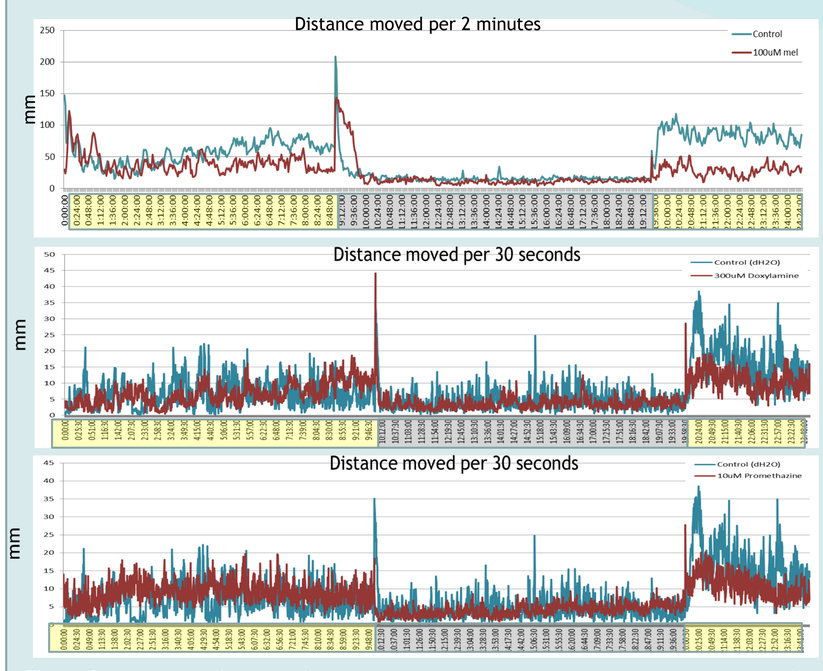

Figure 2:graphs showing the distance moved by controls and treated embryos during a tracking of 24 hours. A: Melatonin treated embryos; B: Doxylamine treated embryos; C: Promethazine treated embryos.

Figure 3:graphs showing sleep duration per hour spent by controls and Doxylamine treated embryos (A) and Promethazine treated embryos (B) during a tracking of 24 hours.

In the case of Melatonin there was a clear delay in the habituation to the dark period when the lights were switched off, problably related to the increased arousal threshold (Fig 4).

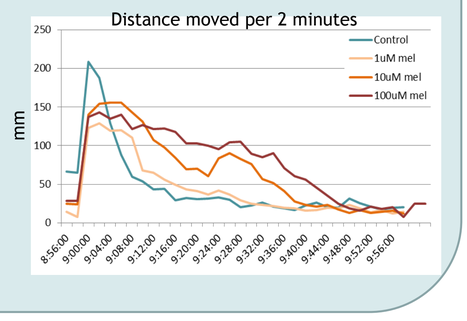

Figure 4:graphs showing the transition from daytime to night of control and Melatonin treated embryos. Transition is represented by the distance moved per 2 minutes during the swithced off of the lights. It is shown that treated embryos spent more time to reach the night basal activity, effect that is clearly dose dependent.

Awake promoting -Stimulant compounds

In case of awake promoting or CNS-stimulant compounds the effect was detected in the distance moved parameter (Fig. 6) but not in sleep duration (Fig. 7). There is a remarkable difference between the two stimulants used. Caffeine stimulation was detected at night period and Modafinil activated embryos locomotor activity only during the day.

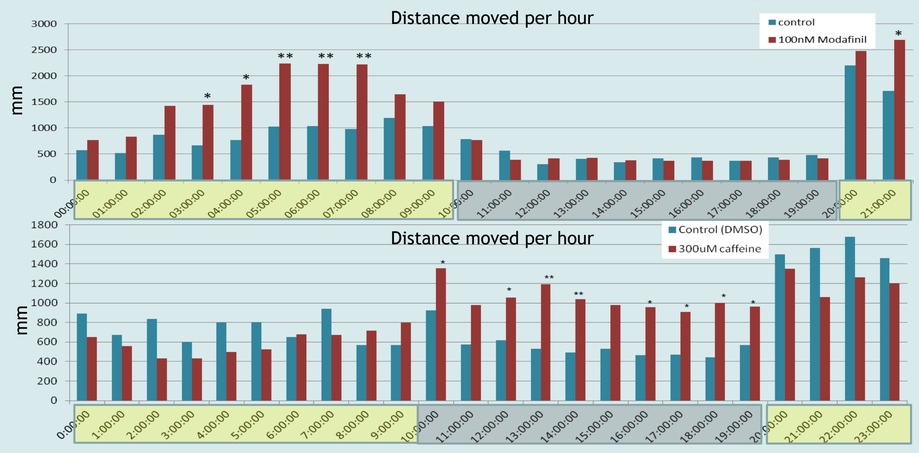

Figure 6: distance moved per one hour by embryos treated with Modafinil (upper image) and embryos treated with Caffeine (in the bottom) comparing to controls. *p>0.05, **p<0,005.

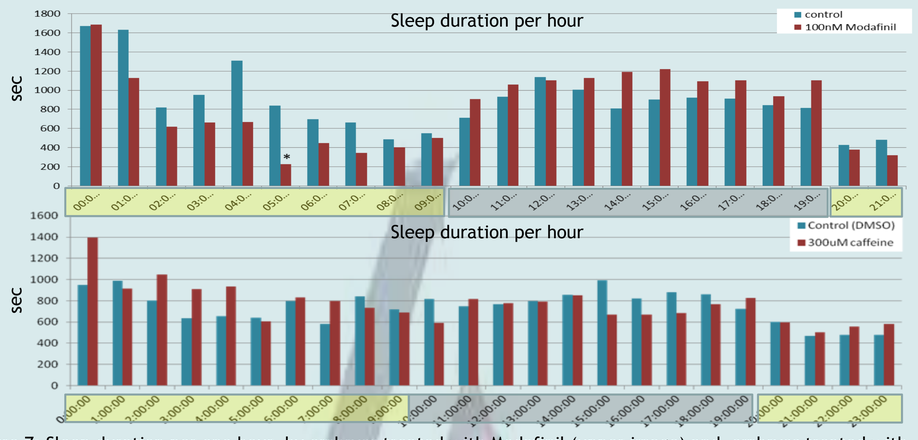

Figure 7: Sleep duration per one hour by embryos treated with Modafinil (upper image) and embryos treated with Caffeine (in the bottom) comparing to controls. *p>0.05.

Table 2: SUMMARY of results. The effect induced in 5dpf zebrafish embryos by tested compounds. Locomotor activity is measured by total distance moved and sleep duration is calculated by the total duration of sleep bouts, defined as more than 6 seconds in immobility state.

Compound	Locomotor activity		Sleep duration	
	day	night	day	night
Melatonin		-	n.a	n.a.
Doxylamine		-		
Promethazine				
Caffeine	-		-	-
Modafinil		-	-	-

	decreased
	increased
-	no differences
n.a.	not analyzed

CONCLUSIONS

- Although more compounds should be analyzed, the obtained results indicate that zebrafish embryos constitute a good model to analyze alteration in sleep-wake state.
- In case of Sleep Duration endpoint, probably adjustment of some analysis parameters would help to detect stimulant compounds.
- Sleep-wake alteration can be easily assessed by locomotor behavior analysis in a High Throughput system with non-invasive techniques.

References

Sigurgeirsson et al., 2011. Effects of Modafinil on Sleep-Wake Cycles in Larval Zebrafish. *Zebrafish* 8(3):133-40
Zhdanova. 2011. Sleep and its regulation in zebrafish. *Rev. Neurosci*. 22 (1): 27-36