CHARACTERIZATION OF ZEBRAFISH CIRCADIAN RHYTHM

A. Alzualde[1], O. Holgado[1], P. Delagrange[2], A. Dekeyne[2]

[1] BBD BioPhenix SL – BIOBIDE; San Sebastian, Spain. alzualde@biobide.es

[2] Unité de Recherche de Découverte en Neurosciences, Institut de Recherches Servier, France

OBJECTIVE

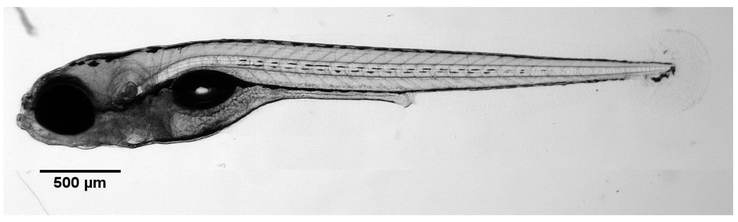
Find an animal model to screen compounds with potential to reduce synchronization time needed to adjust circadian rhythms

INTRODUCTION

A circadian rhythm is any biological process that displays an endogenous oscillation of about 24 hours linked to the light–dark cycle. Circadian rhythms persist in conditions without external periodic input (thus, endogenous). However, the rhythm can be reset by exposure to external stimuli, a process called entrainment. Cycles are adjusted (entrained) to the local environment by external cues called zeitgebers (or time-giver) which include light and temperature. Travel time zone is an example of the ability of the human biological clock to adjust to the local time; people will usually experience jet-lag before their circadian clock is synchronized to local time.

The focus of this work was zebrafish rhythm, thus, requirements for circadian rhythm entrainment and potential zeitgebers in zebrafish embryos were evaluated.

Locomotor activity pattern was considered as indicative of circadian cycles.

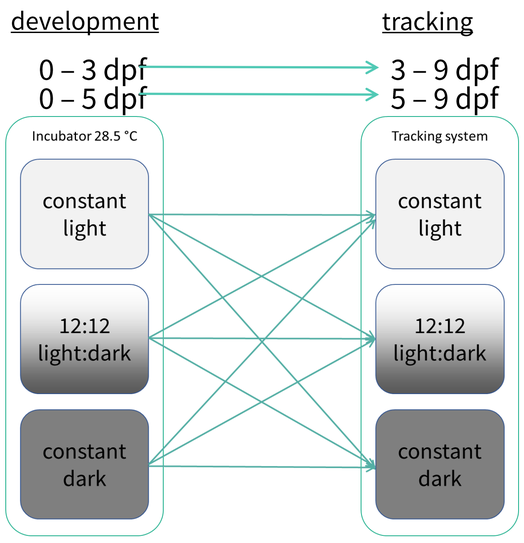


MATERIAL AND METHODS

- ✓ *Danio rerio*: wild type AB strain
- ✓ Locomotor activity: automated larvae tracking
- ✓ Tracking temperature: 20º C
- ✓ 96-square well plates
- ✓ Animal Ethics Committee Approval
- ✓ Variables:
  - lighting conditions during development
  - lighting conditions during tracking
  - advance and delay circadian phases
  - drug administration

RESULTS

Circadian rhythm entrainment analysis



Zebrafish do not entrain to circadian cycle unless they are exposed to light after a darkness event.

Tracking under 12:12 **light:dark:**

- embryos grew in all the conditions entered into circadian cycles at 5 dpf (or 4.5 dpf).

Tracking under constant **light:**

- embryos that did not stay under darkness did not enter circadian rhythm.
- those that grew under constant dark or 12:12 light:dark showed intrinsic circadian cycles.

Tracking under constant **darkness:**

- embryos that grew in constant light did not enter circadian rhythm.
- those grew under constant darkness or 12:12 light:dark showed an intrinsic but not well-defined circadian cycles.

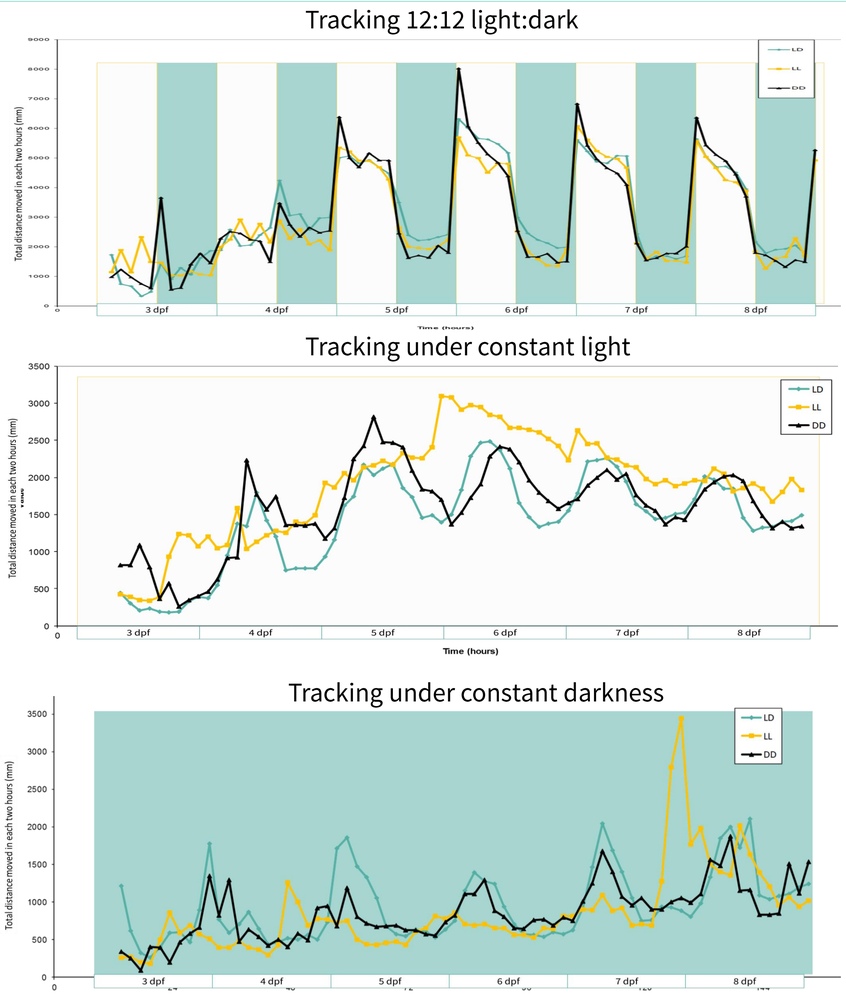


Figure 1: graphs representing total distance moved (mm) in each hour of tracking. Dark periods are shadowed in blue and light ones wihout any shadow.

Phase shift

- 12L:12D from 0dpf to 5dpf
- Tracking from 5dpf at 12L:12D (except at 8 dpf)
- Phase advanced or delayed at 8 dpf

High capacity to adapt so they do not suffer from jet-lag

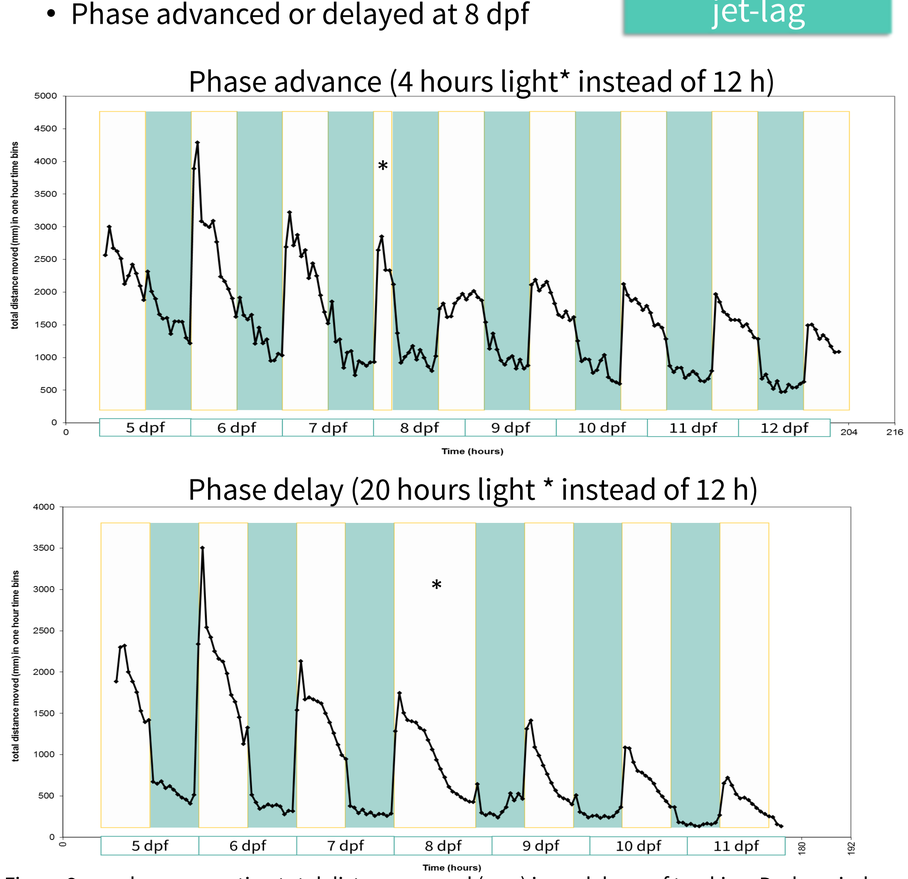


Figure 2: graphs representing total distance moved (mm) in each hour of tracking. Dark periods are shadowed in blue and light ones wihout any shadow.

Chemically induced circadian rhythm alteration

- 12L:12D from 0dpf to 5dpf
- Tracking from 5dpf under constant light & 12:12 light:dark
- At 8 dpf, melatonin treatment
- At 12dpf, end of tracking

Exposure to melatonin affects zebrafish circadian activity decreasing its amplitude

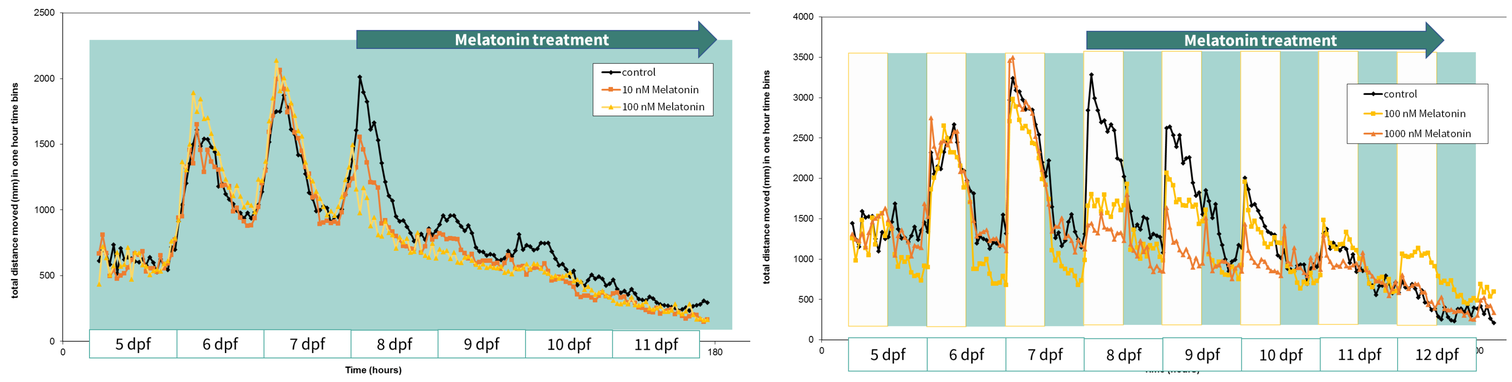


Figure 3: graphs representing total distance moved (mm) in each hour of tracking. Dark periods are shadowed in blue and light ones wihout any shadow.

CONCLUSIONS

- ✓ Zebrafish is a good model for studies focused on circadian rhythms
- ✓ Zebrafish can be used for screening compounds with potential to alter or adjust circadian rhythm
- ✓ Locomotor activity could be indicative of endogenous circadian rhythm