

EXTENDED ABSTRACT

The light/dark cycle is necessary to maintain temporal homeostasis in the hypothalamus-pituitary-interrenal axis of goldfish

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Daily changes in vertebrate physiology and behavior are orchestrated by circadian clocks located in a wide variety of tissues, which are entrained by external zeitgebers (time givers), such as light/dark or fasting/feeding cycles. As a response, they generate rhythmic outputs (i.e. metabolic, hormonal or behavioral oscillations) that persist in the absence of environmental cues. This ability to keep track of time relies on circadian clocks, that consist on transcriptional-translational loops of clock genes and their products, which oscillate with a 24h-long period. The products of *bmal* and clock genes compose the positive arm of the loop, forming a heterodimer able to induce the expression of *per* and *cry* genes, which constitute the negative arm of the loop. In turn, the heterodimer PER:CRY inhibits the activity of CLOCK:BMAL, and thereby its own transcription. These cell-autonomous transcript oscillations control the timing of physiological events, conducting the rhythmic expression of genes involved in multiple processes (Isorna et al., 2017).

One of the main endocrine outputs of the circadian system of fish is cortisol, whose secretion is mediated by the HPI (hypothalamus-pituitary-interrenal) axis. According to previous work, the goldfish (*Carassius auratus* Linnaeus, 1758) exhibits daily rhythmicity in

clock gene expression at all three levels of the stress endocrine axis, i.e. the hypothalamus, pituitary and interrenal gland (IG) (Azpeleta et al., 2012; Gómez-Boronat et al., 2018). However, the influence of external cues in these molecular clocks is not fully established. We investigated the role of photoperiod and feeding time on the synchronization of clock gene expression in the hypothalamus-pituitary-interrenal axis of fish.

Fish acclimated to 12-h light and 12-h darkness (12L:12D, lights-on at 8:00h) and fed at 10:00h were divided into three experimental groups (n=49/group). Group 1 remained under above mentioned conditions (12L:12D, fed at 10:00h). Group 2 was exposed to 12L:12D but fed once daily at random times. Finally, in Group 3 animals were exposed to continuous darkness and fed at 10:00h. After one month, fish were sacrificed at 4-h intervals throughout a 24-h cycle to quantify relative clock gene expression (*per1a*, *per1b*, *clock1a*, *bmall1a*) in the hypothalamus, pituitary and IG by RT-qPCR as reported by Gómez-Boronat et al. (2018).

When both zeitgebers (LD cycle and scheduled feeding) were present (Group 1), the four studied clock genes displayed significant daily expression rhythms in the three axis components, hypothalamus, pituitary and IG (Figures 1A, B, C). Genes of the positive arm of the loop were in antiphase (displaced about 12h) with those of the negative arm, suggesting the presence of fully functional local clocks under these circumstances, as previously reported (Azpeleta et al., 2012; Gómez-Boronat et al., 2018). Moreover, clock gene expression “ticked at time” (i.e. synchronic acrophases) in the three components of the axis.

The majority of genes seemed unaffected by the lack of a fixed feeding time (Group 2; Figures 1D, E, F), especially in the hypothalamus and pituitary, although the interrenal clock could be more food-dependent, since the rhythmicity in the expression of both components of the positive arm of the loop (*bmall1a* and *clock1a*) was lost in the IG under random feeding (Figure 1F).

Conversely, the absence of a LD cycle resulted in a dramatic impairment of the pacemakers (Group 3). All genes in all tissues but one exception (*bmall1a* in the hypothalamus) lost rhythmicity (Figures 1G, H, I) under such conditions.

These data suggest that a LD cycle is essential to maintain the temporal homeostasis of the whole stress axis, the hypothalamus and the pituitary being authentic light-entrainable oscillators. However, feeding time could also play a relevant role in the entrainment of the goldfish interrenal clock, in line with previous reports (Gómez-Boronat et al., 2018), in which a 12-h shift in feeding time resulted in an equivalent shift of the IG’s *per1* acrophases.

Still, the IG could differ from other peripheral clocks, such as the one in the liver of this species, which can be entrained by feeding under constant light (Sánchez-Bretaño et al., 2015). Taken together, data show that both light and feeding time take part in the IG entrainment, so that the clock does not oscillate optimally when these cues are discordant or one of them disappears.

Further studies are required to help determine if the disruption of these oscillators by an unfavorable environment could imply endocrine alterations affecting animal welfare.

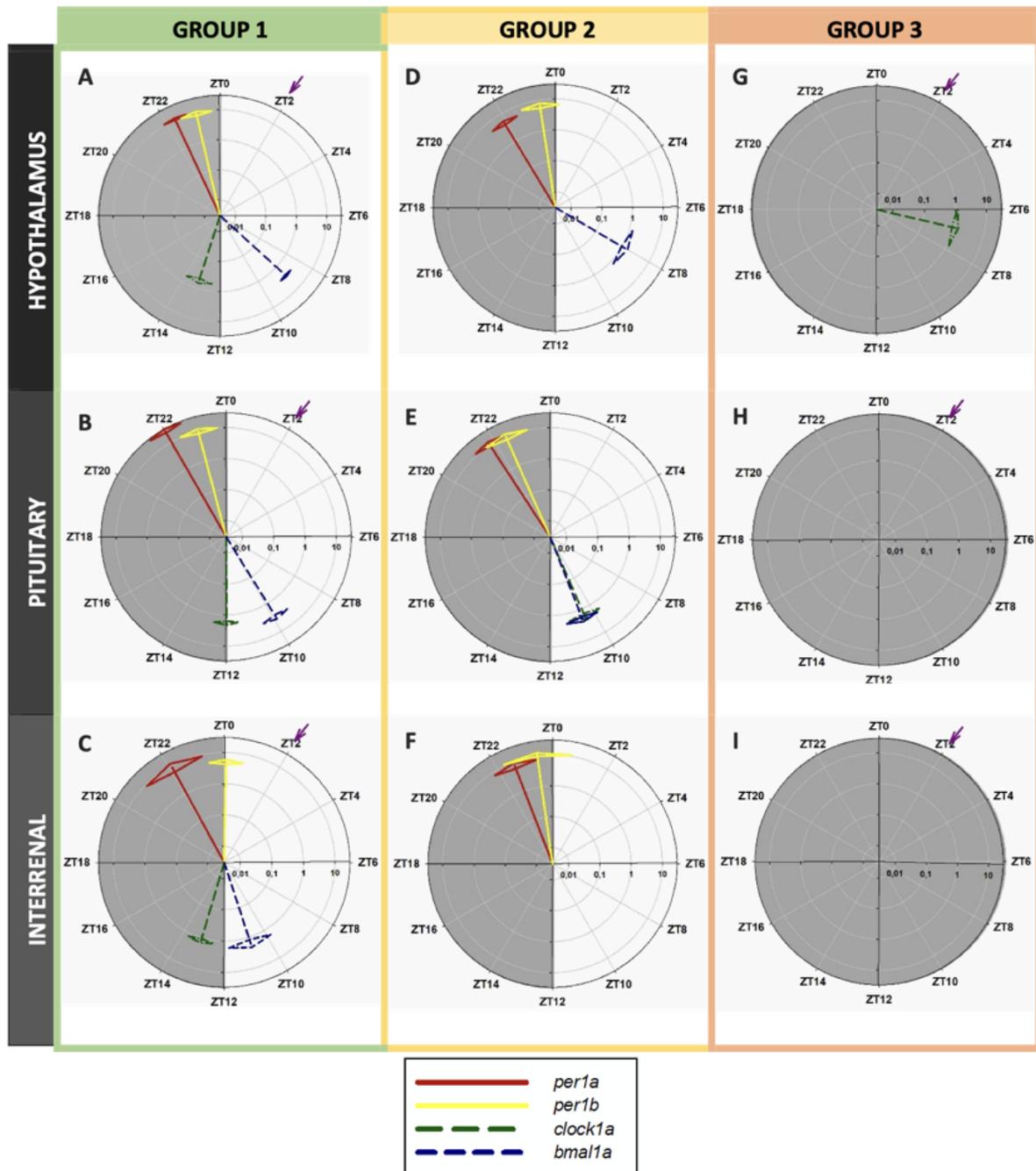


FIGURE 1. Polar representation of the 24-h significant rhythms of target genes in the hypothalamus, pituitary and IG. The length of the vector (radial axis) indicates the value of the amplitude of the rhythm (relative expression, fold change), represented in a logarithmic scale. The angular position indicates the acrophase (i.e., the hour of maximal gene expression). Time (angular axis) is shown as zeitgeber time (ZT, hour). The rhombus at the end of each vector represents the SE of these two parameters. The arrows point to feeding time whenever it was fixed.

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