EXPRESSION PATTERNS OF THE KISSPEPTIN SYSTEM AND GnRH1 CORRELATE IN THEIR RESPONSE TO GONADAL FEEDBACK IN FEMALE STRIPED BASS

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Introduction:

The regulatory role of sex steroids in the neuroendocrine control of vertebrate reproduction is very complex [1]. One key steroidal target is the GnRH1 system, the main regulator of the hypothalamic-pituitary-gonadal (HPG) axis. GnRH1 is responsible for releasing gonadotropins from the pituitary, which stimulate gonadal development and steroid production [1]. However, GnRH1 neurons lack estrogen receptor alpha [1], the steroid receptor implicated in steroid feedback, and thus the link between gonadal feedback and the HPG axis had been unclear. The kisspeptin system has recently emerged as a central processor for relaying signals from the periphery (e.g. gonadal feedback) to GnRH1 neurons, as shown in many mammalian studies [2]. Also, in fish, kisspeptin has been shown to be involved in the regulation of the reproductive cycle [3]. There are two different kisspeptin systems reported in most fish species. Both have been implicated in the control of the HPG axis in different fish species [3].

The present study tests the potential mediatory role of the kisspeptin system between the gonads and the HP axis in striped bass, during two distinct stages of the reproductive cycle of females: recrudescence and mid-vitellogenesis.

Methods:

Bilateral gonadectomies were carried out and control fish were given a sham-operation. After surgery, the gonadectomized fish were divided into 3 groups. During early recrudescence, one group was given a vehicle

Figure 1: Brain kiss1, kiss2, kiss2r and gnrh1 tranript levels in recrudescent (rec) and mid-vitellogenic (vit) female striped bass. Results are presented as copy numbers x 10^4 / 50 ng total RNA ± SEM; * $p < 0.05$.

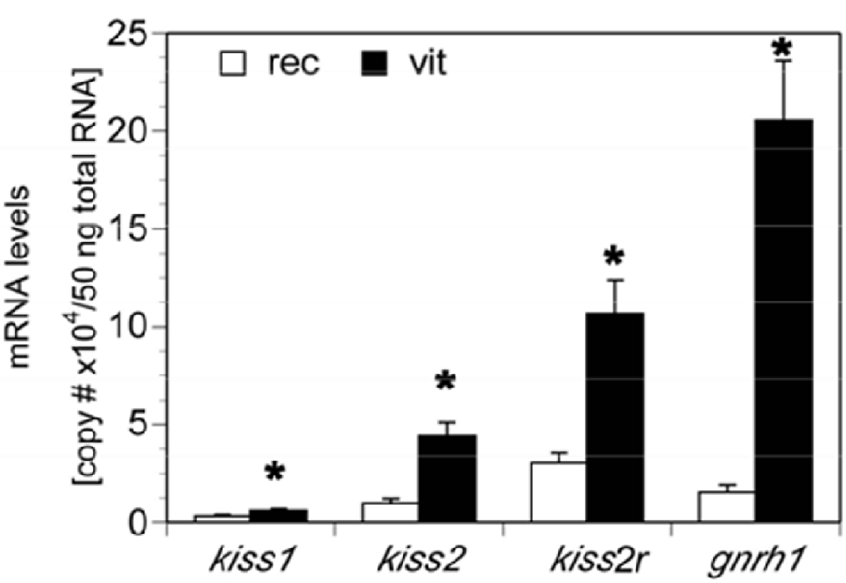
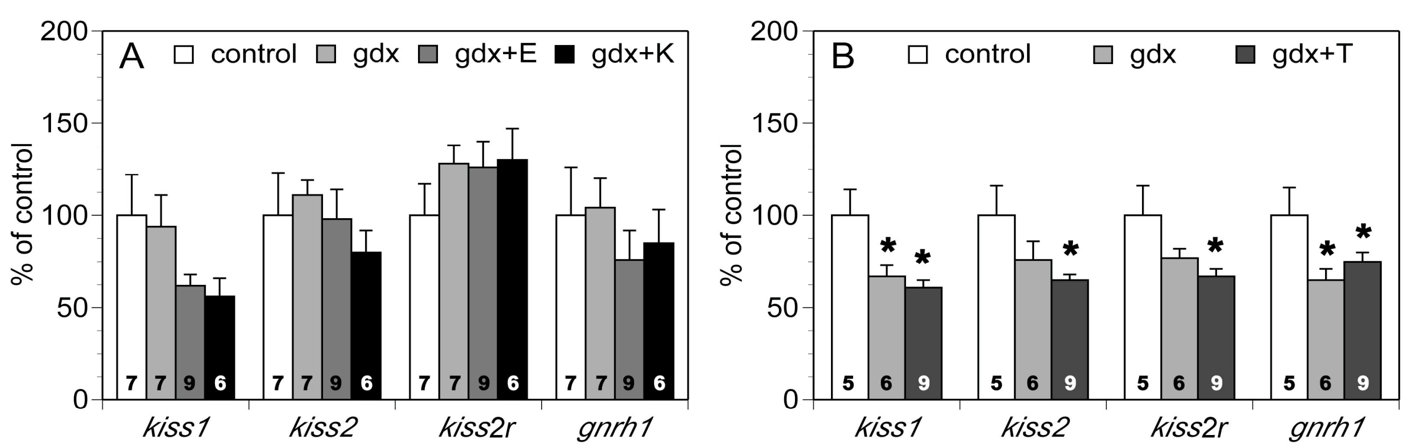


Fig 2: Effects on transcript levels of the *kiss*/*gnrh1* system due to (A) gonad removal [gdx] and steroid replacement [gdx+E and gdx+K] during early recrudescence and (B) gdx and testosterone replacement [gdx+T] during mid-vitellogenesis. Results presented as % of control ± SEM, numbers within bars = number of fish/group (n); * $p < 0.05$.



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injection (gdx), while the second group received 17β-estradiol (gdx+E; 2 mg kg^{-1}) and a third received 11-ketoandrostenedione (gdx+K; 2 mg kg^{-1}) via microspheric delivery systems. During mid-vitellogenesis, one group was given a vehicle injection (gdx), while the second group received testosterone (gdx+T; 4 mg kg^{-1}) via microspheric delivery systems. Fish were sacrificed on day 10 post-surgery. The transcript levels of *kiss1*, *kiss2*, *kiss2r* and *gnrh1* were measured in the brain using real-time fluorescence-based quantitative RT-PCR. To determine differences between experimental groups, the data were subjected to Analysis of Variance (ANOVA) followed by a post hoc test (Scheffe's). Statistical significance was set at $p < 0.05$.

Results:

In female striped bass, steroid levels gradually rise throughout the reproductive cycle. During early recrudescence, when primary oocytes are entering the growth phase, steroid levels are very low compared to the mid-vitellogenic stage, when steroid hormone production increases to stimulate vitellogenin production [4]. To investigate the responsiveness of the kisspeptin/GnRH1 neuronal network to steroid feedback regulation at these stages, we tested the effects of gonadectomy and estrogen/androgen replacement on expression levels of *kiss1*, *kiss2*, *kiss2r* and *gnrh1* expression in the brain. In the control group, we observed significantly higher transcription levels of all four genes when comparing the recrudescent stage with samples from mid-vitellogenic females (Fig. 1). During recrudescence, expression of the investigated genes was not significantly different from controls in any of the treatment groups (Fig. 2A), indicating no gonadal feedback to the hypothalamus at this stage. However, at mid-vitellogenesis gonadectomy significantly reduced expression of *kiss1* by ~30% and *gnrh1* by ~35%, while the reduction in *kiss2* and *kiss2r* (both ~25%) mRNA levels did not reach significance (Fig. 2B). T-replacement further reduced transcript levels of *kiss1* (~40%), *kiss2* (~35%), and *kiss2r* (~30%). These data indicate that during mid-vitellogenesis scgonadectomy removed a positive gonadal feedback from the kisspeptin/GnRH1 system. However, testosterone treatment further reduced gene expression, suggesting a negative steroidal feedback effect on the kisspeptin system.

Conclusion:

Our data supports the conclusion that the hypothalamic kisspeptin/GnRH1 system in female striped bass is not under gonadal control during recrudescence, while later in the reproductive cycle, the kisspeptin/GnRH1 system is regulated by the gonadal factors. There appear to be two simultaneous regulatory mechanisms controlling the kisspeptin/GnRH system: a positive feedback and a negative feedback, the later in the form of T/E_2. The positive effect may be exerted by another gonadal factor such as activin. The close correlation between the *gnrh1, kiss1/2,* and *kiss2r* response in this study may suggest that the kisspeptin system plays a mediatory role in gonadal feedback regulation of the HPG axis in female striped bass.

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