A NOVEL PEAK OF FUNCTIONAL NEUROSTEROIDOGENESIS AND ESTROGEN SIGNALLING IN THE EARLY BRAIN DEVELOPMENT OF ORANGE-SPOTTED GROUPER *EPINEPHELUS COIOIDES*

Nagarajan G. **Aruna A.** and **Chang C.F.**

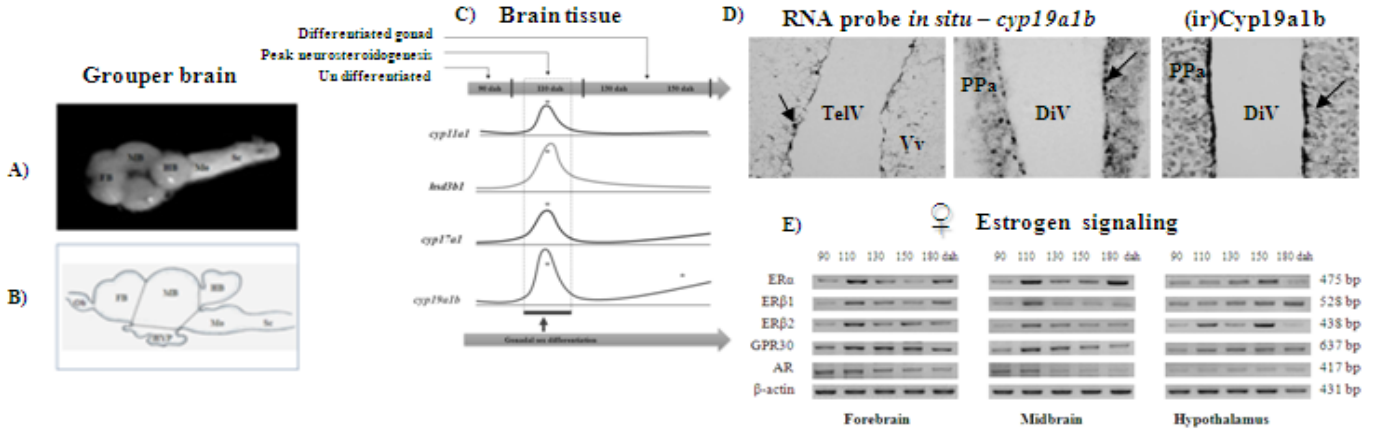
Department of Aquaculture, National Taiwan Ocean University, Keelung, 20224, Taiwan
Fax: +886-2-2462-1579 e-mail: nagarajangi@yahoo.com

Introduction:

Despite neurosteroidogenic enzymes are playing important roles in the regulation of brain development and function, the potential link between brain and gonad by the action of steroid hormones during gondal sex differentiation is still matter of debate in teleosts [1]. In recent years, we focused on the early brain development in response to the synthesis of neurosteroids and receptor activation during gonadal sex differentiation in orange-spotted grouper *Epinephelus coioides* (a mono-female sex development at the juvenile stage) and black porgy *Acanthopagrus schlegeli* (a mono-male sex development at the juvenile stage) [2,3]. The mechanism of brain sex differentiation and/or brain activation during early ages of female grouper fish is not fully understood yet. Moreover, there is an important lack of information regarding the expression of four key steroidogenic enzymes *cyp11a1* (P450scc), *hsd3b1*, *cyp17a1* and *cyp19a1b* in the early brain development and their potential links with gonadal sex differentiation. Hence, these phenomena support the selection of orange-spotted grouper as a model for neuroendocrine research, principally to understand the molecular mechanism of sex differentiation in female fish. Therefore, in the present study we aimed to test the hypothesis that 1) brain of orange-spotted grouper fish has the capability for *de novo* steroidogenesis and the production of these neurosteroids may have a correlation to the occurrence of gonadal sex differentiation and 2) we compared the expression of key genes and signalling in the protogynous grouper and protandrous black porgy in order to know whether this expression pattern has gender related event or not during gonadal sex differentiation. Here, we investigated the temporal expression pattern of the genes related to the neurosteroidogenesis and estrogen signaling in different days after hatching ages (dah) in the teleosts brain.

Methods:

In order to compare the gender effect on steroidogenic ability in the early brain, the dissection of grouper brain (Fig. A and B) was followed to our previous studies published in the male black porgy [3].



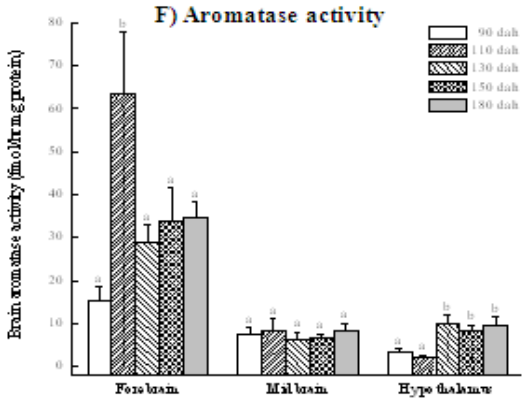


Fig. A. Dorsal view and **B.** lateral view of an adult orange-spotted grouper brain showing different parts in the brain.

Fig. C. Variation in the relative amount of mRNA encoding for *cyp11a1*, *hsd3b1*, *cyp17a1* and *cyp19a1b* in grouper brain at different days after hatching ages.

Fig. D. Transfer section of grouper forebrain showing expression of cyp19a1b in the radial glial cells by ISH and IHC.

Fig. E. RT-PCR analysis of the expression of the grouper estrogen signaling genes at different dah and **F.** aromatase activity in the grouper brain at different dah.

Neurosteroidogenic enzymes and estrogen related gene expressions were quantified by quantitative real-time PCR (qPCR) and RT-PCR analyses. We also measured brain aromatase activity by radiometric method and brain steroid hormone levels (brain estradiol 17β, E2 and testosterone, T) at different dah by enzyme immunoassay (EIA). Further, we demonstrated the anatomical localization of four key genes by *in situ* hybridization (ISH), and immunohistochemistry (IHC) for Cyp19a1b using our grouper brain Cyp19a1b specific antibody. Effects of exogenous E2 on the mRNA expression of steroidogenic genes were also reported.

Results and Discussion:

The brain is considered to be an important sex steroid producing tissue in teleosts [2, 3]. RT-PCR and qPCR analyses indicated that the mRNA expressions of *cyp11a1*, *hsd3b1* and *cyp17a1* were similarly increased in the brain around the period of gonadal differentiation (Fig. C). *cyp19a1b* mRNA expression and aromatase activity showed significant increases in the forebrain at 110 dah (Fig. C and F). mRNAs for ERs (*ERα*, *ERβ1*, *ERβ2*) and *GPR30* but not *AR*, were significantly increased at 110 dah (a time close to gonadal sex differentiation) in the forebrain and midbrain (Fig. E). Brain E2 levels, but not T, were increased in the forebrain at 120 dah. Similarly, a synchronous peaked expression of *StAR*, the key enzyme genes (*cyp11a1*, *hsd3b1*, *cyp17a1* and *cyp19a1b*) and estrogen receptors were reported at 4 months of black porgy [3]. Using ISH, *cyp11a1*, *hsd3b1* and *cyp17a1* genes were highly expressed in several discrete brain regions with overall similar expression pattern, and most of the hybridization signal seems to correspond to the neuronal cells. Whereas, expression of *cyp19a1b* studied by ISH and IHC showed that most prominent expressions were correspond to the radial glial cells (Fig. D). These data well matched with that of zebrafish brain aromatase studied by ISH and IHC. Exogenous E2 (1 µg/g BW) induced significant effects on the steroidogenic enzymes in the grouper brain.

Conclusion:

Therefore, we identified a peak of functional steroidogenic activity and estrogen signaling in the early teleost brain. Moreover, *pcna* transcripts (a marker for cell proliferation activity) were higher in the early brain at 110–150 dah. The early expression of these genes (including *cyp19a1b*) and aromatase enzyme, concurrent to gonadal sex differentiation, provide that these genes are functional during early postnatal development and gonadal sex differentiation, and have distinct contribution to different aspects of sex hormone, but not gender-related brain differention and/ or development.

References:

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