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Research report

Early life low intensity stress experience modifies acute stress effects on juvenile brain cell proliferation of European sea bass (*D. Labrax*)

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HIGHLIGHTS

- Juvenile sea bass brain is characterized by extensive active cell proliferation.
- Early life adversity (UCLIS) did not change juvenile brain cell proliferation.
- Acute stress reduced mitotic activity in teleost hippocampus, amygdala, cerebellum.
- Acute stress increased plasma cortisol in all cases (non-UCLIS, UCLIS juveniles).
- Early UCLIS modified acute stress plasticity of brain cell proliferation responses.

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ABSTRACT

Early life adversity may be critical for the brain structural plasticity that in turn would influence juvenile behaviour. To address this, we questioned whether early life environment has an impact on stress responses latter in life, using European sea bass, *Dicentrarchus labrax*, as a model organism. Unpredictable chronic low intensity stress (UCLIS), using a variety of moderate intensity stressors, was applied during two early ontogenetic stages, flexion or formation all fins. At juvenile stage, fish were exposed to acute stress and plasma cortisol, brain mRNA expression of corticosteroid receptors' genes (*gr1*, *gr2*, *mr*) and brain cell proliferation (using BrdU immunohistochemistry) were determined in experimental and matched controls. UCLIS treatment specifically decreased brain *gr1* expression in juveniles, but had no effect on the juvenile brain cell proliferation pattern within the major neurogenic zones studied of dorsal (Dm, Dld) and ventral (Vv) telencephalic, preoptic (NPO) areas, periventricular tectum gray zone (PGZ) and valvula cerebellum (VCe). In contrast, exposure to acute stress induced significant plasma cortisol rise, decreases of cerebral cell proliferation in juveniles, not previously exposed to UCLIS, but no effect detected on the expression levels of *gr1*, *gr2* and *mr* in all groups of different early life history. Interestingly, juveniles with UCLIS history showed modified responses to acute stress, attenuating acute stress-induced cell proliferation decreases, indicating a long-lasting effect of early life treatment. Taken together, early life mild stress experience influences an acute stress plasticity end-point, cerebral cell proliferation, independently of the stress-axis activation, possibly leading to more effective coping styles.

Abbreviations: ANOVA, analysis of variance; AS, acute stress; BLBP, brain lipid binding protein; BrdU, bromodeoxyuridine; DAB, 3.3-diaminobenzidine; Dl, lateral zone of the dorsal telencephalic area; Dm, medial zone of dorsal telencephalic area; dph, days post-hatch; GABA, gamma-aminobutyric acid; GR, glucocorticoid receptor; *gr-1*, glucocorticoid receptor 1 gene; *gr-2*, glucocorticoid receptor 2 gene; *mr*, mineralocorticoid receptor gene; HPA, hypothalamus-pituitary-adrenal; HPI, hypothalamus-pituitary-interrenal; NPO, nucleus preopticus; NPOpc, parvocellular part of the parvocellular preoptic nucleus; PB, phosphate buffer; PBS, phosphate-buffered saline; PBS-T, Triton X-100 in phosphate-buffered saline; PGZ, periventicular gray zone of optic tectum; RT, room temperature; SD, standard deviation; SEM, standard error of measurement; TOAD, turned on after division; UCLIS, unpredictable chronic low intensity stress; VCe, valvula cerevellum; Vv, ventral nucleus of ventral telencephalic area.

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1. Introduction

Developmental events such as, cell proliferation, migration, cell death, neuronal maturation, neuronal outgrowth, formation and modification of neural circuitry determine the adult brain structure and function. These events are under the influence of genetic, epigenetic and environmental influences and possibly underlie individual differences. Indeed, early developmental period emerges as a highly sensitive period to environmental stimuli. Exposure to stressful events during this period may disrupt the programmed brain development, thus altering brain maturation endpoints, and consequently, increasing the risk for aberrant behavioral outcomes that may lead into adult psychopathology [1,2]. In addition, studies in mammalian and avian animal models, as well as in humans, have acknowledged the major impact that early life stressors exert, supporting a relationship between early-life stress with learning ability [2–7] with even trans-generational effects [8].

Generally, when stress is kept at low levels, it is suggested to have positive effects, leading to a decreased stress response with more efficient adrenocortical negative-feedback system, improved performance of offspring and enhanced learning and memory [9–11]. In support, early stressed birds tested in an associative learning paradigm made more correct choices, suggesting a higher coping ability in stressful situations [8]. Early life stressful experience in mammals is suggested to control the hypothalamus–pituitary–adrenal (HPA) axis, reducing glucocorticoid receptor (GR) levels at adult age by epigenetic programming of GR promoter [12,13]. Similarly, unpredictable chronic stress in Atlantic salmon resulted in down-regulation of hypothalamus-pituitary-interrenal (HPI) axis and glucocorticoid receptors' mRNAs in the preoptic area [14]. Such changes are in line with the stress concept of allostasis [15,16] that is the adaptive progresses for actively maintaining stability through change.

Recently, early life environment is suggested to influence adult neurogenesis in mammals [17–20]. Specifically, decreases in neurogenesis have been proposed as one of the possible mechanisms, underlying the psychophysiological effects of stressful early life experiences [21–23]. Early life adverse experiences in mammals are using maternal deprivation and gestational stress models, mainly focusing on long lasting effects in hippocampal neurogenesis, an area characterized by adult plasticity and involved in learning and memory processes. Interestingly, cell proliferation in hippocampus is enhanced or reduced, depending on the application time and the strength of the stressors [24].

In contrast to mammals, teleost brain is characterized by extensive adult neurogenesis [25–28], providing an excellent model for adult brain plasticity. Moreover, open substrate fish spawners lack maternal care and are particularly vulnerable to unfavorable conditions and thus provide a complementary model to study the impact of early developmental stress later in life. To address the hypothesis that early life mild adversity could be beneficiary for the organism resulting in better coping to future environmental challenges, we used a teleost as an alternative model, the European sea bass, a marine species widely used in aquaculture of great economic importance, sensitive to ordinary husbandry stressors and characterized by high blood cortisol levels in response to stress [29–31]. European sea bass is known to respond to an early chronic low intensity stress (UCLIS) protocol, with high water cortisol release rates in the rearing tanks of stressed larvae in all developmental phases applied [32]. Evidence provided on the long-term effects of early life stress influences on juvenile sea bass responses to aquaculture stressors, would further advance our knowledge on long-term stress effects on brain plasticity data. For this, the present study questioned whether the acute stress responses in juvenile sea bass (*Dicentrarchus labrax*) are modified by previous early life UCLIS experience, using plasma cortisol levels, brain mRNA expression of genes *(gr1, gr2 and mr)* regulating corticoid response and cell proliferation pattern within six major brain neurogenic zones of stress-sensitive limbic areas, including hippocampus, amygdale and preoptic area, as stress plasticity endpoints.

2. Materials and methods

2.1. Animals and husbandry conditions

Batches of fertilized eggs were obtained from a private fish farm and transferred to the installations of the Institute of Marine Biology, Biotechnology and Aquaculture, HCMR (Heraklion, Crete). Depending on temperature, fertilization to hatching lasts several hours, and specifically at 17.0 °C it lasted 72+/−2 h. Larval rearing was performed applying the pseudogreen-water technique [33], according to the protocol described [34]. Briefly following first feeding, the water renewal in the tanks was set to 20% h-1 and was gradually increased to 170% at the end of the experimental period. Aeration was provided by means of a wooden diffuser located in the tank center at a rate of 150–200 ml min^{-1}. Larvae were held during the whole experimental period under mean (±SD) water temperature of 18 (±1.6) °C, dissolved oxygen levels of 7.2 ± 0.8 mg l^{-1}, salinity of 36 and pH of 7.9 ± 0.3. During hatching and until mouth opening, tanks were kept in complete darkness, while a 12D:12L photoperiod regime (lights on at 08:00) was applied during the rest of the experiment. Following mouth opening and eye development, sea bass larvae were exposed to low light intensity conditions (5–10 lx) in the absence of food for a period of 2 to 4 days to ensure normal swim bladder inflation, while the water surface was also kept free from any oily film by the use of an air-blower skimmer. Larvae rearing period lasted for 60 days and then fish were moved into weaning tanks (volume: 2 m^3 each) and kept for about five months under similar husbandry conditions. In particular, borehole water of constant temperature (19 ± 1 °C) was used while photoperiod was natural. Feeding was based on artificial diets (INVE SA) delivered to satiation with automatic distributors at the beginning and then with demand feeders.

2.2. Experimental design of behavioral stress protocol

The experimental design of the developmental early life low intensity stress exposure at flexion or formation of all fins stages, as well as, the acute stress (AS) application at juvenile stage is shown in Fig. 1. The flexion stage is identified 29 days post hatch (dph), 70% of fish have completed the flexion of the notochord, while at the formation of all fins stage, 45 dph, all fins have been fully developed similar to the adult fish.

2.2.1. Unpredictable chronic low intensity stress (UCLIS) protocol during early life

A chronic low intensity stress protocol was applied, in duplicate, for a period of 14 days, as described previously [32], starting at the beginning of two different developmental phases; the flexion or the formation of all fins. In parallel, during the same period, two more groups of larvae were kept undisturbed and served as controls. Specifically, the UCLIS protocol consisted of optical (increase in light intensity from 60 to 200 lx for 15 min; lights on for 0.5 h during night; lights off for 0.5 h during day; exposure to blue or red spectrum for 0.5 h), mechanical (high aeration for 90 s) and unfamiliar environment (presence of object for 0.5 h) low intensity stimuli. Two different types of stressors were applied randomly on a daily basis for the total period of 14 days, so that fish were kept under a low unpredictable chronic stress (UCLIS) that minimized the potential for habituation. Full spectrum lights (Phillips, TLD 36W) were used to approximate natural light and transparent filters to produce the blue (maximum absorption spectrum 450–475 nm) and