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Exposure to continuous light disrupts retinal innervation of the preoptic nucleus during parr–smolt transformation in Atlantic salmon

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Abstract

High quality salmon smolts are essential for aquaculture, enhancement programs and wild populations. However, intensification of aquaculture smolt production and changes in natural habitats can cause sub-optimal environmental conditions, which may result in poor smolt quality. The salmon brain, as the integrator of environmental information, plays a focal role in relaying this information through the light–brain–pituitary axis, which includes retinal and pineal innervation of the hypothalamus. Here we investigated the effect of rearing juvenile Atlantic salmon, *Salmo salar*, under constant light (LL) on optic nerve fiber growth into the hypothalamus. This was compared with the normal increased fiber growth in fish reared under a simulated-natural photoperiod (LDN). Parr were sampled from the LDN group in February and from the LDN and LL groups in May (peak smolt status for the LDN group). Retinohypothalamic projections to the preoptic area were traced using 1, 1′-dioctadecyl-3,3, 3′,3′-tetramethylindo-carbocyanine perchlorate (DiI) and confocal laser scanning microscopy. Data showed that parr exposed to LL did not develop the same extensive retinal innervation to the preoptic nucleus (NPO) observed in control salmon smolts raised under LDN. Since the cells in NPO are central pituitary regulatory neurones, the increased retinohypothalamic innervation during normal smoltification may be responsible for the increased endocrine response to photoperiod information. The deprivation of photoperiod information, during continuous light exposure, may inhibit the natural developmental program to proceed during the parr–smolt transformation.

1. Introduction

The light–brain–pituitary axis (LBP) is the means by which photoperiod information is conveyed to the endocrine system. Light is detected by the retina and pineal organ, which transmits this information through neurones projecting to central brain regions, one of which is the preoptic area (POA). Here the information is integrated with other neural inputs and then relayed to the pituitary (Holmqvist et al., 1992b; Holmqvist et al., 1994), regulating hormone release (Holmqvist et al., 1994; Holmqvist and Ekström, 1995; Baker et al., 1996; Holloway and Leatherland, 1998; Ágústsson et al., 2000). Of the wide range of hormones known to be involved in smoltification, cortisol, growth hormone and thyroid hormone are key regulators, often acting in synergy (McCormick et al., 1995), e.g. hypoosmoregulatory capacity, morphological changes (silvering of scales and skin), metabolic changes and schooling behaviour (McCormick and Saunders, 1987).

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In recent years, structural and chemical changes in the brain during smoltification have been discovered leading to the insight that the brain plays an important role in smoltification. Some of these changes include the increased growth of retinal and pineal fibers into the POA and new brain regions as shown by neural tract tracing (Holmqvist et al., 1994; Ebbesson et al., 2003) and a specific period of cell differentiation shown by transient growth-associated protein-43 (GAP-43) immunoreactive cells and fibers (Ebbesson et al., 2003). This structural reorganisation of the brain occurs prior to the major increases in circulating levels of some hormones, pivotal in the physiological processes of smoltification (Ebbesson et al., 2003). This supports the view that an increased endocrine response to photoperiod information depends on an increased retinal innervation of the POA during this stage of smolt transformation.

Constant light (often referred to as LL or LD24:0) is frequently used to enhance juvenile growth during their first autumn and winter, however, extended use of such extreme light regimes into late winter and spring deprives the juvenile salmon of information about environmental time (seasonal cues). In absence of such photoperiod signals, the parr–smolt transformation becomes disrupted on several levels. Evidence suggests that the endocrine system remains underdeveloped, with reduced circulating levels of several key hormones (growth hormone, GH, thyroid hormones, TH, cortisol, (Stefansson et al., 1991; Björnsson et al., 1995; McCormick et al., 1995; Björnsson, et al., 2000). Examples of physiological consequences of such 'hypo-endocrine' status include impaired development of gill Na/K-ATPase (and on the individual level, reduced hypo-osmoregulatory ability, (Björnsson et al., 1995; Björnsson, 1997; McCormick, 2001), reduced silvering and high condition factor, suggesting disruption of key metabolic changes associated with protein and lipid turnover. Such 'pseudo-smolts' are generally unable to adapt and perform well in seawater.

Thus, deprivation of photoperiod information clearly disrupts the parr–smolt transformation by preventing the natural developmental programme to proceed. Photoperiod disruption has wide ranging physiological consequence, suggesting that the photoperiod acts on key elements of the central regulatory system through its key role in the light–brain–pituitary axis. In the experiment we report on here, we tested the hypothesis that unnatural photoperiods such as continuous exposure to LL prevents the stimulation of increased retinal innervation into the preoptic area associated with smoltification.

2. Materials and methods

2.1. Fish

Ten month old juvenile Atlantic salmon of the Vosso river strain (South-western Norway, see (Nilsen et al., 2003) were brought from the hatchery at Voss to the Industrial and Aquatic Laboratory at the Bergen High Technology Centre in October 2001. On arrival in the lab the fish were distributed into three 1-m^2 tanks with a rearing volume of 400 L, with two tanks for the simulated-natural photoperiod (LDN) group and one tank for continued exposure to constant light (LL). The tanks were supplied with flow through pH adjusted fresh water with a temperature of approximately 8 °C for the duration of the study which lasted until mid-June 2002. The control tanks received a simulated natural photoperiod of 60°N from fluorescent light tubes installed in the tank cover, while the LL tank had lights on constantly. Both groups were fed a commercial dry diet during the light hours of the control tank.

2.2. Retinal tract tracing

For analysis of retinohypothalamic projections, six parr were sampled from the LDN group in February and from the LDN and LL groups in May (the peak smolt status for the LDN group). Brains were fixed by perfusion with 4% buffered formaldehyde after terminal anesthesia. Previously observed changes in tracing experiments during smoltification indicate that the differences are so great that only small sample sizes are needed (Ebbesson et al., 2003). Three brains from each group were excised and embedding in agarose, the optic nerve exposed and isolated by agarose in preparation for applying 1, 1′-dioctadecyl-3,3, 3′,3′-tetramethylindocarbocyanine perchlorate (DiI). After sealing the application site with agarose, the brains were incubated at 41 °C in 1% buffered formaldehyde for 19 days for complete retrograde labelling by DiI. Then, the brains were dissected, embedded in agarose, and sectioned at 100–150 μm with a Vibratome, briefly inspected for successful labelling in a fluorescence microscope (Holmqvist et al., 1992a), followed by confocal laser scanning microscopy for detailed analysis (BioRad MRC 1024, Lasersharp v 5.2 software).

3. Results

3.1. Fish development

Parr in the LDN group expressed normal morphological, endocrine and physiological development into classic smolts while the LL group showed only an increase in growth. The mean weight of the fish from each sample group were as follows: LDN in Feb (29.4 g) and May (44.6 g), and LL in May (54.9 g). Circulating thyroxine data has been redrawn here to illustrate the sampling points described and lower circulating hormone levels in the LL group compared to the controls (Fig. 1). A similar trend was also observed in circulating growth hormone and cortisol levels as well as

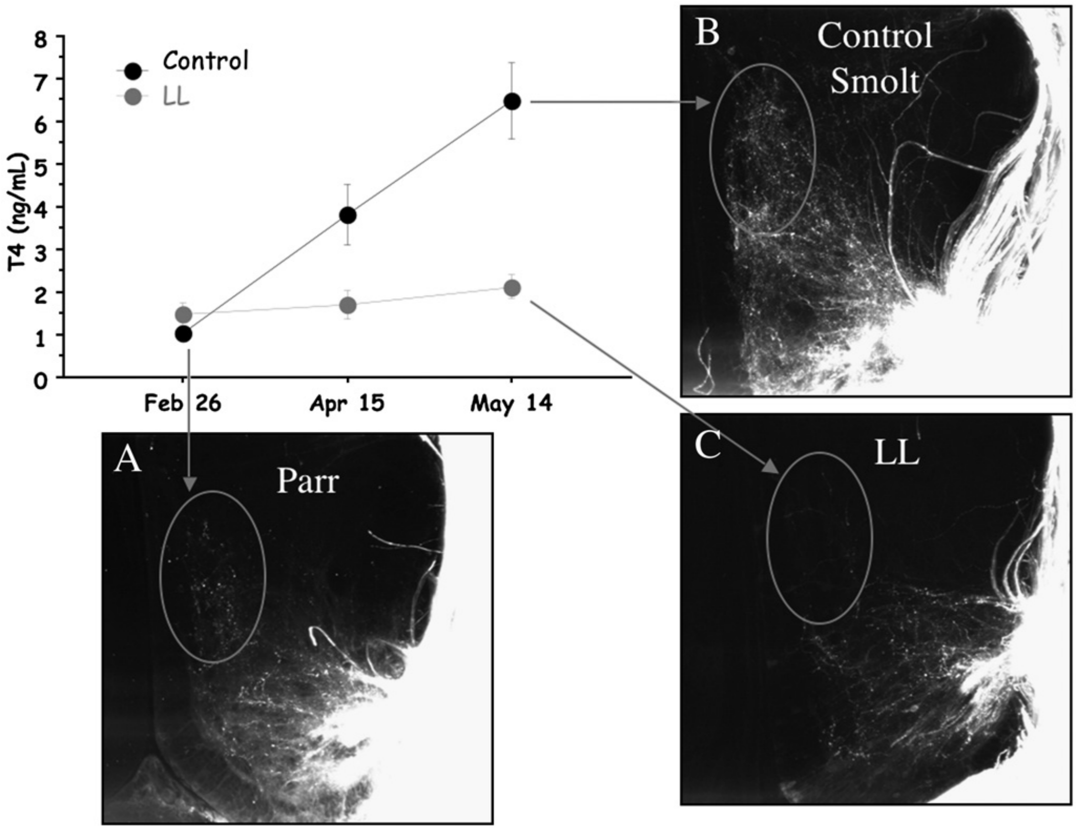

Fig. 1. Retinal innervation into the preoptic nucleus (oval) in Atlantic salmon increased from parr in February (A) to control smolt (B). This increase was not observed in fish exposed to continuous light (LL) in May (C). Note circulating thyroid hormones do not increase in the LL group as they do in the controls in May (Graph redrawn from Stefansson et al., this issue).

hypoosmoregulatory capacity development. For an extensive description of the trajectories in morphology, endocrine and physiological parameters associated with the fish in this study see Stefansson et al. (this issue).

3.2. Methods

The DiI tracing gave consistent results with an optimized exposure time of the tracer, 19 days from the optic nerve. The tracing did not produce any noticeable trans-neuronal diffusion of the tracer. The combination of using adjacent 100–150 μm vibrotome sections and confocal microscopy (2 μm optical slices) has allowed for a detailed analysis of the entire preoptic nucleus. Extensive retinal projections to the hypothalamic optic nucleus was visualized in the Atlantic salmon consistent with previous work using DiI (Holmqvist et al., 1994) and with earlier descriptions in Pacific salmon smolts using other tracing techniques (Ebbesson et al., 1988).

3.3. Tracing

Retinohypothalamic projections are extensive. Here we present only data on the contralateral preoptic nucleus and use the differences in retinal innervation to this region as a measure of structural change between groups. The results show an increase in retinal innervation into the preoptic nucleus from parr in February (Fig. 1A) compared to smolts in May (Fig. 1B) reared under a simulated-natural photoperiod (LDN). Parr exposed to continuous light (LL) showed no increase in retinal innervation into the preoptic nucleus in May (Fig. 1C).

4. Discussion

The present study shows that retinal innervation into the preoptic nucleus in Atlantic salmon increases from parr to smolts reared under a simulated-natural photoperiod, similar to what we have shown in Pacific salmon (Ebbesson et al., 1988; Ebbesson et al., 2003). We further demonstrate that when parr are reared under continuous light the smolt-related increased innervation to the preoptic nucleus does not occur, suggesting that the normal developmental program has been disrupted. The consequences are seen in the lack of increases in circulating hormone levels and development of hypoosmoregulatory competence (Stefansson et al. this issue).

Here, we have used changes in retinal projections to the preoptic nucleus as an indicator of ongoing neural plasticity associated with this midlife developmental period and that this can be disrupted by long term exposure to constant light. If we consider the extensive neural changes that have been described during parr–smolt transformation in salmon, e.g. olfactory imprinting, behavioural and neuroendocrine, a wider view of this impact on development can be realized. In brief, we have previously demonstrated a period of structural neural plasticity in coho salmon during smoltification. This period is characterized by transient GAP-43 immunoreactive cells (an indication of cell differentiation) and fibers in the preoptic area (which also receives new retinal inputs) in addition to many other regions, e.g. olfactory bulb, telencephalon, hypothalamus, thalamus. This occurs prior to the major increases in circulating thyroid hormones and growth hormone levels (Ebbesson et al., 2003). In addition to the above mentioned structural reorganisation, the brain undergoes sequential changes of select neurotransmitter systems, e.g, dopamine, serotonin, gonadotropin releasing hormone, gamma-aminobutyric acid, glutamate, glycine, norepinephrine (Lewis et al., 1992; Ebbesson et al., 1996b; Parhar and Iwata, 1996), some of which are affected by thyroid hormones (Morin et al., 1997) and intensive rearing conditions (Ebbesson et al., 1994). Transient serotonergic neurons in the habenula and lateral preoptic area (Ebbesson et al., 1992) appear, and opiate receptors (Ebbesson et al., 1996a) and TH receptors (Kudo et al., 1994) sequentially surges in specific brain regions. This suggests that changes in the neuroendocrine system, both retinal recipient areas and others, may be necessary for the subsequent endocrine surges which then trigger the smoltification related behavioural and physiological changes (Ebbesson, 2000; Ebbesson et al., 2003). Thus, it is highly likely that, in addition to the lack of increased retinal innervation presented here, other aspects of the neural development described above are also disrupted in fish exposed to LL.

Improper use of photoperiod signals can interfere dramatically with the completion of parr–smolt transformation (Saunders et al., 1985; Stefansson et al., 1991; Berge et al., 1995; McCormick et al., 1995). The parr–smolt transformation of Atlantic salmon (*Salmo salar*) can be considered a synchronisation and integration of a wide range of physiological, morphological and behavioural changes, pre-adapting the juvenile salmon for entry into seawater (Hoar, 1988). Photoperiod is recognised as the major long-term regulator of smoltification in Atlantic salmon (Stefansson et al., 1991). Under aquaculture conditions, photoperiod manipulations are routinely used to control growth rate and the timing of smoltification to seasons other than spring (Stefansson et al., 1991; Solbakken et al., 1994; Berge et al., 1995). Often in salmon aquaculture, freshwater rearing conditions are created to maximize the development of hypoosmoregulatory competence and subsequent growth in seawater. A common strategy is to rear the fish under LL, switch to a period (4–6 weeks) of 12 L:12D to 14 L:10D and then back to LL, which is followed by the developmental increases in morphological, endocrine and physiological parameters (Björnsson et al., 2000). Does this rearing strategy provide a complete smolt development or does it mainly allow for the key desired developmental traits to appear? Other environmental factors are also important for proper brain development such as environmental stimuli (Kihslinger and Nevitt, 2006). Knowing this should be of special interest to salmon enhancement programs where other traits are also of interest such as imprinting and behavioural development.

A key question that remains, what does the short photoperiod provide that triggers the brain to develop? The balance between light and dark, the presence of melatonin, daily rhythms in circulating hormones, and earlier development in the LBP allow the system to respond to photic information. Knowing the answer to this question will add greatly to our understanding of seasonal development periods and allow us to make advances in our rearing strategies.

We conclude that exposure to constant light prevents the normal development of the light–brain–pituitary axis associated with smoltification. Further, the lack of new and extension of existing retinal fibers into the POA in the LL group may result in the reduction of hormonal surges and development of hypophysiological competence. Further research is necessary to ascertain that the lack of development in the LBP resulted in the underdevelopment in the endocrine and physiological parameters. The experiment and conclusions reported on here support the hypothesis since the LBP regulates hormone release and hypoosmoregulatory development. This supports the hypothesis that the increased retinal innervation to the POA may be *the permissive event* allowing increased endocrine response to photoperiod information.

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