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Effect of Melatonin on the Physiological Colour Change in the Blinded Fish, *Clarias batrachus*

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ABSTRACT

The photo neuroendocrine system of the teleost fishes known to regulate rapid aggregation and dispersion of melanosomes within the chromatophores. The present study, throws light on the melatonin effect on the physiological color change in experimentally blinded fish, *Clarias batrachus*. In this experiment, the blinded fish after acclimation were divided into two groups namely experimental group containing blinded fishes and control group containing fishes with intact eyes. The cryptic response observed in scales of experimental and control groups at different time intervals i.e., 00, 05, 10, 20, 30 and 60 minutes after their recovery from the effects of anesthesia (Tricaine). The result was microscopically analysed, showing significant dispersion and contraction of melanosomes in fish skin, which changed to dark colour during dispersion and again reversed to lighter shade due to contraction. Experimentally blinded fish failed to respond to changes in its body color in relation to its backgrounds. After melatonin injection fish stated paling within 5 min. and remained so for 20 min. after that they acquired complete dark shade within an hour. It is concluded that both pineal and extra-pineal melatonin hormones perform different physiological processes.

***Key words :** Clarias batrachus, Melatonin, Physiological colour change*

Introduction

Fish are known to exhibit cryptic color changes according to their background of habitat (Cal *et al.*, 2017) caused by rapid aggregation and dispersion of chromatophore's pigment (Fujii, 2000). The melanophore response to light may be divided in two categories, primary (non-visual) and secondary (visual). The non-visual responses do not involve the eyes as receptors and they are coordinated through an extra- ocular reflex, a response involving nervous/endocrine coordination between a stimulus received by a receptor other than the eye and the melanophore or uncoordinated response in which the melanophore or possibly a skin receptor close to it directly respond to light stimulus (Bentley, 1976). In contrast the visual responses are the result of reception of light by the lateral eyes and usually depend upon the nature of the background, often spoken as black (dark) and white (light) background responses and are coordinated by nerves or hormones or more commonly by both the mechanisms. Differences in the expression of genes (mch, pomc encoding MSH, and sl) associated with pigment migration in skin or scale chromatophores were observed (Cal *et al.*, 2017; Cánepa *et al.*, 2006, 2012; Fukamachi *et al.*, 2009; Takahashi *et al.*, 2014, Kasagi *et al.*, 2020).

Materials and Methods

Blinding of the fish

The acclimatized fish of either sex were kept in a beaker containing 0.01% of Tricaine to anaesthetize them. Both the eye balls were made free from their sockets by making an incision in the skin around the eyeball, after that the optic nerves were transected. After their recovery from the effects of anesthesia these blinded fishes were placed under natural light in glass aquaria and white/black painted polyethylene troughs. The chromatic response of natural background was studied in the same aquarium without any physical disturbance to the fish. The initial and subsequent observations were carried out at definite time intervals i.e., 0, 5, 10, 15 and 20 minutes.

Preparation of dose and injection of melatonin

Melatonin,10 mg/Kg of body weight of fish was injected into the fish intra-peritoneally.

Obtaining of skin bit from blinded fish

The few skin bits containing chromatophores/melanophores were picked up carefully with the help of fine forceps from the dorsal trunk region of the fish after each time interval of their exposure and were observed under light microscope to see the melanopbore responses.

Melanophore response in blind fish kept in natural/black/white backgrounds

The blinded fish, were kept in glass aquaria at natural light condition. The melanophore responses (aggregation/dispersion) were observed through skin bits in blinding fish with light microscope. The initial and subsequent observations were carried out at definite time intervals.

Melanophore response in blind fish after melatonin injection kept in natural background

Just after the recovery of blinded fish from anaesthesia, at 0 min. time, a dose of melatonin of 0.05 mg (0.25 ml) was injected intraperitoneally into the fish, and subsequent observations were made.

Melanophore response in black background adapted intact fish after melatonin exposure in black background

Normal intact fish was kept in dark background to obtain black adapted fish. After that the same dose of melatonin (0.05 mg/body weight) was injected. The exposed fishes were kept in dark illuminated background and observed the melanophore responses at the same definite time intervals.

Results

Significantly aggregation and dispersion of melanophore was observed. Fig 1 and 2 shows the normal fish and melanophores in the skin. After blinding the fish, the fish became jet black containg fully dispersed melanosomes (Figs 3 and 4). Fig, 5 throws the light on the melanophore responses after exposure with melatonin in blinded fish at different time intervals. when dark blinded fish was kept in natural, black and white backgrounds, it failed to respond to changes in its body color in relation to its backgrounds. It remained black in each background, even after 24 hours or days. When melatonin was

Fig. 1. The fish showing its natural color

Fig. 2. Skin of fish showing melanophores in its natural color

Fig. 3. Blind fish just after its recovery (0 min. time scale)

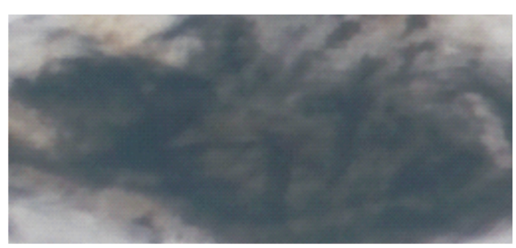
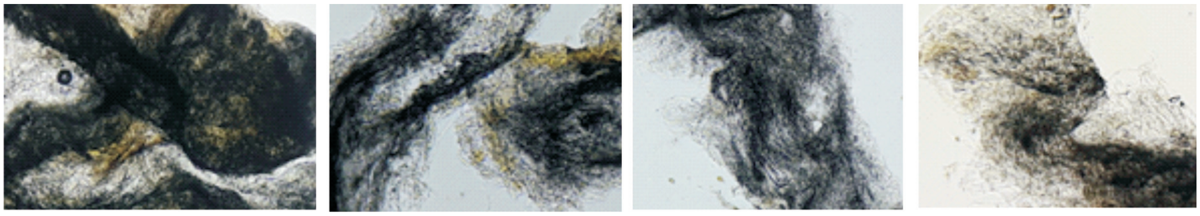


Fig. 4. Skin of blind fish just after its recovery (0 min. time scale)



A (05 min. time scale) B (10 min. time scale) C (15 min. time scale) D (20 min. time scale)

Fig. 5. Skin of blind fish after melatonin exposure in natural background at various time

Fig. 6. Intact black background adapted fish

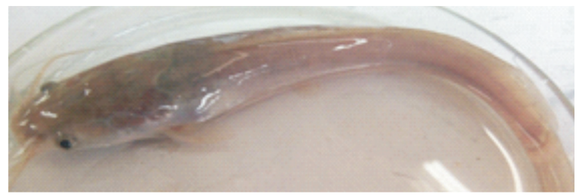


Fig. 9. Normal fish in natural background after melatonin exposure.

Fig. 7. Skin of black background adapted intact fish.

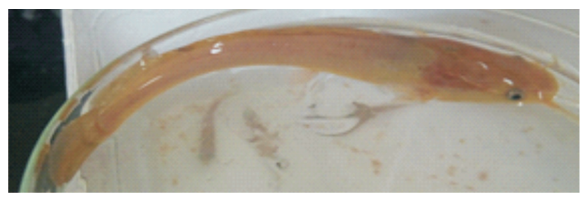
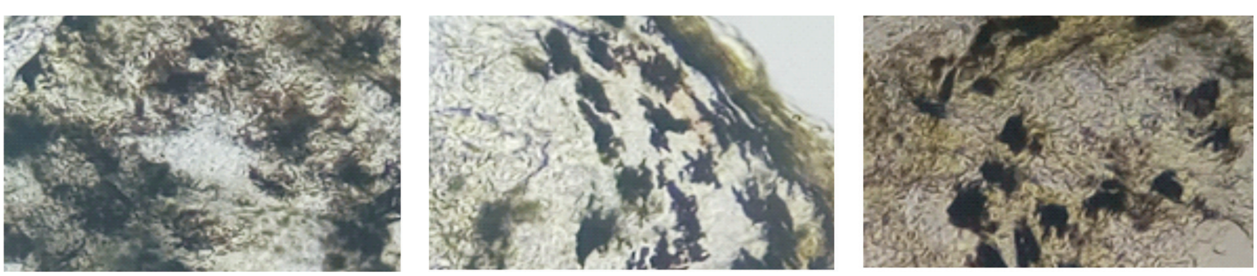


Fig. 10. White adapted fish in white background after melatonin exposure

tested at a dose of 0.05 mg/body weight, the black adapted intact fish over a black background paled notably within 5 min. The paling was maintained by the fish for 15 min. after the treatment. After that, they start to return to their original dark body shade and within 01hr of post-injection stage, they acquired complete dark shade (Figs 6, 7 and 8). The same dose of melatonin was also applied to the jet black blinded fish keeping them in black background and observations were made at 5, 10, 15 and 20 minutes of exposure. It was observed that rate of paling in skin colour was comparatively low as that of paling in exposed black adapted fish (Figs 4 and 5).

Discussion

As the blinded fish recovered from the anaesthesia, it adopted immediately the jet black colour. It might be due to the absence of pineal hormone that lightens the skin colour. Melatonin is also produced from



A (05 min.) C (15 min.) B (10 min.)

Fig. 8. Skin of intact black fish after melatonin exposure kept in black background

retinal and gastro-intestinal passage but was incapable to lighten the skin colour. Generally GI melatonin is associated with the feeding activity of the fish and retinal melatonin acts locally and not released into the blood. When this fish was kept in light and dark backgrounds, it remained dark which also proves the present findings. The blinded fish was exposed to melatonin (0.05 mg/body weight) intraperitoneally, paling in skin was observed up to 20 minutes only (Fig. 5) and after that it again acquired its jet black colour within one hour. Again the same dose was administered, but the result was found same. It appeared that melatonin of previous dose was degraded in liver. It can be said that pigmentation in skin was so dark that more concentration of melatonin is required to lighten the skin up to its natural shade.

Conclusion

It can be concluded that Pineal and extra-pineal melatonin hormones are two different systems set to perform different physiological processes. In respect of colour change, only pineal melatonin is responsible to lighten the skin colour along with activity of pituitary MCH, and to adapt dark colour is associated with MSH.

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