General and Comparative Endocrinology 181 (2013) 265–270

Contents lists available at SciVerse ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Sexual bipotentiality of behavior in male and female goldfish

Seiji Saoshiro, Yutaro Kawaguchi, Youichi Hayakawa, Makito Kobayashi *

Department of Life Science, International Christian University, Mitaka, Tokyo 181-8585, Japan

ARTICLE INFO

Article history:
Available online 4 September 2012

Keywords:
Androgen
Brain sex
Goldfish
Prostaglandin
Sexual behavior
Sexual plasticity

ABSTRACT

It is known that in goldfish *Carassius auratus*, a non-sex changing fish, prostaglandin (PG) treatment can induce female-typical sex behavior in males, and androgen treatment can induce male-typical sex behavior in females. These facts suggest that goldfish have a sexually bipotential brain even after attaining sexual maturity unlike mammals which have sexually differentiated brain. In the present study, in order to further characterize the brain function of goldfish, whether hormonal treatments which induce heterotypical sexual behavior suppress the occurrence of sex-typical behavior and whether sex-typical and heterotypical behavior can be induced in a relatively short time were examined. In the first series of experiments, male goldfish were shown to retain their ability to perform male-typical sex behavior within a week after being induced to perform female-typical behavior. Likewise, female goldfish were also shown to retain their female-typical sex behavior a week after being induced to perform male-typical behavior. In the second series of experiments, when PG-injected experimental males were placed with both PG-injected females and sexually mature males, the experimental males performed male- and female-typical behavior alternately with the females and the males, respectively during 90 min test period. When methyltestosterone-treated experimental females were injected with PG and placed with both PG-injected females and mature males, the experimental females performed male- and female-typical behavior alternately during 90 min test period. The results of the present study are consistent with the current knowledge that goldfish possess a sexually bipotential brain that can regulate both male and female-typical sex behaviors.

1. Introduction

It is well known that sexual differentiation of the brain in birds and mammals is induced by *organizational* effects of sex steroids during the critical periods of early development. In turn, sex steroids exert *activational* effects on the brain of sexually mature individuals for certain acts of sexual behavior to occur. Once brain sex is determined, it is irreversible in birds and mammals throughout the remainder of their lifetime and the brain regulates the sexual behavior of the respective sexes [1–3,19]. However, field and laboratory observations suggest that brain function of some fish species does not conform to the paradigm observed in birds and mammals [6,14,16]. Hermaphroditism is observed in many fish species in nature [7,14], and individuals of these species perform sexual behavior of either sex at some point during their lifetime. Hermaphroditism in fish is classified into two categories: sequential hermaphroditism and simultaneous (synchronous) hermaphorditism. In sequential hermaphroditism, the ovary and the testis develop at different times resulting in a male phase and female phase in each individual. These sequential hermaphrodites are known to change their gonadal sex and also their behavioral sex. Sequential hermaphrodites are further classified into three types according to the direction of sex change: protandrous hermaphroditism (i.e. male to female sex change); protogynous hermaphroditism (i.e. female to male sex change); bi-directional sex change (serial sex change, both-way sex change, i.e. sex change in either direction) [17,24,25]. In simultaneous hermaphroditism, both the ovary and the testis develop at the same time in a single individual fish. Two ways of fertilization have been observed in simultaneous hermaphroditism, mating (egg trading) and self-fertilization. In fish which exhibit mating for fertilization, two partners perform the appropriate sexual behavior, switching between male and female roles and taking turns fertilizing each other's egg [4,5]. These species exhibit quick behavioral sex change repeatedly during each spawning bout. In the self-fertilizing type, the fish releases fertilized eggs without performing any sexual behavior [15]. In these hermaphroditic fishes, except for the self-fertilizing one, individuals will perform both male-typical and female-typical sexual behavior during their lifetime.

Gonochoristic (i.e. non-sex changing) fishes normally do not exhibit heterotypical reproductive functions, but heterotypical sexual behavior can be induced in some species of fishes by hormonal treatments [16]. Male-typical behavior is induced in females of some species by androgen or cortisol treatments [9,10,16], and

* Corresponding author. Fax: +81 422 33 1449.
E-mail address: makito@icu.ac.jp (M. Kobayashi).

0016-6480/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ygcen.2012.08.022

female-typical behavior is induced in male goldfish *Carassius auratus* by prostaglandin (PG) injection [16]. These behavioral studies suggest that both hermaphroditic and gonochoristic fishes possess sexual plasticity of the brain unlike mammals.

Goldfish is a small cyprinid species and intensively used for environmental and physiological studies of behavior. As a result the regulatory mechanism of sexual behavior in goldfish is probably the best understood among fish species [16,20,23]. Female sexual behavior (egg releasing act) is induced by prostaglandin $F_{2\alpha}$ (PGF) produced in the ovary at the time of ovulation. PGF and its metabolites are released into the water as sex pheromones which stimulate male sexual behavior (chasing and sperm releasing act). For the occurrence of sexual behavior, estrogens are not required by females [11], but androgen is considered to be essential for males [22]. When PGF is injected into non-ovulatory females or males, these fish start to perform female-typical sex behavior with normal males within several minutes after the injection although no egg release is accompanied in this case [21]. When a capsule of androgen, such as 11-ketotestosterone (KT), is implanted into females, these females respond to PG-pheromone and exhibit male-typical sex behavior without actual sperm release [22]. Thus, using PGF and androgen, sex-typical and heterotypical sexual behavior can be induced easily in goldfish. What has not been elucidated to date in goldfish is whether hormone treatments for the induction of heterotypical behavior can suppress the occurrence of sex-typical behavior or how rapidly goldfish can change behavioral sex from sex-typical to heterotypical as shown in some hermaphroditic fishes [4,5,18].

2. Materials and methods

2.1. Fish

Goldfish *C. auratus* were obtained from a local dealer in Saitama Prefecture. Fish were kept in 800-l stock tanks maintained at 20 °C under 16L/8D photoperiod. Fish were freely fed with commercial goldfish feed once a day. It is known that gonadal maturity of goldfish is generally maintained under these environmental conditions. Most of the stock males were spermiating and had tubercles on their pectoral fins (male secondary sexual characteristic), and females were found to have vitellogenic oocytes in the ovary when randomly sampled and dissected. Fish weighing 10–34 g were used for the experiments.

2.2. Sexual behavior of goldfish

Natural and PG-induced spawning (sexual) behaviors are well documented in goldfish [16,20,23]. In brief, during natural spawning, ovulated females produce PGF in the ovary, and this PGF acts on the brain and triggers the spawning act (egg releasing act) in the females. PGF and its metabolites are released into the water as sex pheromones which triggers male spawning behavior that are characterized as chasing and culminating in sperm release (i.e. male spawning act). Male chasing is persistent and interspersed with the spawning acts. Spawning acts (complete spawning act) are initiated by the entry of an ovulated female into the floating aquatic vegetation near the surface of the water and where the male follows the female. The female and the male turn on their sides and swim quickly through the vegetation, releasing eggs and sperm. The male always positions itself underneath and in contact with the female during this act. Then, they flip their tails to mix spawned eggs and sperm. Released eggs are characteristically sticky and quickly adhere to the vegetation. Female spawning will continue until most of her ovulated eggs are released, and this may involve hundreds or more spawning acts over several hours. Another type of spawning act of goldfish is called an incomplete spawning act. An incomplete (attempted) spawning act is similar to a complete spawning act except that the fish leave the vegetation without performing gamete release and tail flipping. In the present study, we considered both complete and incomplete spawning acts as normal behavior and were counted equally.

Female goldfish injected with PGF are induced to perform the female spawning act as do ovulated females with sexually mature males, although eggs are not released. Males do not distinguish between ovulated and PG-injected females. In the present paper, the female spawning act (egg releasing act) is referred to as female-typical behavior or female sexual behavior, and chasing and the male spawning act (sperm releasing act) is referred to as male-typical behaviors or male sexual behaviors in goldfish.

For the behavior experiments, fish were transferred from stock tanks to 60 l glass aquaria and acclimated at 20 °C under 16L/8D photoperiod over the course of 1–10 days. Behavior tests were conducted in 60 l glass observation aquaria provided with artificial floating vegetation made of acrylic yarn, gravel, and an aerated box filter and water temperature maintained at 20 °C and a 16L/8D photoperiod.

2.3. Behavioral experiments

2.3.1. Hormonal treatments for the induction of spawning behavior

Prostaglandin $F_{2\alpha}$ (PGF) (Panaseran Hi, Meiji Seika, Tokyo, Japan) was intramuscularly injected into fish with a microsyringe for the induction of female-typical spawning act in male and female goldfish. PGF was injected into experimental fish at a dose of 0.1 μg/0.1 μl saline/g body weight, and to partner females at a dose of 10 μg/2.0 μl saline/fish [11].

For the induction of male-typical spawning act in females, capsules containing methyltestosterone (MT) were prepared and implanted into experimental females [12,13]. Crystalline MT (20 mg) (Sigma–Aldrich Japan, Tokyo, Japan) was first dissolved in ethanol (400 μl) and then mixed with sesame oil (4.5 ml). After the ethanol was evaporated overnight at room temperature, the MT solution was injected into 20 mm length of silicon tubing (SH No. 2, Silascon medical tube, 2.0 mm id, 3.0 mm od, Kaneka Medix, Osaka, Japan). The ends of the tubing were sealed with adhesive (KE45T, Shin-Etsu Chemical, Tokyo, Japan). For implantation of the capsules, an incision approximately 3 mm long was made on the left side of the body near the anus. The capsules were inserted into the body cavity through this incision after anesthesia with 0.02% ethyl 3-aminobenzoate methansulfonate salt solution (Sigma–Aldrich Japan). No sutures were used after the implantation procedure. For individual identification of MT-implanted females, tags (TX-1400L, Destron Fearing, South St. Paul, MN, USA) were also inserted into body cavity. About a month later, when tubercles (male secondary characteristic in goldfish) were visible on pectoral fins, these MT-implanted females were used for experiments. Other experimental fish were individually identified by the use of fin clippings.

2.3.2. Sexual bipotentiality of behavior in male goldfish (Experiment 1)

This experiment was conducted to examine whether male goldfish retain the ability to perform sex-typical behavior after the performance of heterotypical behavior. Experimental males were placed with PG-injected partner females in observation aquaria (one experimental male and one partner PG-injected female per aquarium), and male-typical spawning activity was quantified over a period of 90 min in order to confirm male competence of the experimental males (Week 1). One week after the first test, the experimental males were injected with PGF and placed with sexually mature "stud" males in observation aquaria (one experimental PG-injected male and one stud male per aquarium), and