BioResource Now !

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Investigating the Mechanism of Love by Using Medaka!

Teruhiro Okuyaka
Research Fellowship (Superlative Postdoctoral Fellow), Japan Society for the Promotion of Science, Massachusetts Institute of Technology, and National Institute for Basic Biology

It All Started from Here

When I entered graduate school, the laboratory was just like a "zoo." This is not a made-up story suggesting that graduate students in the laboratory were actually gorillas and monkeys; it is a description based on the presence of seven species of model animals, including honeybees, in the laboratory. Various research projects, such as behavioral genetics, intracellular symbiosis, and limb regeneration, were being simultaneously pursued. Moreover, experimental strains of medaka (*Aplocheilus latipes*) had been newly established to begin the study of behavioral neuroscience. One day in the laboratory, when graduate students were able to freely select species of animals and research projects, I was asked, "You should find an interesting behavior that you will never get tired of, and that may be able to connect actions, nerves, and genes, and launch a new project in which you will study the behavior." After agonizing over this for a few months, I made up my mind to challenge the molecular-neural basis of a certain feeling. When I searched for this term using Google, 1.8 billion search results were returned.
The molecular biology of "LOVE" began.

The Feeling of Love in the Animal Kingdom

When the feeling of love is considered in the animal kingdom, morphological features and mating strategies are often used for mate selection, similar to the strategies used by humans.
For example, a guppy with many red spots on the ventral fin is considered good-looking and is easily accepted by females. In the case of the bowerbird who makes a beautiful nest, a male that can beautifully decorate a nest is popular with females. Such anthropomorphic and mysterious phenomena were named female mate- choice behavior by Charles Darwin, and have since attracted the attention of many behavioral ecologists. However, the neural basis of these behaviors remains unclear.

I found it mysterious that although the criterion of value differed according to the species, such as the ventral fin for guppy and the nest decoration for bowerbird; females examined the value of each individual male and decided which to accept based on the results of the examination. These characteristics were common to all of the animals that I investigated. I considered that there may be a "mechanism underlying the feeling of love" that is evolutionarily common to all animals, so I attempted to answer this question using genetically modified medaka.

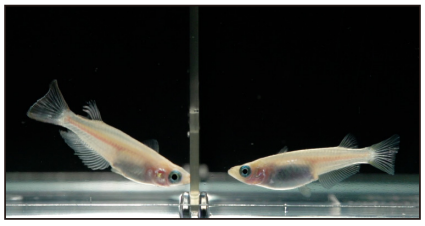

Fig. 1:
A male (left) and a female (right) in a glass tank separated by a transparent board.

Rules for Love in Medaka

Medaka is familiar to Japanese people. As the term "a school of medaka" is well-known in Japan, medaka lives in a group and forms a society. When breeding medaka, we often observe that a male and a female swim together at the corner of the tank. Observing the partner seems to be an important behavior. It has been shown empirically that if a male and a female are separated by a transparent board in a glass tank, prior to the initiation of mating behavior (Fig. 1), eggs can be efficiently collected. Therefore, I first prepared two groups: with and without matchmaking. In the group with matchmaking, a male and a female were separated by a transparent board in a glass tank. In the group without matchmaking, a male and a female were separated by a white board in a glass tank. The behavior was then compared between these two groups. Consequently, the time required for a female to accept a male was shorter in the group with matchmaking than in the group without. The same result was obtained in a similar experiment, in which two males were used instead of one. Even though several males were present in a tank, a female selected a male that was familiar. By making full use of this new behavioral assay system, I attempted to approach the molecular-neural basis of matechoice behavior.

Females Accept Any Male

Fortunately, the National BioResource Project (NBRP) Medaka (http://www.shigen.nig.ac.jp/medaka/) has provided not only transgenic strains, but also mutant libraries. Therefore, I could easily search for a mutant that exhibited abnormality in its behavior to "quickly accept a male familiar to them." Consequently, I found that the time required to accept an unfamiliar male was shorter in females carrying mutations in the *cxcr7* or *cxcr4* genes than in normal females. Based on the expression analysis of these two genes, I became interested in GnRH3 neurons in the terminal nerve, which might be involved in reproduction, as candidate nerve cells for this abnormal behavior.
GnRH1 neurons in the preoptic area (Fig. 2) are considered to be important in the control of the estrus cycle by modulating the release of luteinizing or follicle-stimulating hormones. The function of GnRH3 neurons in the terminal nerve (Fig. 2) that expresses the *gnrh* gene has not been investigated in detail. In particular, the relationship between gene function and mating behavior has been an interesting question to study. Therefore, I produced female individuals, in which only cell clusters of GnRH3 neurons in the terminal nerve were destroyed by infrared laser, and investigated the actions of these individuals. Consequently, these females exhibited a phenotype that was similar to that of a *cxcr7* or *cxcr4* mutant.

↳To the next page

Thus, I had made some progress in the study of the neural basis of mate-choice behavior. In addition, the microscope system using infrared laser can be used for joint research projects in basic biology.

Mechanism Underlying the Excitement of Love

Is information derived from observing a potential mate actually inputted into these neurons? To answer this question, I next performed an electrophysiological analysis, and found that after match-making, the frequency of regular neuronal excitation of GnRH3 neurons in the terminal nerve significantly increased in female medaka. When the spontaneous firing rate was low, the female medaka was in the "refusal mode." After finding a mate, the spontaneous firing rate gradually increased and the refusal mode switched over to the "acceptance mode" (Fig. 3). Finally, I looked at GnRH3 peptides secreted from GnRH3 neurons in the terminal nerve, because my collaborators had already suggested a positive feedback loop, in which GnRH3 peptides were autoreleased due to an increase in neuronal excitation, and neuronal excitation of GnRH3 neurons in the terminal nerve increased due to the exposure of GnRH3 peptides. The NBRP Medaka has provided a screening system to produce medaka mutants called "targeting induced local lesion in genome" (TILLING).

Next, I produced a *gnrh3* mutant and performed a behavioral assay. Consequently, it was revealed that *gnrh3* female mutants did not switch from the refusal mode to the acceptance mode, even in the presence of a familiar male, and the spontaneous firing rate was continuously low. Therefore, the neural mechanism of the mode switch via the autorelease of GnRH3 peptides was revealed to exist. When a man or a woman "falls in love," the excitement of love may be switched on. Is the GnRH3 "switch" the mechanism of love in humans? There are many questions to be answered, including how to associate a specific individual of the opposite-sex with the feeling of love.

In Conclusion

Before starting my research, I knew nothing about medaka. I could not pursue my research, including the screening of medaka mutants and the production of transgenic medaka, without support from the NBRP Medaka and the National Institute for Basic Biology. I would like to sincerely express my deep gratitude to both. Currently, I am studying in the United States, and it was the international training course provided by the National Institute for Basic Biology that made me consider going overseas for the first time. Although the course only ran for one week, I had an exciting experience with graduate students and post-doctoral researchers from many countries in the world. Without this experience, I suspect that my present attitude toward research may differ significantly. I ask all graduate students who have read this article to participate in the international training course and to enjoy the experience of living an extraordinary life.

Reference
Okuyama *et al.*, *Science* 343:91-94 (2014)

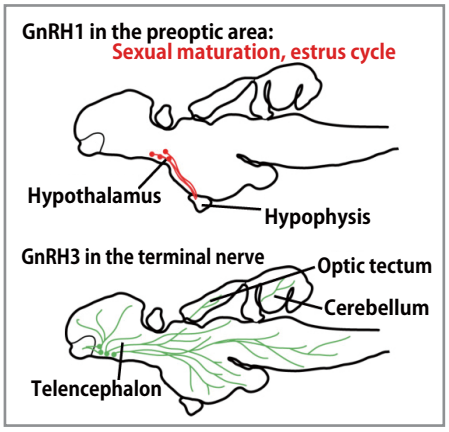

Fig. 2 : GnRH1 neurons in the preoptic area (upper part) and GnRH3 neurons in the terminal nerve (lower part)

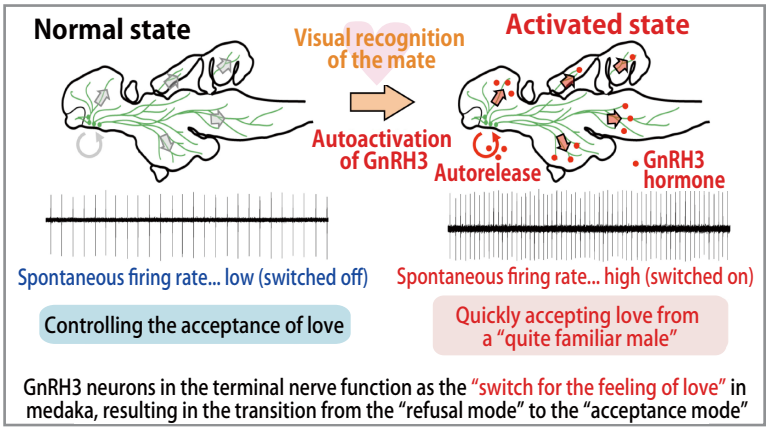

Fig. 3 : GnRH3 neurons in the terminal nerve that are activated by the "switch for the feeling of love"

Database of this Month

National BioResource Project "Morning Glory"

Number of strains: 1,146
Number of genes (alleles): 67(28)
(As of July 2014)

DB name : NBRP Morning Glory
URL : http://www.shigen.nig.ac.jp/asagao/
Language : Japanese, English
Original contents :
- Information about strain resources for research and images
- Information about phenotype classes
- Information about genes, alleles, and mutations
- Linkage maps, etc.

Features : Information about strains can be searched from phenotypes and genetic information. Many images of seeds, leaves, and flower organs are available to the public.
Cooperative DB : Research Resource Circulation (RRC)
DB construction group : NBRP Morning Glory, NBRP Information
Management organization: Genetic Resource Center, NIG
Year of first DB publication: 2009 Year of last DB update: 2014

Comment from a developer: The NBRP Morning Glory is constructed based on information about morning glory resources preserved in Kyushu University, a core organization, and the National Institute for Basic Biology, a sub-organization. Morning glory is a garden plant specific to Japan, many mutants of morning glory were generated during the Edo period (1603–1868), and a large amount of information about this plant has accumulated. The phenotype of the Japanese version of morning glory is described in the traditional notation, in which leaf color is described first followed by leaf shape, flower color, and flower shape, in that order, so you can glimpse of the beauty of the Japanese language. We have repeatedly amended the database for ease of access. At present, the database is connected with the Research Resource Circulation (RRC), the database used to access and submit research papers that have used the NBRP resources. In the future, we will create content with excellent visual effects by making use of a large number of images. Please feel free to use our database and do not hesitate to send us your comments, questions, or opinions using the "Contact Us" option on the top menu.
Japanese Morning Glory in Kyushu University contains not only information about strains of morning glory that were used when this database was constructed, but also a large number of pictorial books and newsletters of morning glory that have been published since the Edo period (1603–1868), so this site is also popular with the general public.

Contact Address
Genetic Resource Center, National Institute of Genetics
1111 Yata, Mishima-shi, Shizuoka 411-8540, Japan
Tel.: 055-981-6885 (Yamazaki)
E-mail : brnews@shigen.info

Editor's Note

Have you enjoyed reading the story of the "switch for the feeling of love" using medaka? This month, Dr. Okuyama, a superlative postdoctoral fellow, discussed a difficult subject in a simple and attractive narrative. Dr. Okuyama has freely set a research theme and steadily pursued that theme in an excellent laboratory environment, while effectively using well-provided medaka bioresources, collaborative research projects, and the international training course of the National Institute for Basic Biology. I suspect that many readers of his article would like to be a researcher like him. Although I'm sure that Dr. Okuyama faced many difficulties in his research, we can feel his strong motivation to overcome them from his article.
I look forward to seeing his work on whether the " switch for the feeling of love" also exists in humans (Y. Y.).

BioResource Information

(NBRP) www.nbrp.jp/
(SHIGEN) www.shigen.nig.ac.jp/
(WGR) www.shigen.nig.ac.jp/wgr/
(JGR) www.shigen.nig.ac.jp/wgr/jgr/jgrUrlList.jsp

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