Discovery of the neural circuit for fear conditioning of fish

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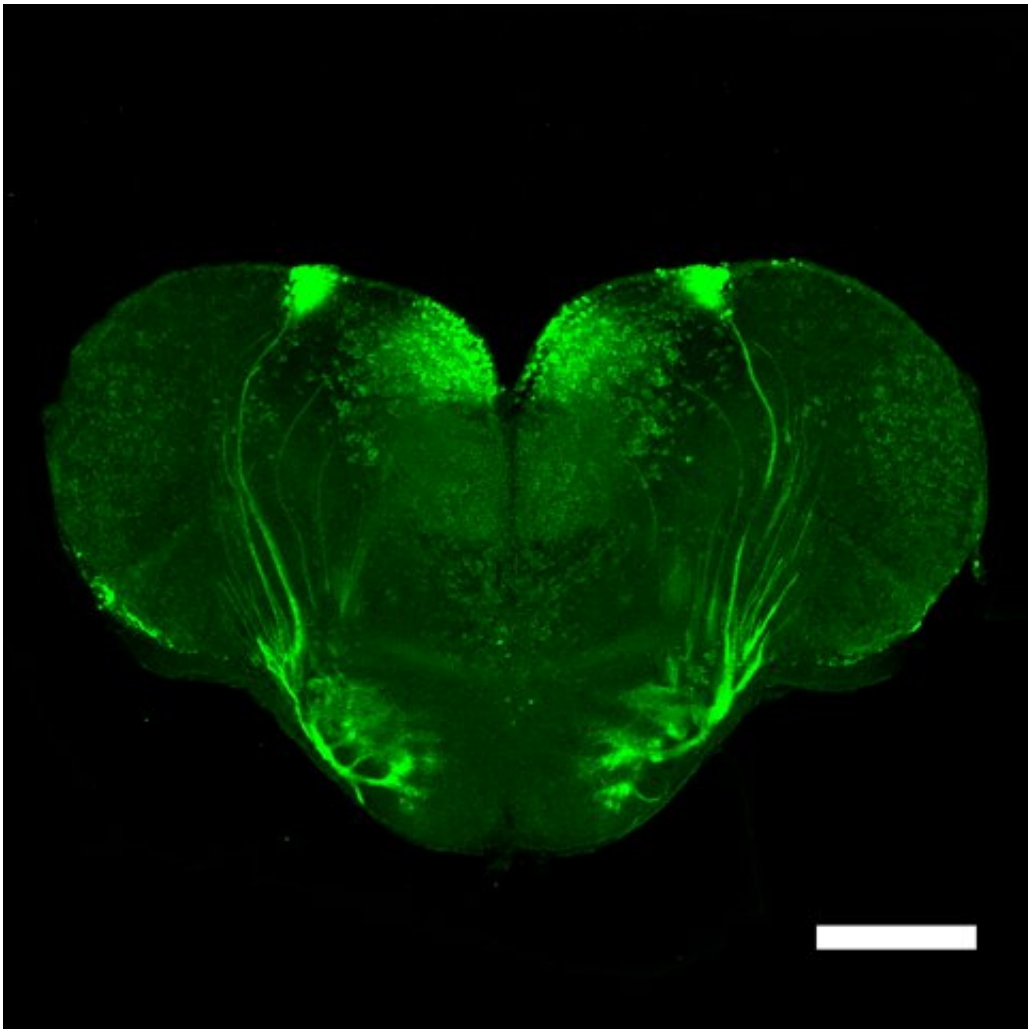

A section of the zebrafish telencephalon. The neurons essential for fear conditioning are illuminated with GFP (green fluorescence protein). Scale bars: 200 μm. Credit: Koichi Kawakami

Animals are often noted sensing signs of danger and reacting. A simple form of this phenomenon is called fear conditioning, which is a type of learning commonly seen in every animal. By manipulating the activity of specific neurons of the zebrafish brain, scientists at the National Institute of Genetics (NIG) in Japan have elucidated a neuronal population essential for fear conditioning in zebrafish. The study, published in the April 25 issue of *BMC Biology*, suggests that such a neural circuit essential for fear conditioning exists and is conserved during vertebrate evolution.

How can animals avoid danger to survive? If animals experienced dangerous events in conjunction with specific signs, animals remember the sign and exhibit fear in response, for instance, reacting with an escape behavior. In mammals including humans, the amygdala, one of the structures of the brain, plays an important role in fear conditioning. However, how the brain structure and neural circuits essential for fear conditioning have been conserved (or changed) during vertebrate evolution was unknown. Zebrafish, a popular model animal in biological studies, exhibit fear conditioning similarly to humans and other mammals. Professor Kawakami's group has succeeded in developing technologies for visualizing and manipulating specific brain neurons in zebrafish by employing the yeast transcription factor Gal4, the green fluorescent protein (GFP), and the botulinum neurotoxin (BoTx). They have generated a collection of transgenic fish lines being used to study brain functions as well as other various organs by other researchers all over the world. Of the nearly 2,000 such transgenic fish lines in his lab, one played an important role in the current study that labels neurons in the dorsomedial (Dm) area of the telencephalon of zebrafish.

"In mammals including humans and mice, fear conditioning is mediated by a brain area called the amygdala. The amygdala integrates information about dangerous events, like electric shock, and some signs such as visual or auditory stimuli. However, in fish, such neurons have

not been found." Prof. Kawakami said.

"It is important to explore such neurons in fish, because we can increase the knowledge about fundamental neural circuits for animals to perform evolutionary conserved fear conditioning."

For this purpose, Dr. Lal, a former graduate student in his lab, developed a behavioral analysis system. Fish were placed with a small tank with two compartments. Ten times a day for five consecutive days, the researchers administered electric shocks while shining green LEDs in the tanks. Finally, in response to the green LEDs, the fish learned to escape from the compartment that was illuminated, and moved to another compartment.

"It is fun to see how smart they are," Dr. Lal said.

Using these technologies and resources, they have found that neurons in the region called Dm of the telencephalon of fish are essential for fear conditioning. These neurons are a functional equivalent of the amygdala of mammals. This result is a clue to clarify the structure and evolution of the neural circuit essential for fear conditioning.

Prof. Kawakami's zebrafish facility has thousands of fish tanks, each of which contains genetically different fish that can turn on, or drive the GFP or BoTx expression in different types of neurons in the brain or in the body.

"This work showcases a successful application of our genetic resources in the study of the brain function. It is also expected to be the basis for clarifying the causes and treatment of diseases involving fear and anxiety and PTSD," Prof. Kawakami said.

More information: Pradeep Lal et al, Identification of a neuronal

population in the telencephalon essential for fear conditioning in zebrafish, *BMC Biology* (2018). DOI: 10.1186/s12915-018-0502-y