frontiers FOR YOUNG MINDS

NEUROSCIENCE
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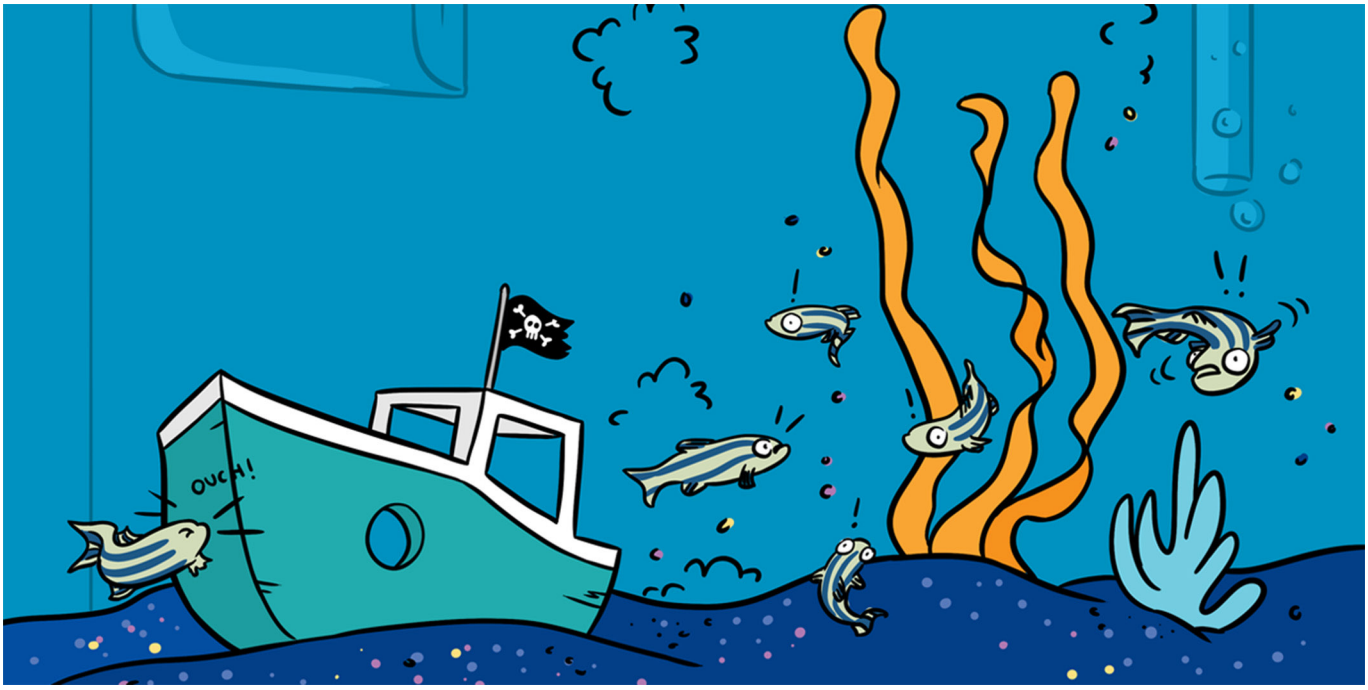

WHAT CAN ZEBRAFISH TEACH US ABOUT FEAR?

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YOUNG REVIEWERS:

DARREN
AGE: 12

JENAE
AGE: 12

Fear can sometimes paralyze us, and it can sometimes be exciting; for some people, fear is so crippling it can significantly mix up their lives. We understand a little bit about how the brain acts when we are afraid, mainly by studying the brains of animals. Recently, surprising findings were made using a humble animal, the zebrafish—a small aquarium fish that, in the past, has helped scientists figure out how our organs develop. Zebrafish are useful because they develop quickly, reproduce easily, and have brains that are similar to ours. They also produce what we call an "alarm substance" that alerts their shoalmates when one of them has been injured. When nearby zebrafish smell this substance in the water, they act as if they are very scared. At the same time, they release a substance called serotonin in their brains, which acts as a light switch, making them less afraid but more cautious—as if they are trying to figure out if a predator is close by or not. Hopefully, finding

out more about how zebrafish brains process this serotonin signal will help scientists develop better treatments for mental disorders that are associated with fear.

WHY IS FEAR IMPORTANT?

FEAR

The mental and physical state humans and other animals feel when they experience something actually threatening.

In our everyday experience, we very often feel afraid: of dangerous stuff, like snakes and other venomous animals, of heights, of bad people, and so on. **Fear** is an emotion observed in all animals, which allows a quick and momentary state of alertness when an animal is perceiving something that can do it harm. An animal's reaction to feeling fear serves to protect it when it is in a dangerous situation. Fear is generated by parts of the brain, such as the amygdala, the cingulate cortex, and a region of the midbrain called the periaqueductal gray area, that act together with the senses (sight, hearing, smell, touch, and taste) to produce a response to danger [1]. Our senses can alert us to the presence of something potentially dangerous, by causing us to feel fear. For example, who among us has not been startled by a loud noise or by a shadow we briefly thought was an animal, no matter how irrational that was? In this context, fear has an important role in the everyday life of animals, because it serves as a type of instinctive protection, helping the animal to recognize both potential and actual risks.

ANXIETY

The feeling of being extremely nervous when expecting something bad to happen.

Fear is also important because, when it is excessive, it can be part of many different mental disorders called **anxiety** disorders. While many researchers argue that fear and anxiety are different things, both involve this negative feeling of danger. Anxiety is what happens when we are expecting something bad to happen; fear is what happens when we actually experience something bad. Anxiety disorders are one of the major health problems in the world today, and the treatments that are currently available are not very good. New discoveries about fear and the brain could help to treat people with anxiety disorders.

USING ANIMALS TO STUDY FEAR

We know a little bit about fear from studying humans—for example, by using neuroimaging (methods which allow neuroscientists to see what is happening inside the living human brain) to try to figure out what is going in the brains of people while they are feeling scared (Figure 1)—but this is not an easy task. First, current neuroimaging technology can only measure activity up to a certain depth into the brain, so deeper regions of the brain (including regions important for fear, such as the midbrain) are difficult to observe with the current techniques. Also, it is actually difficult to safely make people feel afraid in the lab so

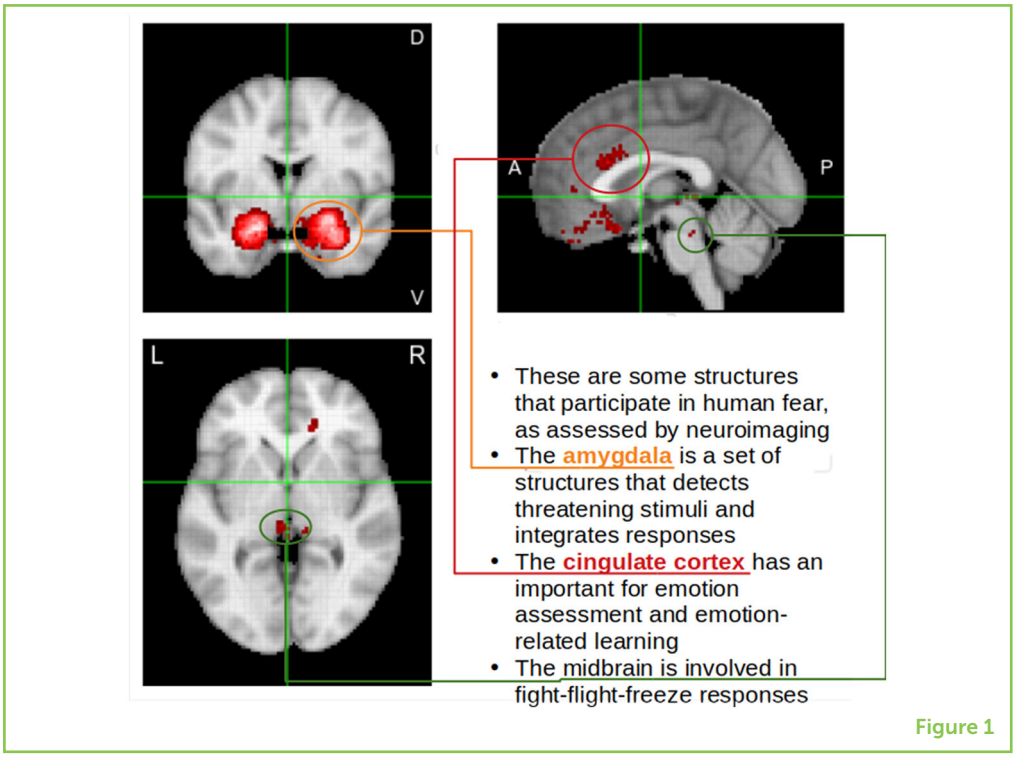

Figure 1

Regions of the human brain that are involved in fear. These images were taken with various neuroimaging tools. The areas of the brain involved in fear are shown in red and circled. D, dorsal ("top"); V, ventral ("bottom"); A, anterior ("in front"); P, posterior ("in the back"); L, left; R, right.

that we can study their responses to fear, because most of the things that make us feel very afraid can also damage us somehow. Finally, it is very hard to find people to volunteer for these kinds of experiments. As a result, much of what we know about fear and how the brain controls this emotion comes from research on animals.

Animals are used in scientific research as an easier way to study behaviors that are also seen in humans. While we tend to think of rats and mice when we think of lab animals, many different species are used for this kind of research, including flies, rabbits, dogs, and fish. Some researchers argue that focusing only on rodents (rats and mice) limits what we can learn about the human brain [2]. These ideas prompted neuroscientists (scientists who study the structure and function of the brain) to look for other animal species that respond like humans. The zebrafish, an animal widely used for research in the fields of genetics, embryology, and behavior, is now "emerging" as a research animal in neuroscience, too. Zebrafish have both genetic (DNA) and physical similarities with humans (for example, similar brains), and these similarities make them a good model for studying human anxiety disorders (Figure 2).

THE ALARM SUBSTANCE OF ZEBRAFISH

One of the many advantages of using zebrafish to study fear is that, like other similar fish, they produce a special alarm substance in their skin

Figure 2

Figure 2

The zebrafish. This small fish is becoming important in neuroscience and pharmacology, due to its small size and rapid reproduction. The animal from the picture has about 4 cm (≈1.6 in) in length! Source: https://commons.wikimedia.org/w/index.php?curid=260841

when they are injured. This substance is produced by cells called club cells, and the purpose of the substance is to signal to other members of the school that a fish has been injured. When the skin of a fish is damaged by a predator, for example, the alarm substance is released, and other fish can smell it. The "smell of danger" causes the other fish to be more cautious and to behave as if they are afraid. When they sense this alarm substance, the other zebrafish swim in tight groups to increase protection. They also swim more erratically (in a zig–zag pattern), to both decrease the likelihood of being eaten and to stir up the sediments (pieces of leaves, sand, or earth on the ocean floor) to make the water cloudy. Sometimes the zebrafish also freeze in place, decreasing the likelihood that the predator will see them [3]. Neuroscientists and behavioral scientists can watch for these behaviors to determine whether the fish are afraid or not, and then use this information to better understand how the brain acts when it is frightened.

SEROTONIN

A neurotransmitter that is released by brain cells to produce effects on other brain cells.

One of the findings made on zebrafish focuses on a substance called **serotonin**. Serotonin—also called "5-HT," shorthand for its chemical name, 5-hydroxytryptamine—is a type of chemical called a neurotransmitter. Neurotransmitters are special chemicals that are released by neurons (nerve cells) and other brain cells when these cells are excited. The neurotransmitters allow nerve cells to communicate with each other and to communicate with muscle cells or cells that release hormones into the bloodstream. Serotonin is perhaps best known as the "happiness hormone," because the medications for depression and anxiety work by stimulating serotonin activity in the brain. However, nothing could be further from the truth: in fact, there is good evidence that serotonin increases anxiety (i.e., we suffer more when we expect something bad to happen), although it seems to decrease fear (i.e., we are less afraid of something that is actually there).

Researchers now believe that serotonin is involved in the fear generated when zebrafish smell the alarm substance. In 2014, researchers discovered that the alarm substance causes the release of serotonin in the zebrafish brains when the fish no longer smell the substance [4],

and this makes the fish more cautious, as if they are trying to determine whether or not there is a predator around. But how does serotonin produce this effect?

In order for serotonin to act in the brain, it must bind to a molecule called a **receptor**—a special protein in our cells that, when bound to a neurotransmitter, begins a response inside the cell, like a key going into a lock. Serotonin has several different receptors that produce different effects. When fish first smell the alarm substance, they act as if they are afraid, swimming wildly and sometimes even freezing in place; when the substance is no longer detectable, they are no longer afraid, but act "extra cautious" (i.e., anxious) to be sure that the danger is no longer there. Researchers found that one of serotonin's receptors, called 5-HT1A, does not seem to be involved in this "extra caution" that appears after the alarm substance is no longer detectable [4]. Evidence for the participation of this receptor comes from another study [5] that found that injecting a drug that block the receptor—meaning they do not allow serotonin to produce its usual effect—made fish more afraid when they smelled the alarm substance, but not after the substance is no longer there. This group also found that blocking other serotonin receptors produces a similar effect, suggesting that serotonin decreases fear, but may also be involved in increasing the subsequent cautiousness that is observed when the substance is no longer present (Figure 3).

RECEPTOR

A protein in our cells that specifically bind to a substance, such as a hormone or a neurotransmitter, making the cell respond to the binding.

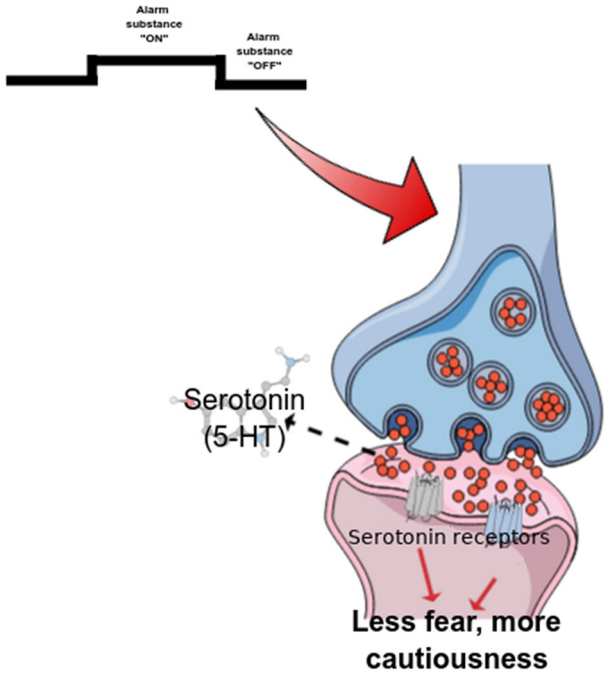

Figure 3

The neurotransmitter serotonin (5-HT) acts as a sign that the alarm substance is no longer present. The black line in the upper left of the figure represents a temporal order, with the lines indicating when the alarm substance is detectable in the water ("alarm substance 'ON'"), and when it is no longer detectable ("alarm substance 'OFF'"). In a special region of the neuron called a "synapse," where one neuron meets up with another neuron, the neurotransmitter serotonin is released when the alarm substance is no longer present ("alarm substance 'OFF'"), but not when it the alarm substance is present. Serotonin then turns on special proteins called receptors, resulting in less fear (in zebrafish, less wild swimming and freezing), but more cautiousness (in zebrafish, more cautious approach and exploration of the tank).

CONCLUSION

Our brains (and the brains of zebrafish) deal with scary situations every day. Your brain and your body are always at the ready to deal with frightening things in a way that protects you but does not make you "cry wolf" when a threat is not certain. With the help of serotonin, we can switch between two strategies for dealing with scary situations: running away from a known danger, or cautiously investigating—and worrying about—whether there is actual danger. It is possible that studying more about serotonin and other neurotransmitters in zebrafish brains will help us produce new medications to treat diseases of fear. Hopefully, these insights will help us find a cure for anxiety disorders.

ORIGINAL SOURCE ARTICLE

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CONFLICT OF INTEREST STATEMENT: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

YOUNG REVIEWERS

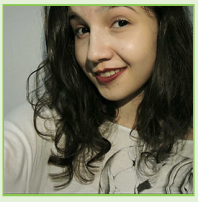
DARREN, AGE: 12

My name is Darren and I am 12 years old. My favorite subjects in school are math and science. I love basketball, especially the Golden State Warriors, traveling and playing video games with my little brother. I also enjoy playing with my dog Mindy.

JENAE, AGE: 12

My name is Jenae and I am 12 years old. My favorite subject in school is science. During my freetime I enjoy participating in sports and dancing. My favorite color is teal and pink, and I love pizza. I also enjoy spending time with family, traveling and playing with my little brother.

AUTHORS



MARYANA PEREIRA PYTERSON

I am a undergraduate student of Psychology at the Universidade Federal do Sul e Sudeste do Pará, currently studying the role of serotonin in panic disorder, using animal models, in the LaNeC (Neurosciences and Behavior Lab). I have a lot of interest in neuroscience, mainly neuropsychology, and I intend to follow this area as my profession. In my free time I like to play with my cats, read, and watch some movies.

PEDRO DE TARCIO GUEDES

I am a Biomedicine undergraduate student at the Universidade do Estado do Pará. I research in the area of neuropharmacology, seeking to elucidate the biological bases of posttraumatic stress disorder, emphasizing the participation of nitric oxide in this behavioral mechanism. I participate in in the research group of the Laboratory

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SAULO RIVERA IKEDA

I am a Biomedicine undergraduate student at the Universidade do Estado do Pará. Currently, I study the biological bases of post-traumatic stress disorder, focusing on the participation of nitric oxide in the consolidation of aversive memories in zebrafish as an animal model in the LaNeF (Laboratory of Neuropharmacology and Biophysics). In the future, I intend to investigate the participation of sleep and the circadian cycle in the formation of memories, especially those that give fear. When I am not in the lab, I watch movies and series or skating.

TAINÁ DIAS

I am studying Biomedicine as an undergraduate at the Universidade do Estado do Pará. Currently I do research on the Laboratory of Neuropharmacology and Biophysics (LaNeF), in which I seek to understand the biochemical bases of Post-Traumatic Stress Disorder (PTSD). My main interests are in neuroscience and everything around it, including specifically behavioral disorders like Post-Traumatic Stress Disorder and types of neuropathology like progressive supranuclear palsy.

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I am an undergraduate student of Biomedicine at the Universidade do Estado do Pará; since childhood I have always had many curiosities about science! Curiosities which I began to unveil when I joined the university in 2017. I am currently an undergrad researcher in the Laboratory of Neuropharmacology and Biophysics (LaNeF), in which I seek to understand the biochemical bases of Post-Traumatic Stress Disorder (PTSD). In my free time I like to walk around the campus and know a little about the line of research of my colleagues.

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I am an Associate Professor of Pharmacology at Universidade do Estado do Pará, in Brazil. I am interested in how the brain copes with stress, and how long-term changes in this capacity can lead to psychiatric disorders, such as post-traumatic stress disorder. When not in the lab, I like to hang out with my kid and engage in craftwork.

CAIO MAXIMINO

I am an Associate Professor of Anatomy and Physiology at the Universidade Federal do Sul e Sudeste do Pará, in Brazil. I want to know what the neural mechanisms of anxiety, fear and stress are, and how these relate to psychiatric disorders. In my spare time, I focus on making my research more accessible for the public, and like roleplaying games, hanging out with my wife and kid, politics, and to play rugby. *cmaximino@unifesspa.edu.br