NEUROSCIENCE
Published: 07 September 2018
doi:10.3389/frym.2018.00042

SHHHH… DANGER IS OUT THERE

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Imagine yourself having a conversation with a friend, and he starts yawning. You think, "My friend is getting bored!" Immediately, you try to be more enthusiastic or … you shorten the conversation. Now imagine a bird in the wild that suddenly hears an alarm call from another bird. What does he do? Most likely, his reaction will be to quickly escape to a safe place. These types of situations, where one animal (including humans) uses the behavior of others to guide his or her own behavior, are constantly happening in our daily lives. Importantly, these behaviors provide a number of advantages that are crucial for survival, like protection against threats. But how do the defense behaviors displayed by one individual who directly detects a threat influence the defense behaviors of others who are unaware of it? And what kind of signals are being used? This is exactly what we are tackling here! And we found out that an important cue is … silence!

INTRODUCTION

Imagine you are swimming in the sea on a warm sunny day, immersed in your thoughts, when suddenly a group of people starts screaming and swimming hurriedly toward the shore. What do you do? Quick! This requires a fast decision!

(A) Continue swimming in a relaxed way.
(B) Stay still, in the same position.
(C) Swim as fast as you can to the shore.
(D) Other—what?

What did you decide to do? And why? Let us take a guess. We predict that you interpreted the screaming and swimming behavior of the group as a sign of danger. And this triggered your own defense response which, most likely, was to start swimming as fast as you could to the shore. Correct?

When you first think about it, this could seem a hasty reaction, since you had no direct information that there actually was a threat in the water. So, why did you decide to swim quickly to shore? Imagine you had decided to continue swimming in the sea, when suddenly you noticed a prominent triangle sticking out of the water, coming straight toward you! Ah, a shark! If the screaming and swimming behavior of the group had not triggered a previous defense response, you would only have detected the shark once it was very close, putting your life in danger. Oh no!

This example shows that, in many situations, waiting until you actually experience the danger first hand can be risky. So, we can see that using information transmitted by others can be a clever strategy to avoid encounters with threats, like predators. Many animal species, from invertebrates (animals without backbones, like flies or crabs) to fish, birds, and mammals use a wide range of social cues to communicate the presence of dangers. These cues can include chemicals or sounds [1–3]. For instance, when fish are exposed to chemical alarm cues (like small pieces of skin) released from other fish that are injured, they display defense responses, such as increased use of a shelter or freezing in place [4]. When pigeons sense a threat and take off in flight, this elicits escape responses in other pigeons [2]. Similarly, the fear response of monkeys that are scared by the presence of a snake (like shaking the cage or screaming) can trigger fear in other monkeys as well [5].

Can you see how powerful this cycle of "social transmission" of fear can be? Let us go back to the first example, when the group of people detected the shark in the water and started screaming and swimming to escape the shark. You used these defense responses as a threat warning signal, triggering your own escape. And, consequently, your own response of swimming quickly to shore could have triggered a reaction in someone else nearby. Like the domino effect, the message about an imminent danger can be broadcast across the environment.

Now, the million-dollar question is: how do the defense behaviors displayed by someone that detects a threat influence the defense behaviors of an observer that has not detected the threat directly? What exactly is the information from the threat-detecting person that the observer is using? Could this information be visual or auditory? Or olfactory? Or tactile?

FROM THE WILD TO THE LAB

To answer these questions, we first needed to recreate this situation in the lab. We needed to expose an individual to an unpleasant event that triggered a defense response that could be detected by others (the observers). Then we could see if the detection of that defense response led to a defense response in the observer, just like the example with the shark.

To recreate this situation in the lab, we used a manipulation called fear conditioning, in which animals learn to predict unpleasant events. What does this mean? Do you know the story of Pavlov's dog, and how it learned to associate a sound or light with getting food? Pavlov was a Russian psychologist who was studying drooling in dogs. He developed a setup in which he would play a sound (or flash a light) for dogs before feeding them, and then he looked at how much the dogs drooled. Now, imagine that, after doing this for a while, Pavlov just played the sound without presenting the food—how do you think the dogs reacted? When Pavlov did this experiment, he discovered that dogs began to drool just by listening to the sound. Why? Because they learned that the sound always came before the food.

Fear conditioning uses the same type of manipulation, except the animals learn to predict unpleasant, instead of rewarding, events. The protocol consists of playing a sound, which is then immediately followed by an unpleasant stimulus—a mild electrical shock (Figure 1A, Step 1). After a set of sound-shock pairs, the sound itself elicits fear responses, because the animal becomes able to predict the shock (Figure 1A, Step 2). In our experiments we used rats, which are social animals that live in groups. This is important, because we wanted to study social transmission of fear between individuals in a group.

So, this was our first step: to condition rats to fear a tone (represented by *bip) after it had been paired with an unpleasant shock (represented by *bzzzt) to the foot (Figure 1A).

Now, which type of fear responses do rats display in this situation? When there is a threat, animals can display different types of defense behaviors such as flight (running away), fight, or freeze. However, when the animals are in a confined space with no way escape, most vertebrates (animals with backbones, like our rats) tend to stop moving. This is a defense response that might allow them to avoid being detected. If the animal does not move, it would not call attention to itself. Since, in our experiment, rats cannot escape from the box, that is exactly the defense behavior that we

FIGURE 1

A. For rats to learn how to associate a sound with a mild foot shock, we placed a rat in a box, and then played a tone (represented by *bip), followed by a mild foot shock (represented by *bzzzt). Once the rat felt the foot shock, it displayed a fear response—freezing (represented by *freeze…; Step 1). After a set of tone-shock pairs, we played just the sound to the rat, without the shock, and we saw that rats froze after only hearing the sound (Step 2). **B.** To study social transmission of fear, on Day 1 and 2 we put two rats both into the conditioning and social boxes, so they could get used to it. On Day 3, one of the rats experienced the tone and the shock, as described in **A.**—the demonstrator (D) rat. The other rat just experienced the foot shock—the observer (O) rat. On Day 4, we placed both rats together in the social box, and we looked at their behavior when we played the tone. **C.** When we played the sound (represented by *bip and respective dashed line), the percentage of freezing increased right after the sound for both the demonstrator (green) and observer rats (blue). What does the percentage of freezing mean? It tells us how much rats froze during a 15 s time window. So, a value of 100% says that, on average, rats froze the whole 15 s period, while 50% means that, on average, rats froze 7.5 s.

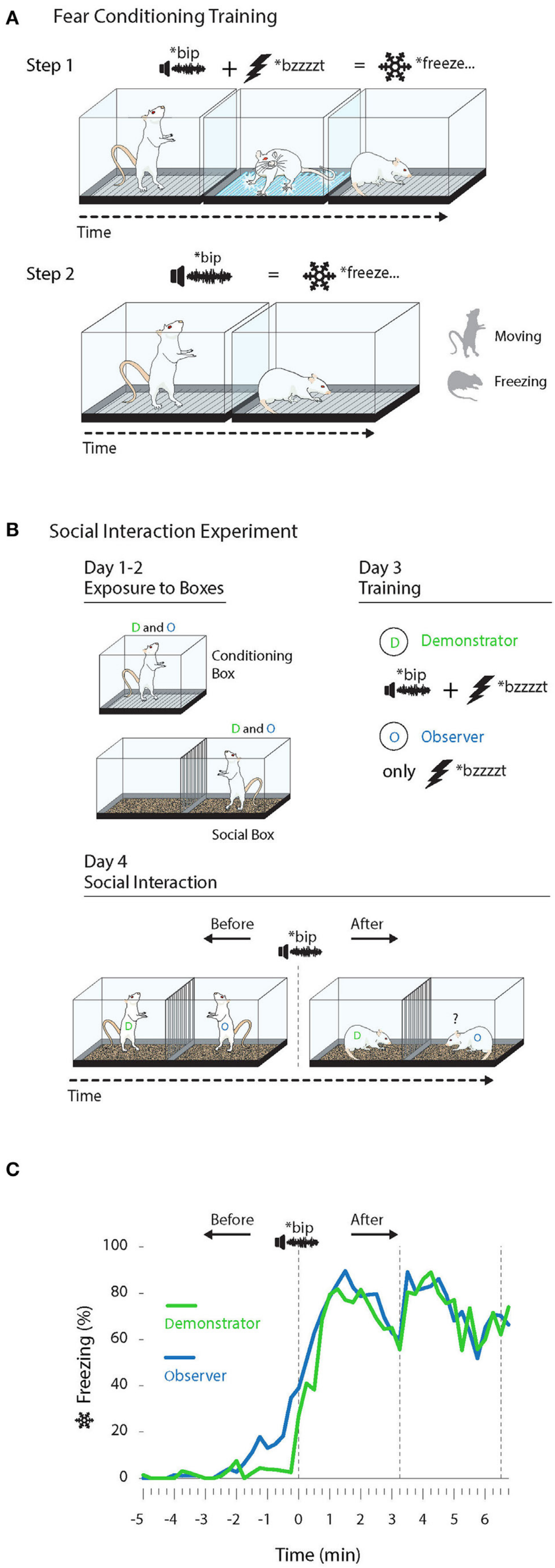

FIGURE 1

observed when the tone was played—rats became motionless (represented by *freeze...; Figure 1A, Step 2).

Looks like we are ready to tackle our question: how does the freezing behavior displayed by rats that have learned to fear the tone, called the demonstrators, influence the behaviors of other rats for whom this sound does not mean anything, the observers?

I FREEZE. DO YOU FREEZE?

To study this question, we put two rats together in a box, called the social box—one demonstrator and one observer. This box had a partition in the middle, separating the rats, but still allowing them to see, hear, smell, and touch each other (Figure 1B, Day 4 social interaction).

Ready to perform the experiment?

Let us just briefly recap: we wanted to look at the defense response of the observer rat while witnessing the defense behavior of the demonstrator, which has previously learned to fear the tone. For that, we placed the rats in the social box, and after a few minutes we played the tone. Next, we looked at the behavior of both the demonstrator and the observer rats (Figure 1B, Day 4 social interaction).

So? What happened? As expected, when the demonstrator rat was presented with the tone (represented by *bip), it froze (Figure 1C). What about the observer rat? How did it react? Once the demonstrator started freezing, the observer rat also displayed a defense response of its own, which was ... any guess? Yes! It also froze (Figure 1C).

Does this remind you of anything? These observations are similar to the example with the shark, where the group of people were the demonstrators, you were the observer, and the defense responses were screaming and swimming hurriedly. But wait! Here is an important catch: not all the observer rats froze—only the ones that previously experienced the foot shock themselves did. What does this mean? Imagine that you did not know that there are dangers in the ocean. Would you have reacted the same way to the crowd? This gives us important information: to be able to respond to the fear response of the demonstrator, observer rats need to have previously experienced the stimulus that caused the fear—the foot shock, in this case.

To summarize what we found, observer rats responded to the defense response of the demonstrator with a defense behavior of their own (freezing). But this only happens if the observer rats had previously experienced the fear-inducing stimulus.

So the next question we asked was, which sensory cues of the demonstrators could be triggering freezing in the observer rats? In other words, how is fear being transmitted from the demonstrator to the observer?

I FREEZE, YOU FREEZE. BUT HOW DO WE COMMUNICATE?

Could it be that the demonstrator is warning the observer through some kind of "verbal communication"—making some kind of sound that warns the observers? Previous work from other scientists showed that rats emit alarm calls in threatening situations, such as when they are in the presence of predators. These calls could potentially warn other rats of approaching threats; however, it is still not clear how important these vocalizations are in fear transmission. So, we decided to investigate this!

The alarm calls of rats cannot be heard by humans, since they are emitted in a frequency above the general range of the human ear. So, to "listen" to what rats were "telling" each other, we used two special microphones, one placed over each side of the social box. When we performed the experiment, we found that only one pair of rats, from the eight pairs tested, emitted distress calls. This shows that, in these conditions, the demonstrator rats do not need alarm calls to communicate fear to the observer rats.

So ... what else could be sending the message to the observer rats? Could freezing by itself be the cue?

Imagine you are in a room with someone else and suddenly that person stops moving. What type of information allows you to detect the transition from movement to immobility? Well, since you can see it, it is the visual information that allows you to detect that the other person stopped moving. So, if that is the case, what would happen if the lights were off?

When we performed the same experiment, but in the dark, we found that both demonstrators and observers *still* froze when the tone was played. In other words, visual cues were not necessary for the observer rats to respond to the fear of the demonstrator. Surprised?

Time for another quick summary: rats were not "talking" to each other, and visual cues were not crucial for the social transmission of fear. What else might be happening to transmit the fear signal, then?

Let us give you a hint. When someone stops moving, there is a transition between sound, caused by movement, and silence, due to freezing. When rats are in the social box, they normally move around, producing rustling sounds.

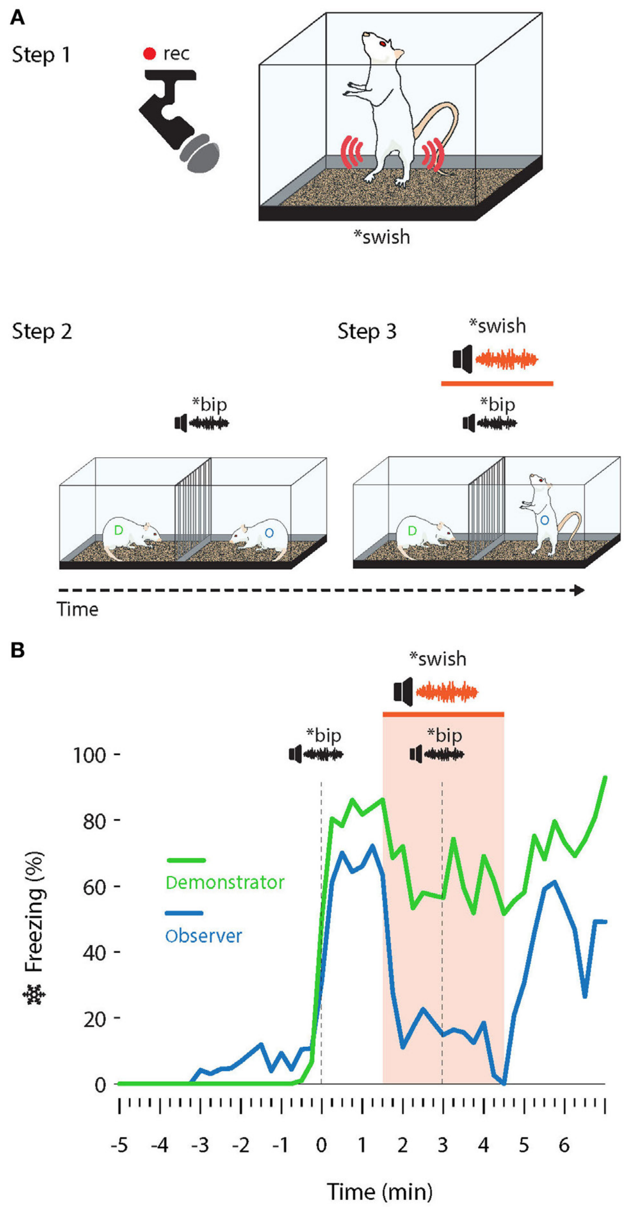

FIGURE 2

FIGURE 2

A. To test if the observer rats were using the change from sound to silence to detect freezing by the demonstrator rat, we did the following: Step 1: we recorded the sound from a rat exploring the box (represented by *swish); Step 2: we placed the demonstrator and observer rats in the social box and played the tone (represented by *bip); Step 3: after the tone, when the demonstrator and the observer were already freezing, we played the sound of the rat moving around (represented by *swish). **B.** The observer rats (blue) started freezing after the tone (*bip and respective dashed line), but when we played the sound of the rat moving (*swish and red bar), the percentage of freezing decreased. After the playback (after the red bar), the percentage of freezing increased again.

But when the demonstrator rat freezes, there is silence, since the sound disappears when there is no movement.

Could it be that the observer rats detected the demonstrator rats freezing because of the transition from the sound of movement to silence?

To test this hypothesis, we first recorded the sound of a rat exploring the box for a few minutes (Figure 2A, Step 1, represented by *swish). Then, we placed the demonstrator and observer rats in the social box and played the tone (Figure 2A, Step 2, represented by *bip). After the tone was played, when both the demonstrator and the observer were already freezing, we played the sound of the rat moving around (Figure 2A, Step 3, represented by *swish).

FIGURE 3

A. To investigate if the transition from sound to silence due to movement termination was sufficient to induce freezing, we placed an observer rat alone in the box and played the recorded sound of movement (represented by *swish), interspersed with moments of silence (represented by *silence). **B.** We observed that when the movement sound was on (*swish and red bars) the percentage of freezing was low. However, during the moments of silence (*silence and white bars), the percentage of freezing showed a big increase.

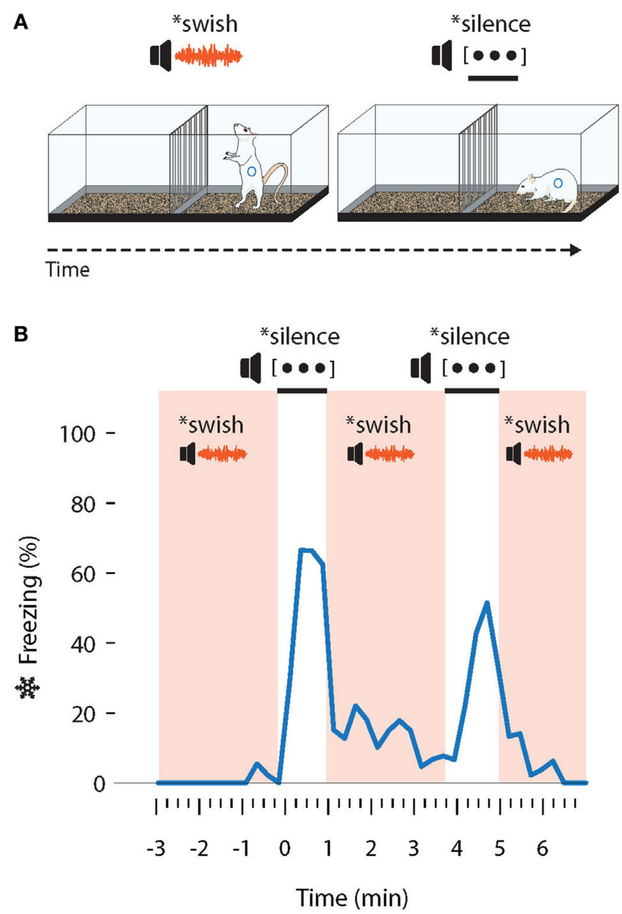

FIGURE 3

This experiment was done in the dark, so that observer rats could not see what the demonstrator was doing.

What happened? What would you expect? If silence was the cue that caused freezing in observers, then you would expect that playing the sound of the rat moving should prevent their freezing. And … that is exactly what happened! When we played the sound of a rat exploring the box (represented by *swish), the observer stopped freezing (Figure 2B, red bar). And importantly, freezing restarted immediately when the sound stopped playing—during silence (Figure 2B, after the red bar).

Aha! Interesting, right? This tells us that the observer rats seemed to be using the "sound" of silence to detect freezing by the demonstrator rat.

Finally, if this is indeed the case, we should be able to cause freezing in observer rats that are alone in the social box, just by simulating the changes in sound produced by the movement of another rat. So, we tried this experiment. First, we placed a rat alone in the social box and played the sound of another rat moving around (Figure 3A, represented by *swish). Then, we played the "sound" of silence, to mimic a rat freezing (Figure 3A, represented by *silence). And, "Eureka"! When we performed this experiment, we observed that rats froze during the moments of silence (Figure 3B, white bars). Cool, is not it? The onset of silence is enough to cause freezing in rats. Let us put the pieces together. Observer rats freeze in response to the display of freezing by demonstrators. And this social transmission of fear does not rely on alarm calls or on visual cues, but instead, on the transition from sound to silence, due to the lack of movement of the demonstrator rats.

NOW, BACK TO THE WILD: I FREEZE, YOU FREEZE, HE FREEZES

Why is this discovery important, especially if you are an animal in the wild?

Animals use auditory cues to detect the presence of a predator through the behavior of other individuals, e.g., alarm calls [3]. However, a drawback of this kind of auditory signal is that, when an alarm call is emitted, the animal sounding the alarm is protecting others but also calling attention to itself. Freezing, on the other hand, is a behavior that animals already use to defend themselves, to avoid being detected, but—as we show here—freezing can also serve as a cue to warn other animals of an approaching threat. Since most vertebrates freeze when threatened, this behavior is a cue that can spread very quickly in an environment. Also, this is not just true for rats—the other animal could be a mouse or a dog. Silence could be used to rapidly spread the news of danger between different kinds of animals.

So, the take-home message of our study is this: be cautious when there is a sudden silence. Shhhh…

ORIGINAL SOURCE ARTICLE

Pereira, A. G., Cruz, A., Lima, S. Q., and Moita, M. A. 2012. Silence resulting from the cessation of movement signals danger. *Curr. Biol*. 22(16):627–8. doi:10.1016/j.cub.2012.06.015

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SUBMITTED: 20 December 2017; **ACCEPTED:** 10 August 2018;
PUBLISHED ONLINE: 07 September 2018.

EDITED BY: Lauren Jantzie, University of New Mexico, United States

CITATION: Vicente MI, Pereira AG, Gomes RA, Costa GM, Moita MA and Ramos C (2018) Shhhh… Danger Is Out There. Front. Young Minds 6:42. doi:10.3389/frym.2018.00042

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.

REVIEWED BY

SAMANTHA, AGE: 12

My name is Samantha and I am in 7th grade. My favorite pastime is reading. I really enjoy going outside and riding my bike. I love to play with my dogs and my siblings, go on hikes, correct people's grammar, and be with my family. My favorite subjects are Spanish, Language Arts, and Science.

AUTHORS

MARIA I. VICENTE

During my PhD, I studied how rats make decisions, which proved to be a super exciting and insightful experience in my life. However, I soon realized that what I really enjoyed was taking the scientific process outside the lab and to show non-scientists how to think about science in their daily lives. And that is exactly what I am doing right now—bringing science closer to society, through the development of scientific educational projects.
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ANA G. PEREIRA

I am a postdoc at Harvard University in Boston, USA. Currently, I am studying the mechanism by which the food preferences of mothers can be transmitted to their babies, and throughout my PhD I investigated the role of auditory cues during social transmission of fear—which is actually the focus of this paper. During this time, I also worked with several high school students, introducing them to the fascinating organ that is our brain and how it drives behavior. During my free time, I like to dance, do yoga, and have a good meal with my friends. And read ... books are my secret addiction.

RAQUEL A. GOMES

I am an educator with a love for science. I am a science education officer at Champalimaud Centre for the Unknown in Lisbon, Portugal. I have a Microbiology degree and a PhD in Neurobiology. In my research, I studied the details of how the nervous system forms during embryonic development. I have also taught high school and college in the US. One of my passions is to think about and design creative ways to share scientific knowledge about the inner workings of living organisms with young people. My other passions are collecting orchids and other unique plants, doing ceramics, and traveling to new places.

GIL M. COSTA

I was born in Sintra, Portugal, and have a Biology degree and a PhD in Neuroscience. My PhD research was done at the Champalimaud Research, in Lisbon, and was focused on the topic of decision making, more specifically on decisions about odors and estimation of confidence in our own decisions. Parallel to my scientific work, I developed skills in graphic design—in 2005, I started creating posters for theater plays. I found my passion in design and currently design for scientists full-time, creating materials for both public outreach and communication amongst scientists.

MARTA A. MOITA

I am a group leader at Champalimaud Research in Lisbon, which means that I am the scientist that coordinates a team of researchers interested in understanding how the brain controls defensive responses to threats, and how the social environment regulates behavior. To study these issues, we use rats and fruit flies, because we know a lot about their nervous systems and we have tools to manipulate or measure the activity of their brains. One of the things that I like the most about this job is thinking together with other people.

CATARINA RAMOS

I am a science communication officer at the Champalimaud Centre for the Unknown in Lisbon, Portugal. My first real lab experience was with yeast in San Diego and, from that moment onward, I knew I wanted to work in science. As a PhD student, I studied a neurodegenerative disorder called Huntington's Disease in Milan, Italy. After that I returned to Lisbon, where I joined a lab dedicated to neurobiology. I was always very enthusiastic about sharing the wonders of science with the public and now I get to do this every single day.