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Backstory

Unraveling the motivational secrets of honey bee foraging during the COVID pandemic

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Honeybees are unique among insects because they deploy impressive learning and memory capabilities during their foraging activities and rely on a sophisticated social organization that includes specialized castes, division of labor, and elaborated communication codes. Their foraging behavior is at the core of the millenary relationship linking them to humans, as we profit not only from products such as honey, pollen, propolis, and wax, but also from pollination services provided by bees to agriculture. Thus, characterizing the neural mechanisms that control the foraging motivation of honeybees represents a strategic goal both from a scientific and an economic perspective.

This ambitious goal can only be reached through the joint action of multiple disciplines tackling the problem from different perspectives. Therefore, we established a consortium of molecular biologists, electrophysiologists, behavioral biologists, electricians, specialists in brain imaging, and beekeepers to characterize the role of a neuropeptide — a protein made of few amino acids and released by neurons to modulate different physiological functions — in the regulation of appetitive responses of honeybees.

In our recent iScience article (Bestea et al., 2022a), we aimed at studying the role of a neuropeptide termed short neuropeptide F (sNPF) in the responses of bees to food (sucrose solution in our experiments) and food-related stimuli (flower odorants, for instance). Interestingly, sNPF is an insect "pendant" of another neuropeptide that can be found in vertebrates, the neuropeptide Y, which regulates responses to food and to stress. Research by **Gabriela de Brito Sanchez**, an electrophysiologist interested in sensory receptors and based at the University of Toulouse, and **Rodrigo Velarde**, a Bolivian molecular biologist based at SOLATINA, a Latin-American research consortium working on bees, had identified this neuropeptide as a potential regulator of bee colony activity. However, no experiments had been performed to address the question of its effect at the level of individual bees. Thus, a research program was conceived by Gabriela to investigate if sNPF controls the feeding decisions made by a foraging bee confronted with food and food-related stimuli. To this end, it was critical to manipulate sNPF levels of individual bees to study if this variation would induce significant changes in the bees' food and stress responses.

A MULTIFACETED RESEARCH GROUP

Along with Gabriela and Rodrigo, we assembled a team of researchers from different backgrounds to carry out our research on sNPF (Figure 1). Firstly, we had to wait for the arrival of a talented young PhD student, **Louise Bestea**, to perform the series of experiments that are the basis of our iScience article, and that relied on this manipulation of sNPF levels.

To design the experiments that addressed the question of the possible effects of sNPF on responses to stressful stimuli (besides food stimuli), an electrician, **Patrick Arrufat** helped conceive the setups, which allowed stimulating the bees with mild electric and thermal shocks. Patrick was incredibly helpful, as he could really think in terms of stimulus effectiveness (conductance, resistance, etc.) for a single bee. Finally, after showing that responses to flower odorants increased upon sNPF elevation, we decided to invite **Marco Paoli** and **Brice Ronsin**, specialists in biphoton imaging, who demonstrated that the observed behavioral effects had a clear neural correlate: olfactory neurons in the bee brain increased their response upon sNPF elevation.

Another person outside the academic world was also important for this work: **Olivier Fernandez**, a professional beekeeper, provided invaluable help to keep our bees and ensured the use of high-quality individuals for our experiments. Speaking with Olivier was particularly exciting as bee scientists and

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Figure 1. Members of the project team
Clockwise from top left – Beekeeper Olivier Fernandez (Occitanie Beekeeper Union); honeybee olfaction specialists Julie Carcaud and JC Sandoz (CNRS, Gif-sur Yvette); molecular biologist Rodrigo Velarde (SOLATINA), electrician Patrick Arrufat (University Paul Sabatier); study team at the Center of Integrative Biology; Marco Paoli, Gabriela de Brito Sanchez, Martin Giurfa, Brice Ronsin, and Louise Bestea (University Paul Sabatier).

beekeepers share the same passion for bees: while scientists test hypotheses, beekeepers are fantastic observers of bee behavior and provide scientists with fascinating facts that may be at the origin of innovative experiments.

"bee scientists and beekeepers share the same passion for bees: while scientists test hypotheses, beekeepers are fantastic observers of bee behavior"

With this in mind, Gabriela and Rodrigo conceived the method of topical application of sNPF and obtained a series of results showing that delivering the neuropeptide via a solvent applied on the thorax of a bee caused important changes in its feeding behavior, consistently with an internal increase of sNPF levels: bees ate more, even if they were satiated. Louise enjoyed testing the bees in many behavioral experiments presenting different sorts of stimuli as much as she enjoys drawing them with an incredible talent. At that time, we had established a research consortium with our friends and partners in Gif-sur-Yvette, **Julie Carcaud** and **Jean-Christophe Sandoz,** both specialists in honeybee olfaction but had never addressed the topic of neuromodulation of bee behavior. To them, the question of whether sNPF would affect odor processing in bees' brain was very exciting. The group managed to create a lively and stimulating discussion atmosphere to imagine how sNPF may affect the foraging behavior of bees. These discussions — and this has to be underlined — occurred mostly during periods in which we all were confined at home because of the COVID-19 pandemic. However, using videoconferences to keep a fluid communication provided a way to keep the project alive, moral high, and a stimulated intellect.

WORKING TOGETHER

The consortium was a lively group that elaborated constantly on the hypotheses and conceived multiple experiments; everyone contributed from their own perspective, thus underlining the richness of

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having convergent expertise onto a common project. Importantly, the constraints imposed by the COVID-19 pandemic certainly affected the possibility of moving forward, but we tried to be creative and imagine ways to overcome isolation and project cessation. Videoconferences were done regularly to bind the team, in particular with Rodrigo, the distant member of the group based in Bolivia. Louise got a special permission granted to PhD students who had to finalize their theses to work in the laboratory, even if successive lockdowns were in place. Her courage in these circumstances, a time when majority of the French citizens were confined to their homes, needs to be acknowledged.

In between lockdowns, we managed to meet in Gif-sur Yvette, close to Paris, to boost the spirit of the team and give a new energy to the project despite the difficult context, but this fleeting experience was followed by new lockdowns and restrictions. Despite this, the project could be achieved and Louise defended her PhD thesis with honors in December 2021.

''In between lockdowns, we managed to meet … to boost the spirit of the team … this fleeting experience was followed by new lockdowns''

At the end, we had demonstrated the fundamental role of sNPF for driving appetitive responses of bees to food, yet being dispensable for responses to stressful stimuli. Critically, we showed that sNPF is so important to determine a bee's foraging motivation that elevating it in a semi-satiated individual turned it into the equivalent of a starved bee. This effect is accompanied by an increase of activity in neural circuits processing food-related information such as the olfactory circuit in the bee brain. In addition, another work by our team confirmed the critical role of sNPF in appetitive motivation, as elevating its levels improved visual learning and memory of free-flying bees rewarded with sucrose solution for discriminating colors in a maze (Bestea et al., 2022b).

PLANS FOR THE FUTURE

Overall, these works uncovered a fundamental component of honeybee foraging motivation: when bees leave the hive searching for food, sNPF levels are high; on the contrary, feeding decreases these levels. Hungry colonies have higher sNPF levels, and their foragers are more efficient at learning and memorizing food-related signals.

These results have a significant potential for apicultural activities. However, we are still far from being able to change the sNPF levels of an entire colony; moreover, investigating the additional physiological consequences that such a variation may induce is mandatory before engaging in the development of applications.

Exchanges with beekeepers should be continued to this end, not only because of their obvious interest for bee research, but also because their commitment with honeybees may be the source for scientific inspiration. Designing experiments at a colony level may benefit from their expertise and skills, which scientists do not have. Interactions between scientists and beekeepers should thus be seen as a two-way communication channel in which both partners obtain significant benefits.

Further questions are yet to be answered and will be the basis for future projects. For instance, considering that bees do not forage to satisfy individual hunger but to satisfy colony needs, what are the social signals that trigger sNPF elevation, and how do they lead to sNPF increase in the nervous system of bees? To answer these questions, the consortium plans a new series of experiments with the same enthusiasm. The world of bee neuropeptides and their influence on their behavior is a box filled with mysteries that awaits to be solved.

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