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CRISPR-Cas9 Knockdown of Octopamine Beta Receptor Subtype 2 in honey bee brains

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ABSTRACT

To understand the role of octopamine neurotransmitter in energy metabolism, we knocked down the octopamine beta receptor subtype 2 using CRISPR-Cas9 that was injected into the brain of forager honey bees. We measured their appetite using the Proboscis Extension Response assay and found decreased appetite levels in starved honey bees three days after injection. The effectiveness of using the baculovirus vector for delivery of CRISPR-Cas9 throughout the brain was confirmed using confocal imaging.

BACKGROUND INFORMATION

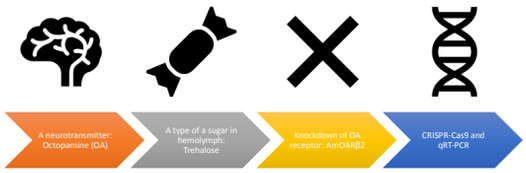

Figure 1. Overview of the background

Forager honey bees rely on their energy metabolism during their activity. Trehalose is the main sugar in the hemolymph of the honey bees, so it is evaluated as an indicator of honey bees' energetic state. Low levels of trehalose correspond to high levels of Octopamine (OA) which is a neurotransmitter in the insects. In Figure 1, main components of the appetite regulation and methodology of the project are demonstrated.

OBJECTIVE

To induce baculoviral transfection in neurons and knockdown octopamine beta receptor subtype 2

METHODS

Honey bee brain injections of CRISPR, GFP and PBS

Behavior test: Proboscis Extension Response (PER)

Brain imaging to map AmOctβ2 and identify infection regions of the baculovirus vector

qRT-PCR analysis to determine genetic alterations

Under dissecting microscope, we performed brain injections which is visualized in Figure 2. To deliver CRISPR system, the baculovirus vector which is schematized in Figure 3 is used. This vector contains Cas9, sgRNA targeting OA and EGFP (Enhanced Green Fluorescent Protein) as a reporter gene. EGFP containing plasmid and PBS (Phosphate-buffered saline) were performed as the control treatments.

We have collected Proboscis Extension Response scores (PER) for different treatment groups. In this assay, gradually increasing sugar solutions are used to score the appetite of honey bees.

We imaged the brain of a CRISPR injected bee 3 days post injection (DPI). Using confocal microscopy, we obtained signals belonging to GFP as high as DAPI and Phalloidin dyes.

Figure 2. Honey bee brain injections

Figure 4. PER assay

Figure 3. pAC-sgRNA-Cas9 plasmid containing CRISPR system to target OA

RESULTS

Treatments/DPI	1	2	3
CRISPR	3.214	2.141	0.927
GFP	3.575	1.500	2.333
PBS	4.000	1.946	2.000

Table 1. GRS values of the honey bees belonging to 3 different treatment groups

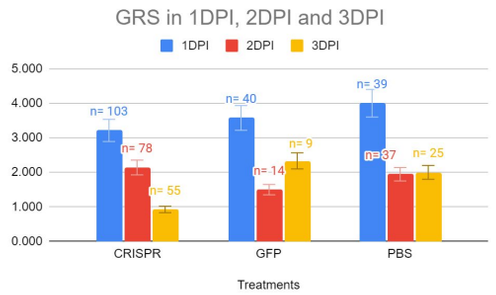

Figure 6. GRS values of the honey bees belonging to 3 different treatment groups

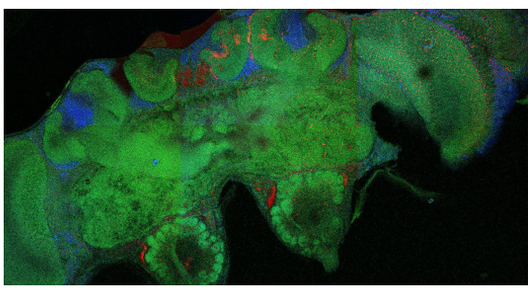

Figure 5. Confocal images of honey bee brain image under 10X magnification and 4x4 Tile Scan. 50 µm of brain sections were stained with DAPI (blue) amd phalloidin (red). Blue, red and green represents cell nuclei, f-actin and baculovirus transfected reigons, respectively.

After the injections of CRISPR, GFP and PBS in the honey bee brains, we measured the appetite levels of the honey bees in the following 3 days after injection. Proboscis Extension Response scores which are presented in the Figure 6 suggested decreased appetite levels in starved CRISPR-injected honey bees three days post-injection. Using confocal imaging the effectiveness of using the baculovirus vector for delivering CRISPR-Cas9 throughout the brain was confirmed. Especially, Calyces and Antennal Lobes have more intense GFP signals compared to surrounding regions which is demonstrated in the Figure 5

FUTURE DIRECTIONS

These data suggest that baculovirus has an impact in appetite regulation. To validate the results obtained at the behavioral level, qRT-PCR analysis is being performed. After completing the 2^-**ΔΔ** (Delta delta Ct) analysis for all samples, Kruskal Wallis test will be performed in R, to test correlation between behavioral data and gene expression analysis.

ACKNOWLEDGEMENT

This research was funded by The Scientific and Technological Research Institution of Turkey (TUBITAK), grant number: 119Z251. We would like to thank undergraduate students Doğa Tuncer and Emre Günaydın for their help with the sample collection and brain injections, Can Sontepe, Faruk Kalyoncu, Gizem Şahin, İlknur Bahçıvan and Zeynep Orhan for their help with RNA-isolations and RNA quality checks.

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