Title: Quantitative Analysis of Antennal Movements Triggering Trophallactic Behavior in Honeybees Using DeepLabCut, a Deep Learning-Based Markerless Pose-Tracking Tool

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Honeybees exhibit trophallaxis, a behavior in which a recipient extends their proboscis to drink a nectar droplet held by the donor's mouthparts. During this process, the recipient's antennae are frequently observed tapping the donor's mandibles. However, analyzing these antennal movements (AMs) is technically challenging because of their high speed and complex overlapping motion. Consequently, the functional roles of AMs in trophallaxis remain unclear. Here, we show that specific AMs in recipients trigger trophallaxis, based on quantitative motion analyses using DeepLabCut, a deep learning-based markerless pose-tracking tool, combined with behavioral assays. By tracking AMs during trophallaxis between harnessed bees using DeepLabCut, we successfully detected antennal orientation and horizontal vibration frequency in both recipients and donors. The recipient's antennae were oriented toward the donor before proboscis extension and exhibited rapid vibrations exceeding 50 Hz during trophallaxis, whereas no specific AMs were detected in donors. To test causality, when the antennae of either live bees or dead bees with immovable antennae were alternately brought into contact with a donor's head, the proportion of droplet offerings toward live bees was significantly higher than that directed toward dead bees. These results indicate that mechanical stimulation generated by recipients' antennal vibration triggers droplet offering by donors. Thus, honeybee antennae function not only as sensory receptors but also as effectors that emit mechanical signals. Simulating trophallaxis-triggering mechanisms based on vibrational stimulus intensity may inform swarm robotics, such as by enabling automated energy transfer between robotic agents and mitigating communication interference.