Honeybees flying over a mirror crash irremediably

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In altitude control in honeybees, the visual inputs encountered in a tunnel mainly consist of an optic-flow pattern generated by the textures and by the edges shaping the tunnel perspective. Flying honeybees *Apis Mellifera* are known to be particularly sensitive to the optic-flow pattern generated by the contrasting features of the tunnel to adjust their altitude (Baird et al., *LNAI*, 2006; Portelli et al., *J. Comp. Physiol. A*, 2010; Srinivasan, *Physiol. Rev.*, 2011; Portelli et al., *Sci. Rep.*, 2017). Recently, honeybees were trained to follow the tunnel ceiling while encountering a "dorsal ditch" in the tunnel configuration midways (Portelli et al., *Sci. Rep.*, 2017). Honeybees coped to this new tunnel's configuration by rising quickly and hugging the new, higher ceiling, by keeping a similar forward speed, similar distance to the ceiling, and similar dorsal optic flow to those observed during the training step. Conversely honeybees trained to follow the floor kept on following the floor regardless of the change in the ceiling height (Portelli et al., *Sci. Rep.*, 2017).

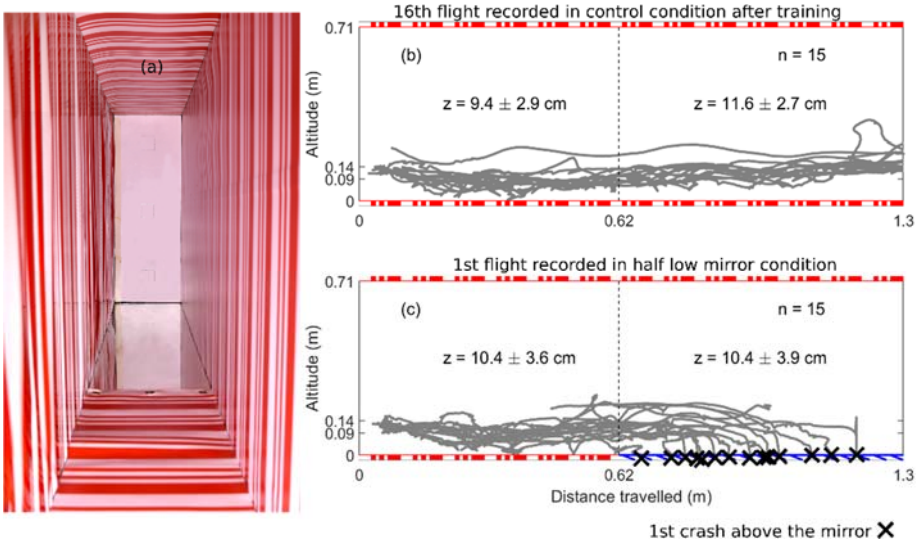

Figure 1. (a) Flight tunnel: 25x71x200 cm (width x height x length) with a mirror covering the second part of the floor. (b) Trajectories of a group of 15 bees trained to collect a sugar reward at the tunnel end. The 16th trial is captured with a camera recorder filming the 1.3 first meter at 100 Hz (c) Recordings of the first trial in half low mirror condition showing that honeybees repeatedly crash themselves at a distance travelled of 91± 15cm.

The present study aims at pursuing investigating the role of dorsal and ventral visual inputs in the honeybees' control of altitude by quantitatively reproducing the seminal experiment of Heran & Lindauer (1963). In such an experiment, they trained honeybees to fly above a water surface. When the water surface was rippled or when a floating bridge provided a visual contrast, honeybees were able to cross the lake. However, honeybees crossing mirror-smooth water during foraging trips flew lower and lower until crashing head first into the water (Heran and Lindauer, *Zeitschrift für vergleichende Physiologie*, 1963). To replicate experimentally such a behavior in a flight tunnel, we used a mirror placed on the floor covered with textures during the training session (Fig. 1a, see video https://youtu.be/KH9z8eqOBbU for experimental procedure). In a first session, honeybees were trained to follow the ground during 16 trials (Fig. 1b). In a second session, the half low mirror was uncovered (Fig. 1a), creating a virtual "ventral ditch", it was observed reputedly crashes on the mirror below (Fig. 1c). We conclude that ventral visual inputs are crucial for altitude control in honeybees (Fig. 1a). Further experiments will be introduced in various optical context.