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Automated high-throughput individual tracking system for insect behavior: Applications on memory retention in parasitic wasps

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Highlights
- Automated simultaneous video tracking of individual insect choice behavior
- Generating various behavioral parameters relevant for memory retention testing
- Simultaneous testing in 36 olfactory T-mazes in a plate of only 18x18 cm
- Standardized method suitable for behavioral comparisons of different species

Abstract

Background: Insects are important models to study learning and memory formation in both an ecological and neuroscience context due to their small size, behavioral flexibility and ecological diversity. Measuring memory retention is often done through simple time-consuming set-ups, producing only a single parameter for conditioned behavior. We wished to obtain higher sample sizes with fewer individuals to measure olfactory memory retention more efficiently.
New method: The high-throughput individual T-maze uses commercially available tracking software, Ethovision XT®, in combination with a Perspex stack of plates as small as 18x18 cm, which accommodates 36 olfactory T-mazes, where each individual wasp could choose between two artificial odors. Various behavioral parameters, relevant to memory retention, were acquired in this set-up; first choice, residence time, giving up time and zone entries. From these parameters a performance index was calculated as a measure of memory retention. Groups of 36 wasps were simultaneously tested within minutes, resulting in efficient acquisition of sufficiently high sample sizes.
Results: This system was tested with two very different parasitic wasp species, the larval parasitoid *Cotesia glomerata* and the pupal parasitoid *Nasonia vitripennis*, and has proven to be highly suitable for testing memory retention in both these species.
Comparison with Existing Methods: Unlike other bioassays, this system allows for both high-throughput and recording of detailed individual behavior.
Conclusions: The high-throughput individual T-maze provides us with a standardized high-throughput, labor-efficient and cost-effective method to test various kinds of behavior, offering excellent opportunities for comparative studies of various aspects of insect behavior.

Keywords: learning, memory retention, parasitic wasps, *Cotesia glomerata*, *Nasonia vitripennis*, tracking system

1. Introduction

Learning and memory formation are universal traits in the Animal Kingdom (Dubnau 2003), which makes it possible to study them in a wide range of animal species with varying levels of brain complexity, including insects, such as fruit flies (*Drosophila melanogaster*), bees (*Apis melifera*) and parasitic wasps (Chen and Tonegawa 1997; Galizia et al. 2011; Margulies et al. 2005; Smid et al. 2007). For ecological and neuroscience studies insects are ideal models due to their small size, behavioral flexibility and enormous ecological diversity.

Memory retention is an important parameter in studies of learning and memory formation, and it is generally assessed by measuring conditioned behavior. Many different bioassays have been used to study memory retention in insects such as the proboscis extension reflex (Bitterman et al. 1983), the two-choice wind tunnel (Geervliet et al. 1998b), the Y-tube olfactometer (Wäckers 1994), the static two-chamber olfactometer (Huigens et al. 2009), the four-quadrant olfactometer (Vet et al. 1983), the locomotion compensator (servosphere) (Vet and Papaj 1992) and the T-maze olfactometer (Hoedjes et al. 2012; Jiang et al. 2016). These bioassays measure memory retention through conditioned behavior in different ways and each has its own strengths and weaknesses. The two-choice wind tunnel, the four-quadrant olfactometer and servosphere bioassays allow for detailed recording of biologically relevant behavioral responses of individual insects, but are time consuming. Wind tunnels also require expensive equipment and ample space. The T-maze olfactometer is used with groups of insects, which is more time efficient, but data points