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## Introduction

Learning visual cues is essential in bees for orientation, as well as for the recognition of nest sites, food sources and mating partners. While research on visual learning in social bees was primarily conducted with free-flying individuals, associative conditioning of visual stimuli using the proboscis extension response (PER) assay in intact bees has just recently been established.<sup>1,2,3</sup> Moreover, research using the PER method has primarily focused on workers, whereas males were

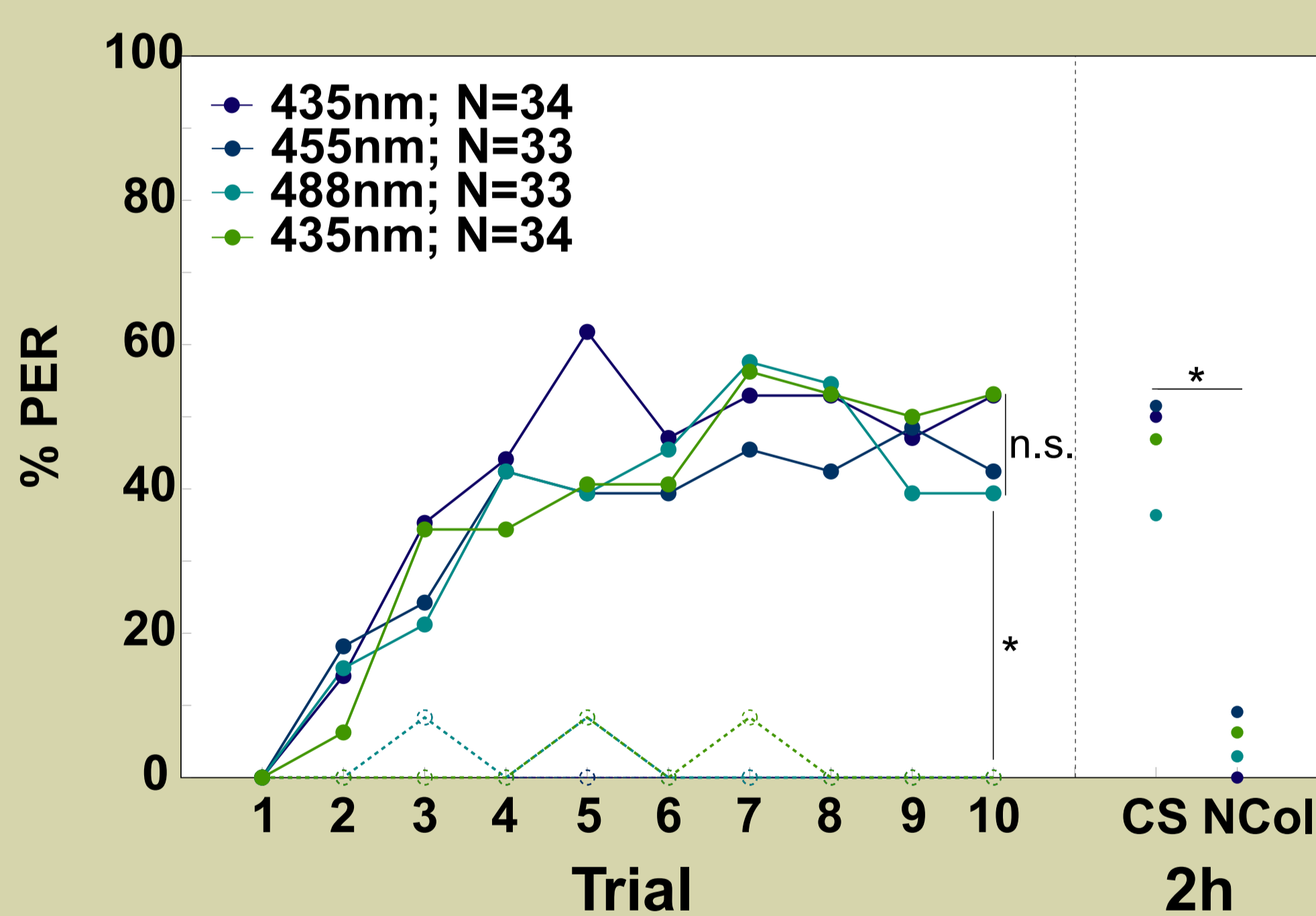
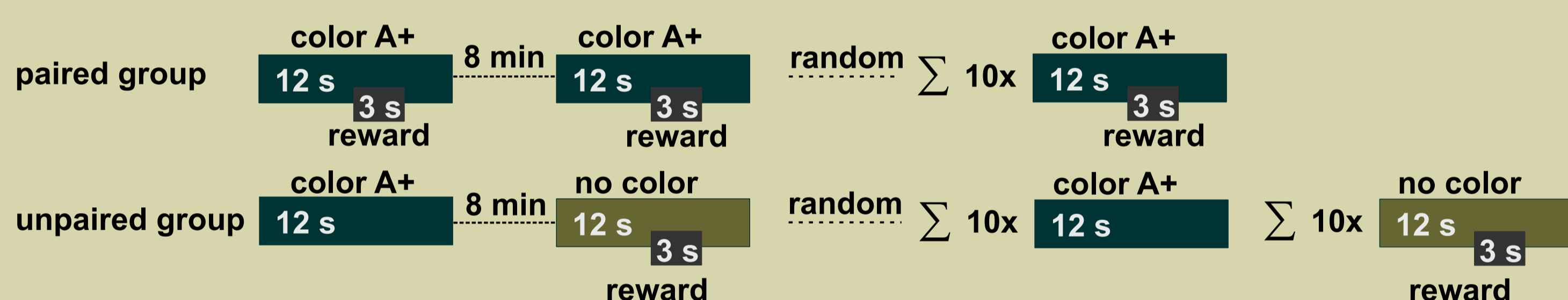
largely neglected. Here we report color learning experiments with *Bombus terrestris* drones. Individuals were confronted with absolute (A+) and differential (A+ vs. B-) conditioning tasks using four different light stimuli (Fig. 1B) in combination with two different grey filters (Fig. 1C) to ensure that the bumblebees must use chromatic information instead of achromatic qualities like light intensities. Furthermore, bumblebees were tested for memory retrieval two hours after conditioning.

## Results

### 1. Absolute color conditioning

To examine, whether bumblebee drones are able to associatively learn a color stimulus as a predictor of a sugar reward, individuals were trained to light stimuli of particular wavelengths (435, 455, 488 and 528 nm; Fig. 1A) in an absolute conditioning paradigm. No differences were found between different color stimuli, neither after a paired presentation of the CS and US, nor after an unpaired pre-

sentation. The paired groups of drones reached a learning performance of about 40-60% and, as expected, differed significantly from the unpaired groups. Moreover, drones were able to retrieve the learned association after two hours and exhibited responses virtually exclusively to the learned CS and not to a novel color (NCol) (Fig. 2).

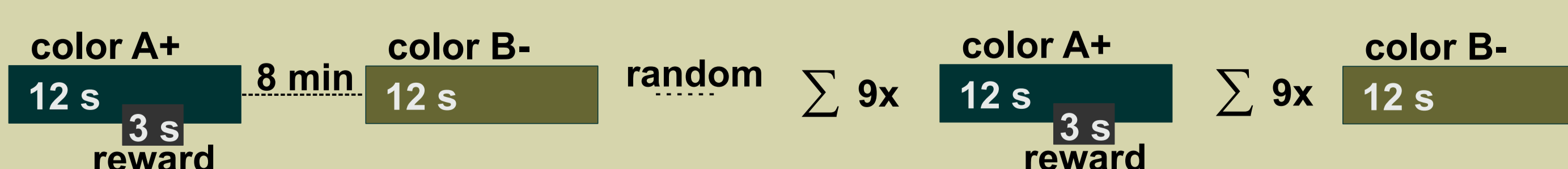


**Fig. 2** Results of the absolute PER color conditioning of drones. Bumblebees were trained over 10 trials and tested for memory retrieval after 2h for the conditioned stimulus (CS) and a novel color (NCol); N=12 for each unpaired group; \* p<0.05; n.s. = no significant difference

### 2. Differential color conditioning

To study if drones can learn to discriminate light stimuli of different wavelengths, individuals were trained in a differential conditioning paradigm. Three different monochromatic light stimuli pairs (435/528, 435/488, 435/455 nm) were presented. To ensure that the bumblebees distinguish the stimuli on the basis of chromatic information but not on achromatic

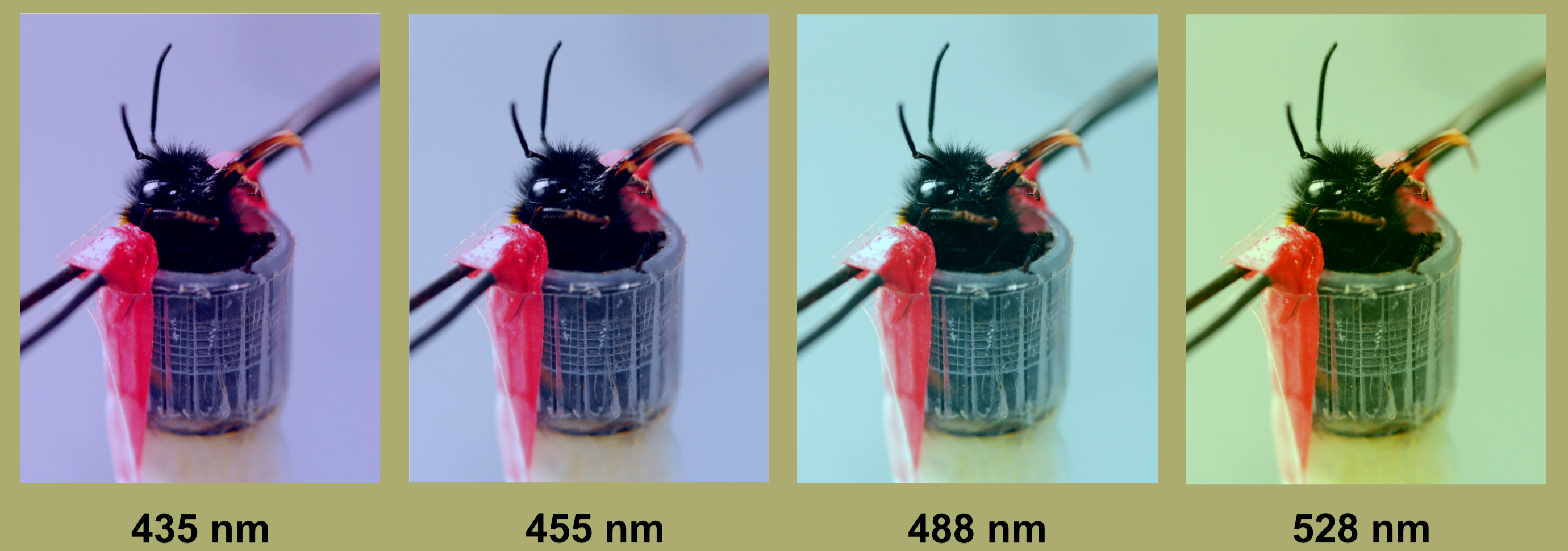
qualities (e.g. differences in light intensity), each light stimulus was either presented alone, or in combination with grey filters with 51% and 13% transmission, respectively (Fig.1E). Drones performed well in discriminating both color stimuli (if the wavelength difference was  $\geq 20$  nm) and were able to retrieve the information two hours after conditioning (Fig. 3).



## References

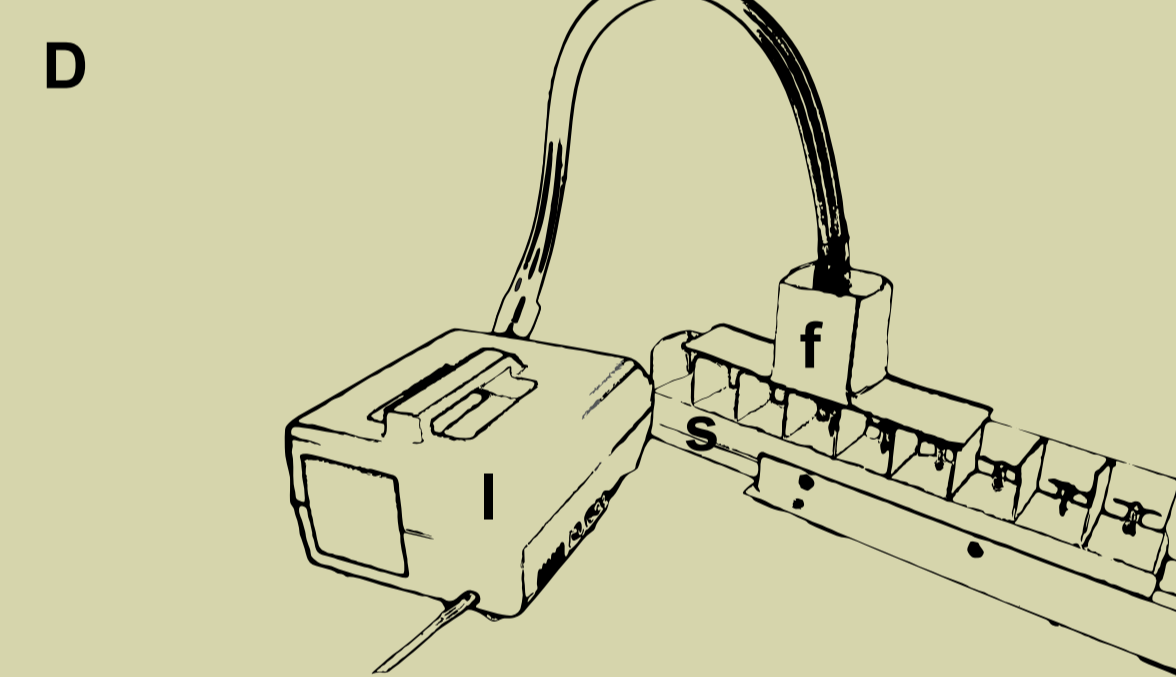
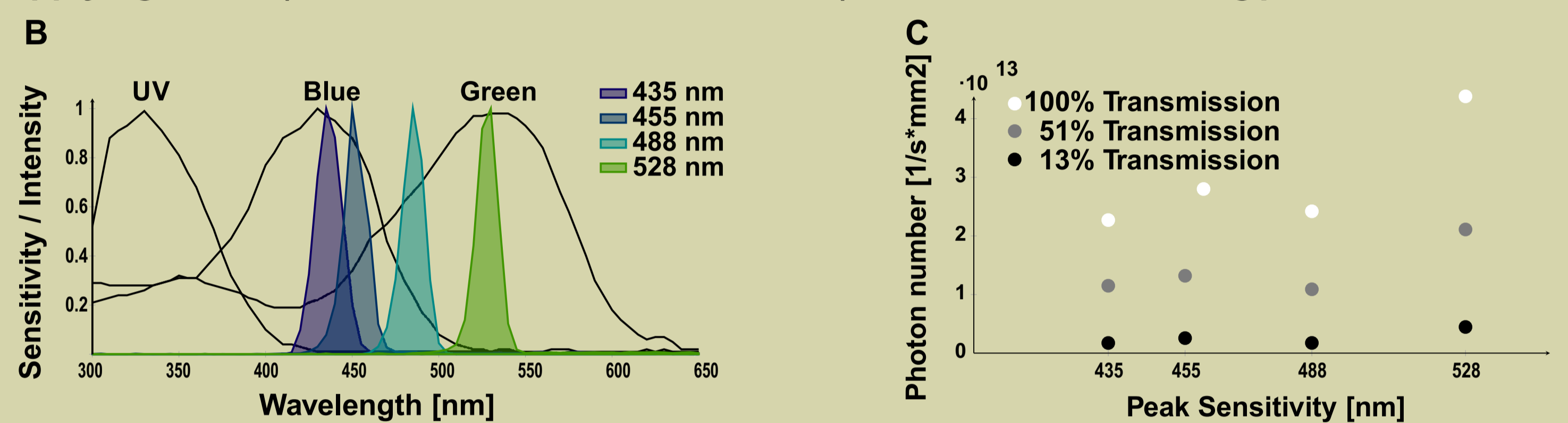
- <sup>1</sup>Dobrin, S. E. and Fahrbach, S. E. (2012). Visual associative learning in restrained honey bees with intact antennae. PLoS ONE 7(6): e37666.  
<sup>2</sup>Riveros, A. J. and Gronenberg, W. (2012). Decision-making and associative color learning in harnessed bumblebees (*Bombus impatiens*). Animal Cognition, pages 1-11.  
<sup>3</sup>Jeringan et al. (2014). Color-dependent learning in restrained Africanized honey bees. The Journal of Experimental Biology, 217, pages 337-343

**Fig. 1A**

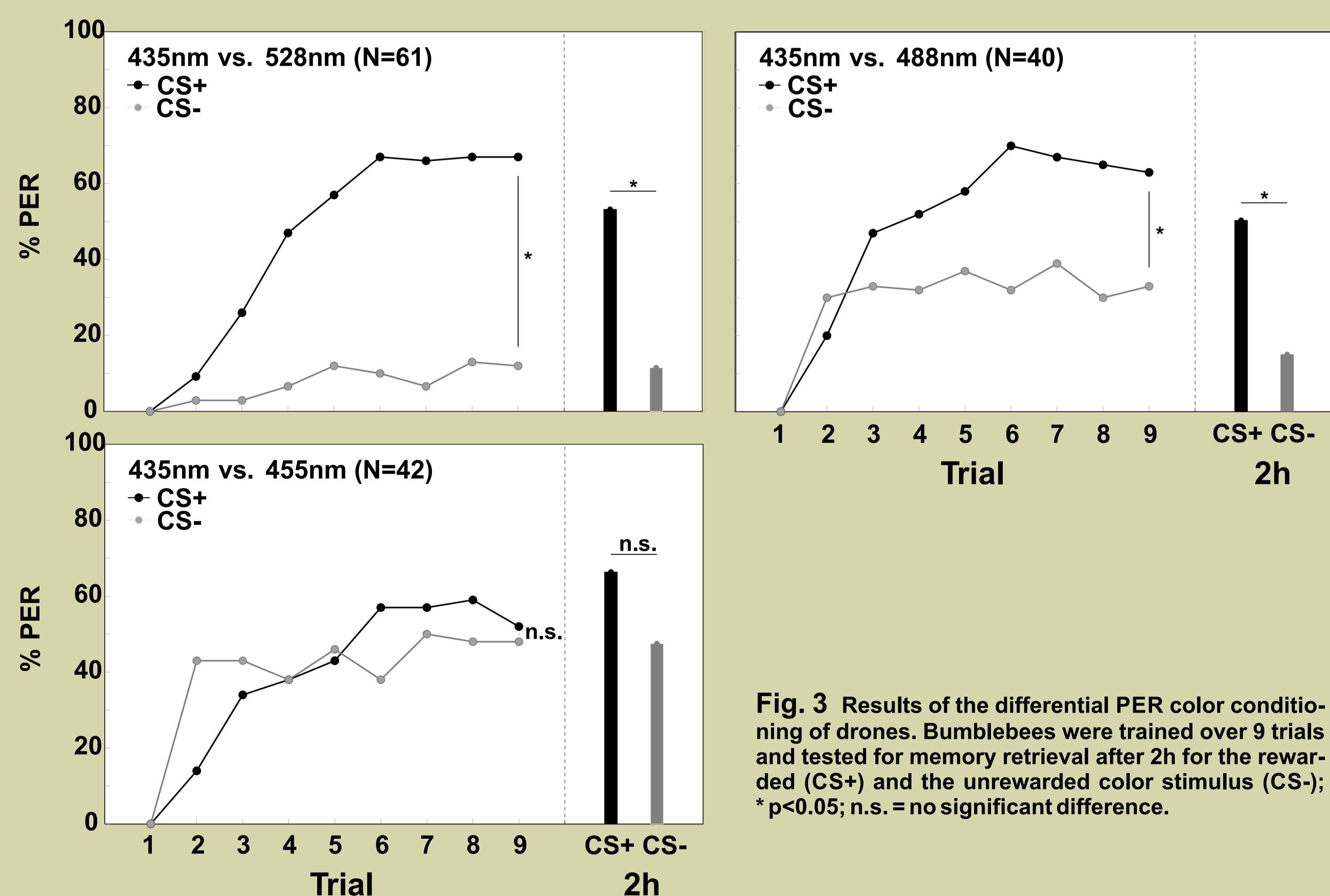


## Material & Methods

Drones from *Bombus terrestris* colonies, raised in a climate chamber (25°C, 75% humidity), were harnessed in small plastic tubes and fixed by neck brackets. After 18h of starvation, bees were tested for a proper PER. Individuals showing an extension of the proboscis after touching the antennae with sucrose solution were used for the experiments. For conditioning, harnessed bees were placed on a sleigh below a color filter box where light could shine through (Fig. 1D). Before and after each conditioning trial, the bee rested for 10 s to become familiar. The trials were performed according to the tasks as described in detail in the results below. For each bee, the PER was recorded during the presentation of the CS (conditioned stimulus; color), and before applying the US (unconditioned stimulus; sucrose), to estimate the learning performance.



**Fig. 1** A: Illustration of the visual conditioning of the PER for the tested color stimuli in harnessed bumblebees; B: Spectral sensitivity of the three photoreceptor-types in *Bombus terrestris*, overlaid by transition spectra of the four tested color filters; C: Intensities of the monochromatic light stimuli used for differential conditioning; D: Illustration of the set-up for visual PER conditioning. Light from a cold-light source (l) passes a color filter (f) and reaches the harnessed bee, placed on a movable sleigh (s);



**Fig. 3** Results of the differential PER color conditioning of drones. Bumblebees were trained over 9 trials and tested for memory retrieval after 2h for the rewarded (CS+) and the unrewarded color stimulus (CS-); \* p<0.05; n.s. = no significant difference.

## Conclusion

Bumblebee drones are able to associate a color stimulus with a food reward in absolute and differential PER conditioning and can memorize it two hours after learning. Furthermore, drones can use chromatic features of the light stimuli during differential conditioning. Overall, bumblebees depict a well suited model organism to study mechanisms of visual learning and memory under controlled conditions.