Supplementary material

Active explorers show low learning performance in a social insect

Eve UDINO, Margot PEREZ, Claudio CARERE, Patrizia d'ETTORRE*

Laboratory of Experimental and Comparative Ethology, University Paris 13, Sorbonne Paris Cité, France

*Correspondence: dettorre@leec.univ-paris13.fr

Material and Methods

Learning task

Each ant was anesthetized on ice for about ten minutes and then harnessed in an ant holder. The holder was an inverted 0.2 ml Eppendorf® standard microcentrifuge tube, from which the apex was cut off. The ant's head was passed through the apical hole of the tube and fixed with adhesive tape, thus leaving the antennae and mouthparts free to move (Figure S2). Ants were left at rest for 3 hours to recover and to habituate to the harness. This harnessing condition allows controlling factors that could influence learning, such as the number and the duration of conditioned (CS) and unconditioned stimuli (US) presentations or the inter-trial interval (ITI) (Guerrieri and d'Ettorre 2010).

A differential conditioning was performed to assess whether ants were able to learn to respond differentially to the two stimuli (one rewarded, one unrewarded) and to measure individual learning performances with accuracy. Three microliters of pure odorant (octanal or hexanol) were applied on a clean strip (1 cm^2) of filter paper before being introduced inside a 10 mL syringe. The syringe, placed 2 cm in front of the ant antennae, allowed presenting the conditioned stimuli by blowing an air puff. During the conditioning procedure, each ant was placed under a stereomicroscope to observe the *maxilla-labium extension* response (MaLER, Guerrieri and d'Ettorre 2010).

The acquisition phase consisted in 10 trials (5 CS+ and 5 CS- presentations in a pseudo-random order). Each trial lasted 1 minute, the ITI was of 10minutes, as 10 ants were trained per session. In CS+ trials, each ant was placed under the stereomicroscope and after 25 seconds of habituation, the CS+ was presented for 5 seconds. From the third second, a sucrose soaked toothpick was delivered

to the ant for 5 seconds, which trigger the MaLER. Therefore, the CS+ and the US overlapped for 2 seconds. Then, after 25 seconds, the ant was put back at its resting place. In CS- trials, the protocol was identical but without reward delivery. An air extractor placed behind the ant removed possible residual odorant molecules during the experiment. Only individuals who responded to the US at least four times during the conditioning trials were included in the statistical analyses (> 90% of individuals tested).

References

Guerrieri FJ, d'Ettorre P, 2010. Associative learning in ants: conditioning of the maxilla-labium extension response in *Camponotus aethiops*. *J. Insect. Physiol.* 56: 88–92.

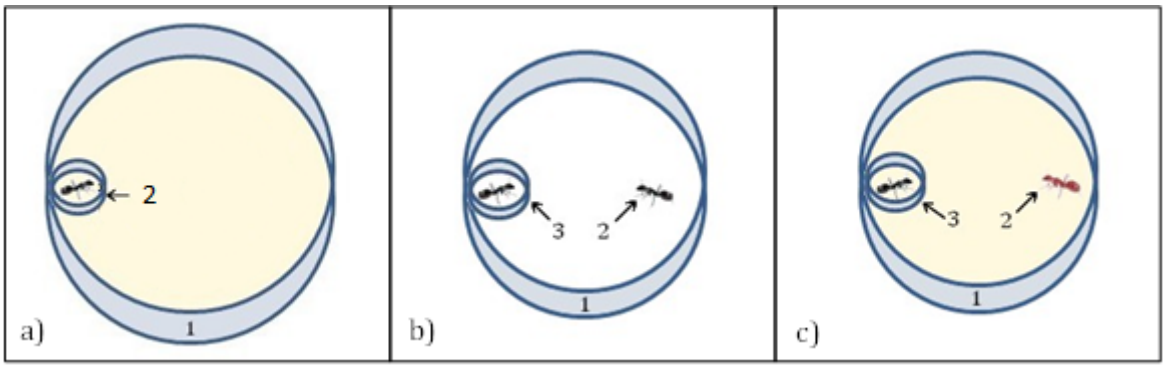

Figure S1. Experimental set-up for personality tests. **(a)** Exploratory activity test in (1) a circular open-field with clean filter paper floor, including (2) an acclimatization tube where the ant is introduced before the test. **(b)** Sociability test in (1) a circular apparatus placed in the foraging area of the nest in which (2) a nestmate target ant is placed and (3) the acclimatization tube with the tested ant; **(c)** Aggression test in (1) a circular apparatus with filter paper floor impregnated with nest odor of the tested ant, and where a non-nestmate target ant (2) and the acclimatization tube containing the tested ant (3) are placed. The walls of the devices were Fluon®-coated to prevent ants from escaping.

Figure S2. Harnessed ant in a Eppendorf® tube ready for the conditioning task (picture M. Perez)

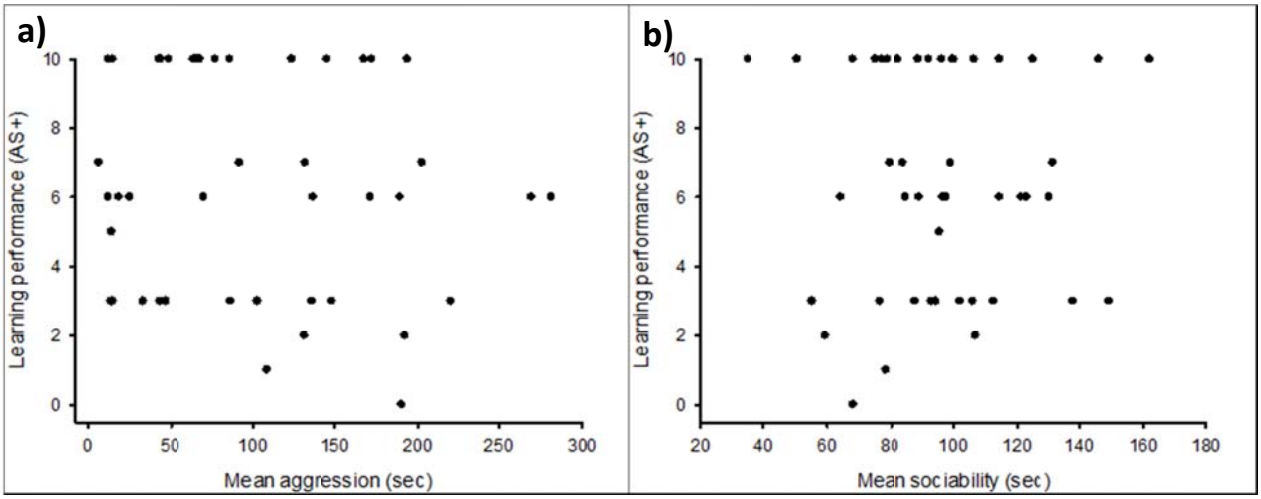

Figure S3. Relationship between aggression, sociability and learning performance (acquisition score, AS+). **(a)** r_s = -0.13, *P* = 0.38; **(b)** r_s = 0.006, *P* = 0.97. For each behaviour the two repeats are averaged.