Corresponding Author: Prof. Patrizia d'Ettorre,

Corresponding Author's Institution: University of Paris 13

First Author: Patrizia d'Ettorre

Order of Authors: Patrizia d'Ettorre; Claudio Carere; Lara Demora;
Pauline Le Quinquis; Lisa Signorotti; Dalila Bovet

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Abstract: Emotional state may influence cognitive processes such as
attention and decision-making. A cognitive judgement bias is the
propensity to anticipate either positive or negative consequences in
response to ambiguous information. Recent work, mainly on vertebrates,
showed that the response to ambiguous stimuli might change depending on
an individual's affective state, which is influenced by e.g. the social
and physical environment. However, the response to ambiguous stimuli
could also be affected by the individual's behavioural type
(personality), a question that has been under-investigated. We studied
the link between individual differences in exploratory activity and the
response to an ambiguous stimulus in the ant Camponotus aethiops.
Exploratory behaviour, quantified with an open-field test, was variable
among individuals but consistent over time within individuals. Individual
ants learned to associate a spatial position to a reinforcement and
another spatial position to a punishment. Once the ants had acquired this
discrimination, cognitive judgement bias was tested with the stimulus in
an intermediate position. Fast explorers in the open-field took
significantly more time to approach the ambiguous stimulus compared to
slow explorers, suggesting a negative judgement bias for fast explorers
and a positive bias for slow explorers. This previously unknown link
between individual difference in exploratory activity and cognitive bias
in a social insect may help understanding the evolution and organization
of social life.

Paris, 21st September 2016

Dear Dr. Guillette,

Thank you for your letter concerning our article "Individual differences in exploratory relate to cognitive judgement bias in carpenter ants"

We have now addressed all your novel points concerning the interpretation, which allowed further clarifying the general discussion. We hope that you are now fully satisfied by our revision.

On behalf of my coauthors

Sincerely

Patrizia d'Ettorre

Paris, 21st September 2016

Editor's comments

Lines 272 - 275 I don't see how a bi-modal distribution shows a speed-accuracy trade-off. This needs to be explained or removed.

AUT: it was explained below (former lines 276-279). We have now rephrased the sentence giving first the explanation and then the statement.

Lines 279-283 The first bit about Verbeek makes sense, that fast explorers form routines. I think you are trying to say that your fast explores may not have approached the ambiguous stimulus because it was not part of their routine? Is this the connection you are trying to make? And if so, does this results then show that cog bias test may not be testing optimism/pessimism in this instance? Can you please clarify this idea in the manuscript? I don't really see the connection to the chickadee work. How does a fast explorer doing worse relate to your study? You need to add a little bit here to make the connection easy for the reader to see.

AUT: We have now clarified why we have given the two bird examples in this part of the discussion (now lines 282-294). We say that fast explorers are in general less flexible and less prone to integrate new information than slow explorers. This has been explained (by others) with the fact that fast explorers form routines. In your study (Guillette et al., 2010), chickadees that are fast explorers have problems with reversal learning, this is totally in agreement with the "routine hypothesis", as clearly explained in Table 1 of Sih and Del Giudice, 2012. This is simply the point we want to make, we hope it is now clear.

Lines 284-292 I'm not sure this bit adds anything to your story. Can you connect it to what you found or remove?

AUT: You are right, it was a bit confusing. We have removed this part.

Lines 302-306 I'm not following this logic. Are you saying that a larger area explored means more options (potentially) the individual has to choose from? Having more options is not the same things as being more selective. The bit about the slow explorers seems to contradict itself: how does spending more time evaluating food mean that you are less selective? Shouldn't this be the other way around?

Line 306 I don't see how this is an example of a speed/accuracy trade-off.

AUT: We agree that the way we formulated this part was potentially confusing, so we have now explained better and cited a relevant paper in bumblebees.

Minor edits for clarification:

Line 113/177 - The inclusion of 'proactive style' and 'reactive style' are out of place here because they have not been introduced. Perhaps delete or explain why there are relevant here. The only other time there two terms is used in once in the discussion but I think this bit should be revised. See my earlier major comment.

AUT: We have added the classical reference in the introduction; we believe it is good to have these terms in our paper because they are currently used.

Line 191 - delete 'at least'.

AUT: done

Line 214 - please add n's for the fast and slow individuals in the cognitive bias test and add the labels 'optimistic' and 'pessimistic' accordingly.

AUT: We added optimistic and pessimistic, as you requested. However, we believe it is not necessary to add the sample size for the two groups, being our group formation based on the median of 74 individuals (n=34 in each group).

Line 216-18. Maybe change to 'Difference in exploratory activity (time spend moving the central area and total time spent in the central area of the open field) between optimistic and pessimistic ants was analysed with a t-test.'

AUT: done, thank you.

Line 244 - maybe change 'slow individual' to 'slow explorer' for clarity, and the same for 'fast individual'.

AUT: done.

Supplementary material - the title needs to be changed here to match the manuscript.

AUT: done.

- Individual ants show consistency in their exploratory behaviour
- Ants learn to associate a positive or negative stimulus with spatially distinct positions
- Cognitive judgment bias was tested with the stimulus in an intermediate position
- Fast explorers showed a pessimistic bias while slow explorers an optimistic bias
- Results suggest a link between personality and cognitive state in eusocial insects

1   **Individual differences in exploratory activity relate to cognitive**

2   **judgement bias in carpenter ants**

3

4   Patrizia d'Ettorre[1*], Claudio Carere[1], Lara Demora[1], Pauline Le Quinquis[1], Lisa Signorotti[1] &

5   Dalila Bovet[2]

6   [1] *Laboratory of Experimental and Comparative Ethology (LEEC) University of Paris 13, Sorbonne*

7   *Paris Cité, France*

8   [2] *Laboratory of Comparative Ethology and Cognition, University of Paris Ouest, Nanterre La*

9   *Défense, France*

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12   **Short title:** exploration and cognitive bias in ants

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15   **Correspondence:** Patrizia d'Ettorre

16   Laboratory of Experimental and Comparative Ethology (LEEC)

17   University of Paris 13, Sorbonne Paris Cité, France

18   E-mail: dettorre@leec.univ-paris13.fr

19

20   **ABSTRACT**

21   Emotional state may influence cognitive processes such as attention and decision-making. A

22   cognitive judgement bias is the propensity to anticipate either positive or negative consequences in

23   response to ambiguous information. Recent work, mainly on vertebrates, showed that the response

24   to ambiguous stimuli might change depending on an individual's affective state, which is influenced

25   by e.g. the social and physical environment. However, the response to ambiguous stimuli could also

26   be affected by the individual's behavioural type (personality), a question that has been under-

27   investigated. We studied the link between individual differences in exploratory activity and the

28   response to an ambiguous stimulus in the ant *Camponotus aethiops*. Exploratory behaviour,

29   quantified with an open-field test, was variable among individuals but consistent over time within

30   individuals. Individual ants learned to associate a spatial position to a reinforcement and another

31   spatial position to a punishment. Once the ants had acquired this discrimination, cognitive

32   judgement bias was tested with the stimulus in an intermediate position. Fast explorers in the open-

33   field took significantly more time to approach the ambiguous stimulus compared to slow explorers,

34   suggesting a negative judgement bias for fast explorers and a positive bias for slow explorers. This

35   previously unknown link between individual difference in exploratory activity and cognitive bias in

36   a social insect may help understanding the evolution and organization of social life.

37

38   **Keywords:** affective state, cognition; exploratory activity; learning; personality; social insects

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40

1 INTRODUCTION

Animals show the ability to feel emotions that may induce long-lasting consequences (e.g., Désiré et al., 2002). For their assessment, indirect methods such as measuring stress hormones or quantifying abnormal behaviour are usually employed (Bateson and Matheson, 2007). It is increasingly acknowledged that affective state (driven by e.g., emotions) may influence cognitive processing in animals as in humans (e.g., Harding et al., 2004). In particular, the positive or negative valence of an affective state may bias cognitive processes such as attention, memory, and judgement (e.g., Novak et al., 2015). This phenomenon is generally referred to as 'cognitive bias'. In the present study, we focus on 'cognitive judgement bias' following the definition by Mendl and co-workers (2009): "Judgement bias in this context refers to the propensity of a subject to show behaviour indicating anticipation of either relatively positive or relatively negative outcomes in response to affectively ambiguous stimuli" (Mendl et al., 2009, p.164).

Cognitive judgement bias can be studied using a simple go/no-go procedure, in which animals are first trained to discriminate between two distinct stimuli, then (after acquisition) their response to ambiguous stimuli is tested. Therefore, animals are requested to categorise an ambiguous stimulus as either positive or negative (see for a classical example in rats: Harding et al., 2004). For instance, European starlings (*Sturnus vulgaris*) were trained on a go/no-go task to discriminate between a white visual stimulus associated with palatable food and a dark grey stimulus associated with unpalatable food. Then, the birds were tested with unreinforced ambiguous stimuli (intermediate shades of grey) with the expectation that their response reflects their affective state (Bateson and Matheson, 2007). A positive cognitive judgement bias is therefore a high expectation of reward following an ambiguous stimulus. This "optimism" is usually observed when animals are in a positive affective state, following for instance the addition of an environmental enrichment. In this case, subjects would approach quickly the ambiguous stimulus. A negative cognitive judgement bias, instead, is a high expectation of punishment (or non-reward) in face of an ambiguous stimulus. This "pessimism" is usually observed when animals are in a negative affective state, triggered by e.g., stress or decrease in environmental quality. In this case, subjects would take long to approach (or not approach at all) the ambiguous stimulus. We adopt the pessimistic/optimistic terminology following operational definitions, such as in Douglas et al. (2012).

Studies of cognitive judgement bias have been carried out in several vertebrate taxa, such as birds (Bateson and Matheson, 2007), rats (Brydges et al., 2011; Harding et al., 2004), and pigs (Douglas et al., 2012). The subjects were tested with ambiguous stimuli after experiencing a change in environmental quality (removal or addition of enrichments). These studies have shown that environmental enrichment may induce a positive judgement bias, while a decrease in the quality of

76　the environment may result in a negative bias. Similarly, stressful conditions induce a negative

77　cognitive judgement bias (Burman et al., 2009; Mendl et al., 2009). In honeybees, to our knowledge

78　the only invertebrate tested, individuals were trained to associate a given scent with a reward, and

79　another scent with a punishment. The bees were then divided into two groups, one undergoing

80　mechanical shaking for 30 seconds as a condition of stress, the other not undergoing any stressful

81　procedure. The two groups were then exposed to an ambiguous stimulus, an intermediate odour

82　(compared to the two learned odours). Stressed bees were more likely to interpret the ambiguous

83　stimulus as predicting a punishment (Bateson et al., 2011).

84　　Studying cognitive judgement biases may help developing new methodologies for

85　evaluating emotions and therefore improving welfare in non-human animals (Mendl et al., 2009).

86　One study, however, failed to clearly demonstrate a cognitive judgement bias: laying hens housed in

87　an enriched environment did not respond positively to an ambiguous stimulus compared to hens in a

88　standard environment. It was therefore suggested that if environmental differences are not large

89　enough, inter-individual differences might exert a significant impact on the responses of animals

90　(Wichman et al., 2012).

91　　Animals show consistent inter-individual differences (personality) in e.g., boldness,

92　aggressiveness, activity, sociability and/or exploratory tendency, and these personality traits are

93　often correlated (behavioural syndrome). For instance, individuals that show high exploratory

94　tendencies, are also highly aggressive towards conspecifics and bold when predators are present

95　(Sih and Del Giudice, 2012). Cognitive processing should be influenced by individual differences in

96　personality because these are predicted to affect fitness outcomes of individuals (Wolf and

97　Weissing, 2012). Indeed, a link between cognition and personality has been postulated (Carere and

98　Locurto, 2011; Sih and Del Giudice, 2012; Wolf et al., 2008), and empirical evidence in this

99　direction is growing despite methodological challenges and limitations of experimental procedures

100　(Griffin et al., 2015). Differences in personality traits could underpin affective states and this

101　relationship could be bidirectional: for instance, individuals may be more prone to develop either

102　positive or negative affective states depending on their personality, resulting in cognitive judgement

103　bias. Such biases could then feedback on personality traits, as shown in humans (Mathews et al.,

104　1997).

105　　To our knowledge, only one study so far tested the link between personality and affective

106　state in animals, suggesting that some personality traits modulate cognitive processing of

107　environmental stimuli (attention bias) in psittacine birds, *Amazona amazonica* (Cussen and Mench,

108　2014). Once assumed a link between individual affective state and consistent inter-individual

109　behavioural differences, a case for its directionality remains to be made. A key aspect of cognition

110　that relates to consistent individual differences along the bold-aggressive-exploratory axis is the

ecologically relevant speed-accuracy trade-off (Sih and Del Giudice, 2012): an individual may either use a fast/inaccurate strategy or a slow/accurate one, but cannot e.g., forage while being fast and accurate at the same time. Accordingly, we predicted that fast explorers ('proactive style', *sensu* Koolhaas et al. 1999), which are rather insensitive to environmental change and prone to set routines, should likely exhibit a negative cognitive judgement bias when facing uncertainty (ambiguous stimulus). These pessimistic individuals would not approach, or would take long time before approaching, an ambiguous stimulus. Conversely, slow explorers ('reactive style') should show a positive cognitive judgement bias, therefore being optimistic and approaching relatively quickly an ambiguous stimulus. We tested this hypothesis by: *i)* assessing the consistency of individual differences in exploratory activity in a large sample of individuals; *ii)* measuring exploratory activity in a separate set of individuals; *iii)* assessing the response of these same individuals to an ambiguous stimulus (cognitive judgement bias). Our study organism is a social insect, the carpenter ant *Camponotus aethiops,* which has been already tested for possible links between personality traits and learning ability (Udino et al., submitted).

2   MATERIAL AND METHODS

2.1   Animals and housing

Five queenright colonies of *Camponotus aethiops,* collected near Toulouse (Midi-Pyrénées, France, latitude 43.5°, longitude 1.516667°), were each housed in two Fluon®-coated plastic boxes connected by a hose. Colonies were kept under laboratory conditions (22±2°C, L12/D12, 40% humidity). One box, the nest (26x19x7cm), had a plaster floor and was darkened by cardboard; the other, the foraging area (29x26x8cm), was exposed to light. Colonies were fed twice a week with mealworms (*Tenebrio molitor*) and a mix of honey and apples, water was provided *ad libitum*.

Camponotus aethiops is a species that lives in hot, dry and open habitats and builds the nest in soil. Colonies are monogynous (only one queen) and medium size (500-3000 workers). They are omnivorous and eat preferentially dead insects, fruits and flower nectar, they also feed on extrafloral nectaries. Foraging workers usually avoid the warmest part of the day and are active early morning and late afternoon (Lenoir et al. 1990). Workers orient well using visual cues, especially when these are present in their horizontal field of view (Laffort et al. 1991) and are capable of visual discrimination learning (Yilmaz et al. 2014). It is not known whether this species uses chemical trails, like some other species of the same genus (Hölldobler and Wilson 1990), but there is evidence that *C. aethiops* workers perform group recruitment with leader, i.e., after having discovered a novel food source, a forager ant comes back to the nest and recruits several workers (1-20), which follow closely behind the leader to the food area (Suzzoni et al. 1991).

146 *2.2 Exploratory activity test*

147 One hundred and twenty five ants foragers were individually marked with dots of paint
148 (uniPAINT©) on their thorax and gaster before the experiments started. Exploratory activity was
149 evaluated in a circular open-field arena (Ø 6 cm), an adaptation of the classical open-field test (e.g.
150 Prut and Belzung, 2003) with a floor of clean filter paper (replaced after each trial), in which an
151 area of 4 cm diameter was considered as the central zone (Figure 1). An ant was taken from the
152 foraging arena and introduced into an acclimatization tube placed in the peripheral zone for 120
153 seconds. Then, the tube was removed and the behaviour of the ant was observed for 300 seconds.
154 We measured the time spent walking and resting in the central zone and in the peripheral by direct
155 recording on a PC equipped with the software EthoLog (Ottoni, 2000). After the test, the ants were
156 immediately returned to their respective colony. One week later, the open-field test was repeated
157 with the same ants (n=125) to assess individual consistency over time.

158 A second batch of ants from the same colonies was used to study the relationship between
159 exploratory activity and cognitive bias. These ants (n = 85) were tested in the open-field arena as
160 described above and then returned to their colony. After about two hours they started the procedure
161 described below (learning phase and ambiguous stimulus test), which was completed on the same
162 day.

163

164 *2.3 Cognitive judgement bias test*

165 *2.3.1 Learning phase*

166 The apparatus consisted of a rectangular arena (18 x 12 x 5.5 cm) with a floor of filter paper
167 (changed after each trial) and a visual pattern on the external part of one of the longest walls. Two
168 microscope cover slips (20 x 20 mm, Menzel-Gläser, Germany) were placed one at each corner of
169 this wall. Each slip had an identical little piece of plasticine as landmark (Figure 2A), but one slip
170 was baited with an appetitive stimulus (a drop of sucrose solution, 30% w/w) and the other with an
171 aversive stimulus (a drop of quinine solution, Sigma–Aldrich, 1% w/w, Guerrieri and d'Ettorre,
172 2010). The position of the two stimuli (left/right) was randomly chosen across individuals but
173 remained the same for a given ant across the learning trials. For each trial, the ant was introduced
174 into an acclimatization tube placed near the wall far from the stimuli at an equal distance from each
175 stimulus for 120 seconds. The first phase of learning consisted of 6 trials in which both stimuli were
176 present. After the removal of the acclimatization tube, the time the ant needed to discover the sugar
177 solution was recorded (the trial was stopped after 10 min if the ant did not reach the sugar solution).
178 Between each trial the ant was put back into its colony for about 5 min, and then it started the next
179 trial. The ant should actively approach and taste both stimuli to ascertain their valence (positive or

180 negative). When the ant reached the appetitive stimulus, it was left undisturbed until it finished

181 drinking the sugar solution to avoid disturbance stress. If the ant did not get to the aversive stimulus

182 at least once during the 6 trials (about 10% of the tested ants), the session was discarded and these

183 ants did not continue to the second phase.

184   The second phase of learning consisted of 6 additional trials in which only one stimulus was

185 present (3 trials with the aversive stimulus and 3 trials with the appetitive stimulus in a random

186 order). The latency time to reach the stimulus was recorded during each trial, which lasted a

187 maximum of 3 minutes. Between each trial the ant was put back into its colony, as in the first phase.

188 We considered that an individual learned the task if the sum of the latency time of the 3 trials with

189 the appetitive stimulus was at least two times smaller than the sum of the latency time of the 3 trials

190 with the aversive stimulus during the second phase of learning. The majority of the ants (90%) did

191 not approach the aversive stimulus once during this phase, in which case they were assigned a

192 latency of 180 seconds.

193

194 *2.3.2 Ambiguous stimulus test*

195 The ants that learned the task (74 of 85 individuals tested) were subjected to the test with the

196 ambiguous stimulus. This test was carried out in the same rectangular apparatus used for the

197 learning trials (with clean filter paper) but this time the cover slip was placed at the center of the

198 wall (same distance from the right and the left corner, Figure 2B). A drop of water was placed on

199 the slip instead of the appetitive or negative stimulus. The ant was placed in the acclimatization tube

200 as usual and after the removal of the tube the latency time to reach the ambiguous stimulus was

201 recorded. If the ant did not reach the stimulus the test was stopped after 10 min and a latency of 600

202 sec was assigned. This is an established paradigm called go/no-go procedure (Harding et al., 2004),

203 in which the response is either approaching or not approaching (or approaching slowly) the

204 ambiguous stimulus.

205

206 *2.4 Data analysis*

207 Intra-class correlation (Lessels and Boag 1987) was calculated to assess individual repeatability

208 across the two sessions of the open-field test. We used LMM-based calculations by R package *rpt*R

209 (Nagakawa and Schielzeth, 2010) and we assessed 95% confidence intervals (CI) by 1000 bootstrap

210 steps. Individual was a random factor. P values were calculated by 1000 permutations (α level =

211 0.05).

212   For the ambiguous stimulus test, we calculated the median of the latency time to reach the

213   ambiguous stimulus (97.01 sec) across all 74 subjects, and we then divided the 74 ants in two

214   groups: fast optimistic (latency < median) and slow pessimistic individuals (latency > median). This

215   was justified by the fact that the distribution of approach time was bimodal, with individual ants

216   approaching the ambiguous stimulus very fast or not at all (Figure 3). Difference in exploratory

217   activity (time spend moving the central area and total time spent in the central area of the open

218   field) between optimistic and pessimistic ants was analysed with a t-test. We also looked at the

219   correlations between individual latency time to approach the ambiguous stimulus and exploratory

220   activity by Spearman correlation (data not normally distributed).

221   To investigate whether cognitive judgement bias might be related to learning ability, we calculated

222   an index of learning performance by comparing the first 3 trials to the last 3 trials of the first phase

223   of learning (these are the 6 trials in which the two stimuli, sucrose solution and quinine solution,

224   were present). Given the sum of the time needed to reach the appetitive stimulus in the first 3 trials

225   = A and the sum of the time needed to reach the appetitive stimulus in the last 3 trials = B, learning

226   performance was calculated as (A-B)/A; the closer is this number to 1, the faster the animal

227   approached the appetitive stimulus, i.e., the better the learning performance. We then looked at the

228   possible correlation between learning performance and latency to reach the ambiguous stimulus.

229

230

231   **3 RESULTS**

232

233   *3.1 Exploratory activity test*

234   Ants showed significant consistency over time in their exploratory activity. The two variables

235   reflecting the level of exploration during the open-field test were significantly repeatable across the

236   two sessions (time spent moving in the central area: R = 0.384; CI = (0.222, 0.517); p = 0.001; total

237   time spent in the central area: R = 0.289; CI = (0.121, 0.441), P = 0.003, Figure S1, supplementary

238   material).

239

240   *3.2 Cognitive bias*
241
242   We observed substantial individual differences in the approach time to the ambiguous stimulus and

243   the distribution of approach time was bimodal (Figure 3).

244   Ants that spent less time in exploratory activity (slow explorers) readily approached the

245   ambiguous stimulus (optimists), while ants that spent more time in exploratory activity (fast

246   explorers) were slow in approaching the ambiguous stimulus (pessimists). This difference between

247   the optimist and pessimist group in relation to exploratory activities is statistically significant (time

248   spent moving in the central area: t = 3.47, p < 0.001, Figure 4A; total time spent in the central area:
249   t = 2.96, p < 0.01, Figure 4B; these two variables are highly and positively correlated: r = 0.78, p <
250   0.001). This is confirmed by the positive correlations between latency to approach the ambiguous
251   stimulus and both time spent moving in the central area (r_s = 0.36, p < 0.01) and total time in the
252   central area of the open-field (r_s = 0.38, p < 0.01): ants that spent more time in exploring the open-
253   field were slower in approaching the ambiguous stimulus during the cognitive bias test (Figure S2).
254   Cognitive bias was not related to learning ability, as shown by the lack of correlation
255   between learning performance and latency to reach the ambiguous stimulus (r_s = -0.122, P = 0.30).
256
257

258   **4 DISCUSSION**
259   The aim of the present study was to test the relationship between cognitive judgement bias
260   (measured as response to an ambiguous stimulus) and inter-individual differences in exploratory
261   behaviour (a personality trait) in workers of the carpenter ant, *Camponotus aethiops*. We observed
262   individual differences in the latency time to approach the ambiguous stimulus, with a clear bimodal
263   distribution: ants were either very fast in approaching the ambiguous stimulus (optimistic) or they
264   were very slow (or did not approach the stimulus at all within the cut-off time), i.e., pessimistic. In
265   our go/no-go procedure, a non-approach is considered a response, as ants previously learned the
266   discrimination between appetitive and aversive stimulus, and therefore not approaching is an
267   appropriate response for the aversive stimulus. This bimodal pattern strikingly resembles the one
268   observed in great tits, *Parus major,* for approach time towards a novel object and latency to return
269   after a startle (indicating risk taking behaviour), which formed the basis to create genetic selection
270   lines for avian personality (Verbeek et al., 1994; Drent et al., 2003; van Oers et al., 2004).
271   In general, proactive individuals are fast explorers but are relatively insensitive to new
272   environmental information, whereas reactive individuals are slow explorers but adjust their
273   behaviour to changes in the environment, or when signals have changed in meaning. Therefore, a
274   trade-off between speed and accuracy occurs (see introduction, Sih and Del Giudice, 2012), which
275   may result in a bimodal distribution. Both strategies (proactive/reactive) might entail their specific
276   individual advantages when performing in stable (fast responders performing better) or unstable
277   (slow responders performing better) environments, while individual with intermediate profiles
278   would have certain disadvantages in both kinds of environment (Verbeek et al., 1994). Our results
279   are consistent with studies in great tits, in which fast explorers appear to form more routines than
280   slow explorers (Verbeek et al., 1994). Forming routines may explain why the behaviour of fast
281   explorers is generally less flexible (Sih and Del Giudice, 2012). Indeed, in black-capped chickadees
282   (*Poecile atricapillus*), fast explorers performed worse than slow explorers in a reversal learning

10

283   task, which requires altering an acquired information (Guillette et al., 2010).

284   Our data suggest that the observed pattern of responses to the ambiguous stimulus could be
285   explained by individual differences in the exploration test (open-field, which individuals performed
286   before starting the learning phase and the cognitive judgement bias test). Individuals that spent
287   significantly more time in the central area of the open-field were those showing a longer latency to
288   approach the ambiguous stimulus. Thus, individuals who took the longest time to approach the
289   ambiguous stimulus were those exploring most. Fast exploring individuals showed a negative
290   cognitive judgement bias (pessimism), while slow explorers showed a positive cognitive judgement
291   bias (optimism). In an ant colony, division of labour is based on age and/or morphology (Wilson,
292   1971). The foragers are older individuals but there is variability in foraging activity (see e.g.
293   Beverly et al., 2009). We predict that fast explorer foragers may spend more energy than slow
294   explorers as they cover a larger foraging area. Therefore, fast explorers are supposed to be less
295   accurate in examining the environment and retain only information about high quality food.
296   Conversely, slow explorer foragers may afford accurate evaluation of food sources (of varying
297   quality) distributed in a relatively small foraging area. This is an example of speed/accuracy trade-
298   off, also reported in bumblebees (Burns and Dyer, 2008).

299   We are not aware of any study relating affective states with personality traits in social
300   insects. The results of the present study are among the first linking consistent individual differences
301   (animal personality) to response to ambiguous stimuli (cognitive judgement bias). Most studies
302   evaluating cognitive biases involved enrichment or impoverishment of the environmental quality,
303   which induced a positive and negative cognitive bias respectively (Bateson and Matheson, 2007;
304   Brydges et al., 2011; Douglas et al., 2012). Other studies have tested the effect of stress on the
305   response to an ambiguous stimulus (Bateson et al., 2011; Burman et al., 2009; Salmeto et al., 2011).
306   These studies aimed at finding a non-invasive and effective way to assess the affective states of
307   animals in captivity. Since cognitive bias is considered an indicator of positive or negative affective
308   states, it may also provide important information to assess and improve welfare in captive animals
309   (Mendl et al., 2009). Our study suggests that personality should be taken into consideration when
310   evaluating the welfare of animals since personality types are differently linked to affective states,
311   for instance some personality types are likely to score more pessimistic than others in cognitive bias
312   tests. Invertebrates are widely used in animal experimentation as well as in zoos and aquaria, and
313   there is a growing interest and concern about their welfare upon realizing that many species possess
314   advanced cognitive abilities, consciousness, individuality, pain suffering ability, etc. (Carere et al.,
315   2011).

316   Finally, our data are in accordance with evolutionary explanations of strategies used by
317   animals to cope with uncertainty (Mathot et al., 2012), which predict that if animals differ in their

relative investment in sampling, individuals that sample more thoroughly would have lower exploration/activity scores, but exhibit greater plasticity than individuals that sample with less accuracy. In our study, readily approaching the novel ambiguous stimulus by slow explorers could be interpreted as sampling in an uncertain context, e.g. when in nature resources fluctuate in space and/or time.

5 CONCLUSIONS

The results support the postulated link between consistent individual profiles of exploratory activity (likely reflecting personality) and individual differences in affective states measured by cognitive judgement biases in response to ambiguous environmental stimuli. To our knowledge, this is the first experimental evidence of such a relationship in an invertebrate species. The next steps should focus on testing the possible consistency of affective states across subsequent trials and their potential impact on individual personality profiles, taking into account ontogenetic changes.

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432

FIGURE LEGENDS

434

435

436 **Figure 1**. Experimental set-up (open-field arena) used to study exploratory activity.

437

438 **Figure 2**. Experimental set-up used to study cognitive bias. A) First phase of learning, with the
439 appetitive stimulus (sucrose solution) and one corner and the aversive stimulus (quinine solution) at
440 the other corner (left/right balanced across individuals). B) Ambiguous stimulus test, with the
441 neutral stimulus (water) placed at the center.

442

443 **Figure 3**. Frequency distribution of the latency time to approach the ambiguous stimulus during the
444 cognitive bias test (n = 74).

445

446 **Figure 4.** Approach time to the ambiguous stimulus during the cognitive bias test (ants are grouped
447 in two categories: slow and fast) in relation to exploratory activity: A) time spent moving (mean and
448 95% CI) in the central part of the open-field; B) total time (mean and 95% CI) spent in the central
449 part of the open-field (n = 74).

450

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452

453

Figure 1
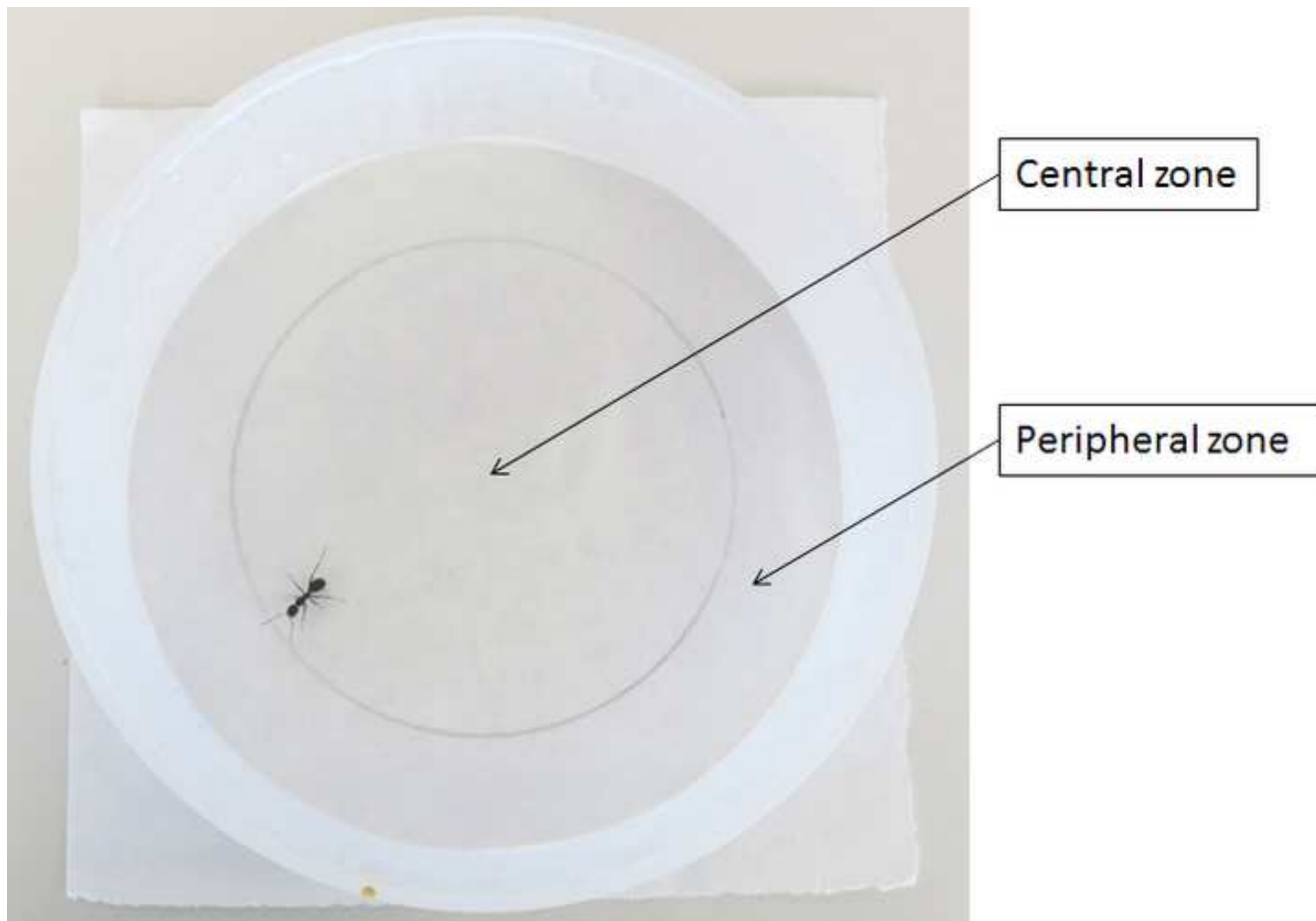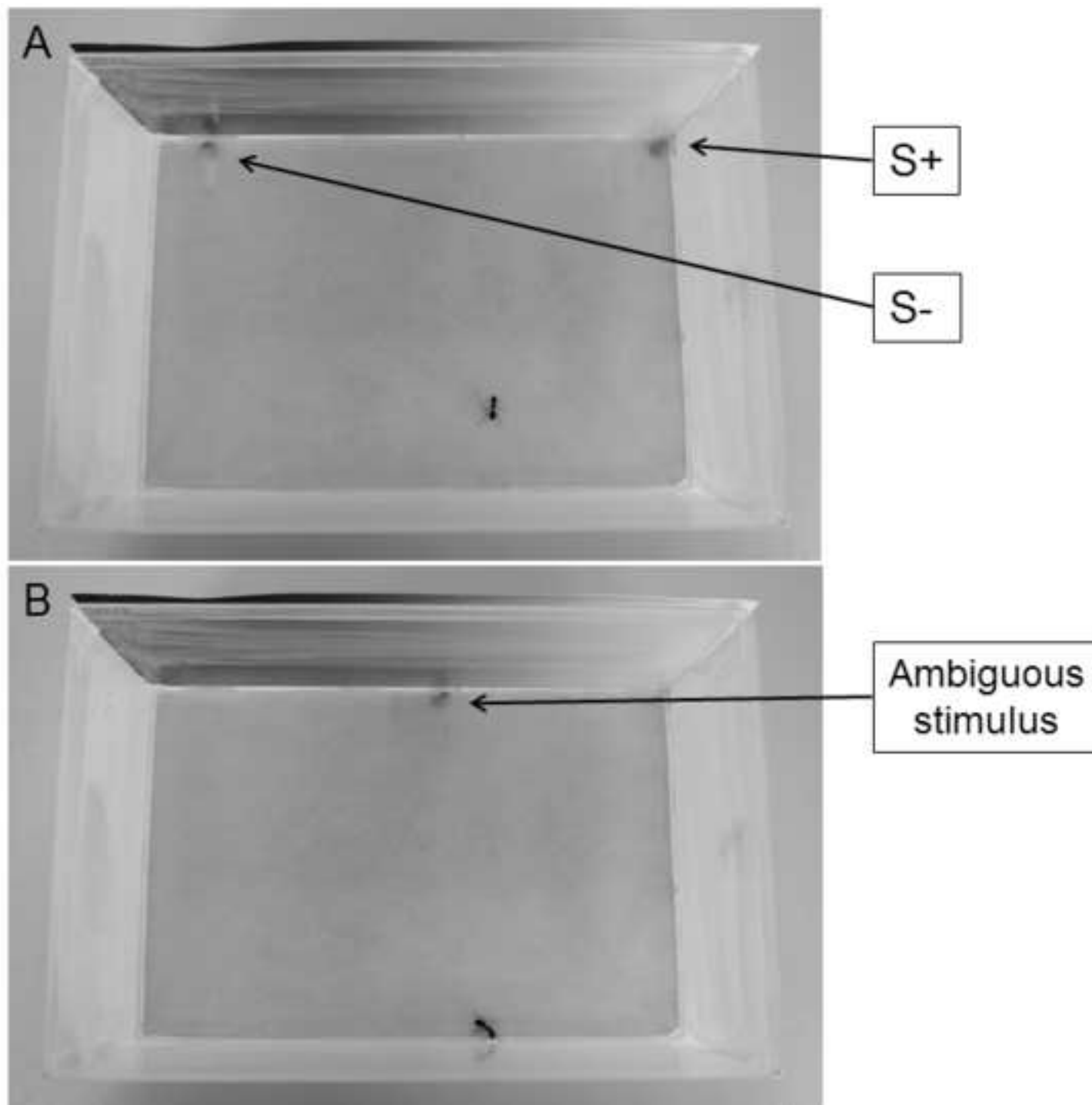
Central zone

Peripheral zone

Figure 2
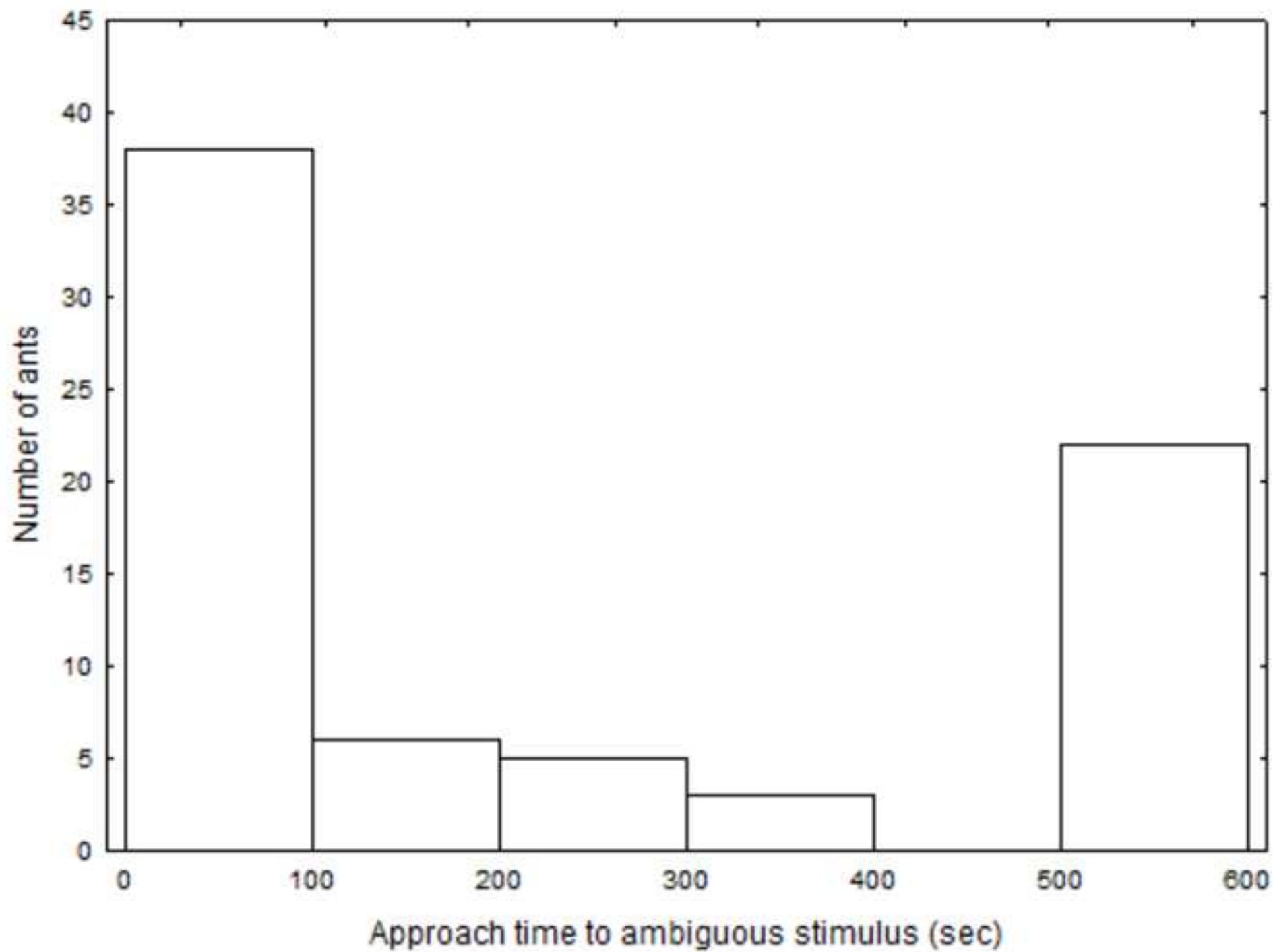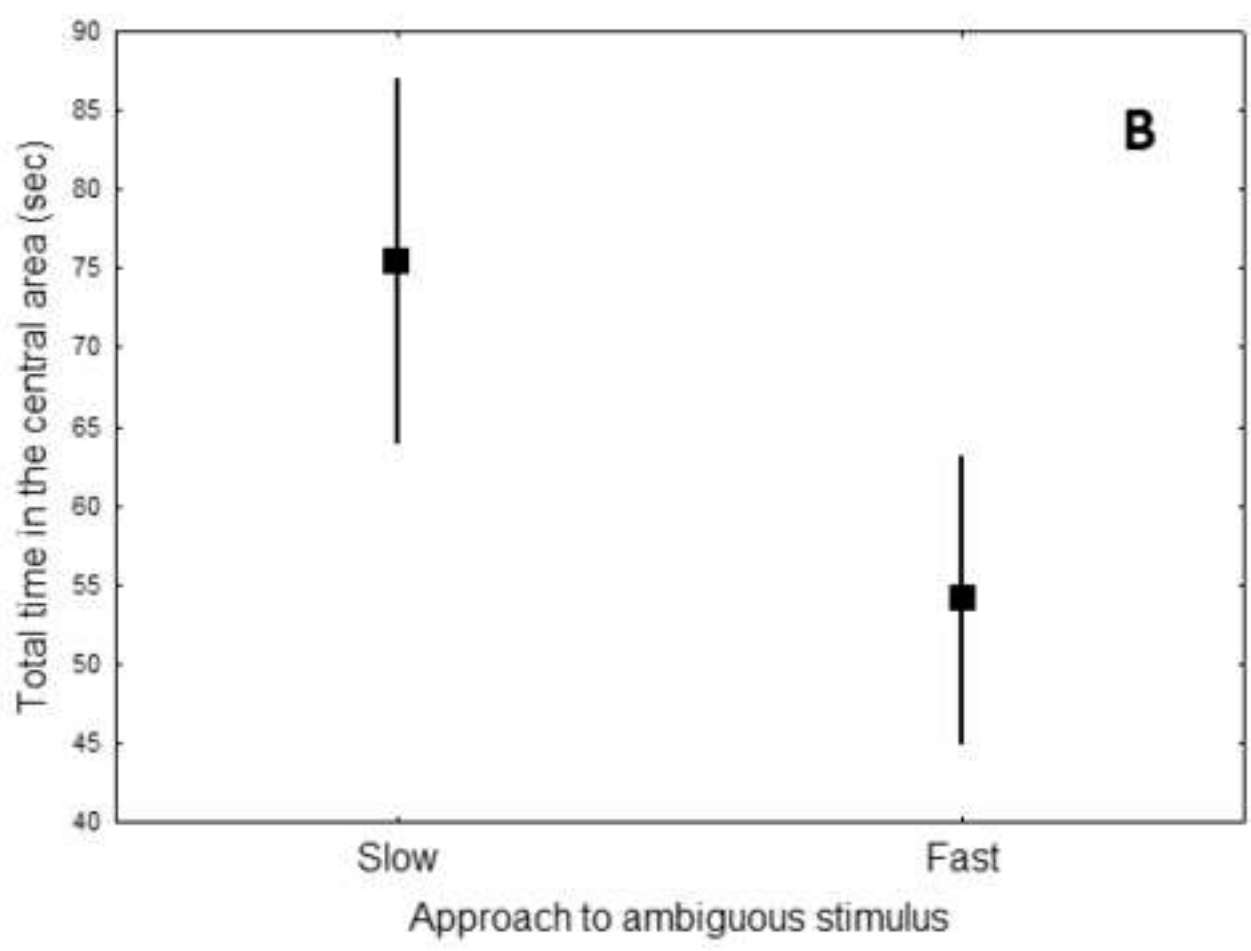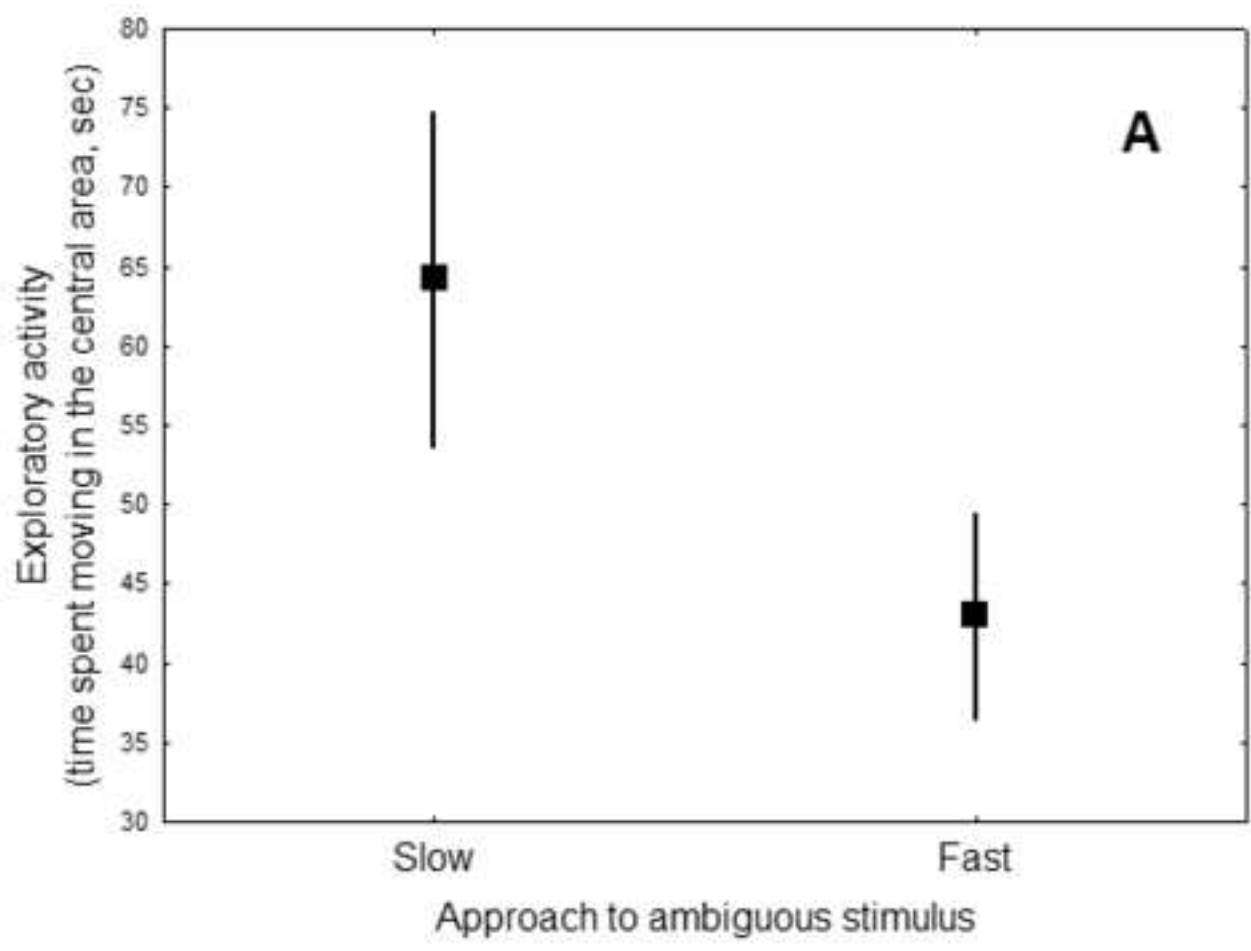[Click here to download high resolution image](#)

Figure 3

Figure 4
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