Encoding lateralization of jump kinematics and eye use in a locust via bio-robotic artifacts

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ABSTRACT

The effect of previous exposure to lateral sensory stimuli in shaping the response to subsequent symmetric stimuli represents an important overlooked issue in neuroethology, with special reference to arthropods. In this research, we investigated the hypothesis to 'programme' jumping escape direction as well as surveillance orientation in young and adult individuals of *Locusta migratoria* as an adaptive consequence of prior exposure to directional-biased predator approaches generated by a robotic leopard gecko representing *Eublepharis macularius*. The manipulation of the jumping escape direction was successfully achieved in young locusts, although young *L. migratoria* did not exhibit innately lateralized jumping escapes. Jumping escape direction was also successfully manipulated in adult locusts, which exhibited innate lateralized jumping escape at the individual level. The innate lateralization of each instar of *L. migratoria* in using a preferential eye during surveillance was not affected by prior lateralized exposure to the robotic gecko. Our results indicate a high plasticity of the escape motor outputs that are occurring almost in real time with the perceived stimuli, making them greatly adaptable and compliant to environmental changes in order to be effective and reliable. In addition, surveillance lateralization innately occurs at population level in each instar of *L. migratoria*. Therefore, its low forgeability by environmental factors would avoid disorganization at swarm level and improve swarm coordination during group tasks. These findings are consistent with the fact that, as in vertebrates, in insects the right hemisphere is specialized in controlling fear and escape functions.

INTRODUCTION

Escape and surveillance responses to predators represent profitable models to study the conversion process of sensory signals into motor outputs in an organism. These behaviours have been found to be implemented by specialized neural circuits in a number of animal species (Wang and Frost, 1992; Graziano et al., 1994; Wicklein and Strausfeld, 2000; Yamamoto et al., 2003; Preuss et al., 2006; Oliva et al., 2007; Fotowat et al., 2009). However, basic knowledge on how these sensorimotor sequences are affected by visual experience and how this influences lateralization is extremely scarce. Lateralization (i.e. the different specialization of the left and right sides of the brain, producing left–right asymmetries in the behaviour) is a fundamental principle of the brain arrangement widely described in vertebrates (Rogers et al., 2013a; Vallortigara et al., 2011; Vallortigara and Rogers, 2005; Vallortigara and Versace, 2017). Interestingly, although invertebrates have a relatively compact nervous system, lateralized traits were also identified in these species and reported by a growing number of studies (Ades and Ramires, 2002; Backwell et al., 2007; Benelli et al., 2015a,b,c; Rigosi et al., 2015; Rogers and Vallortigara, 2008, 2015; Rogers et al., 2013b, 2016; Romano et al., 2015, 2016a,b, 2018a; Benelli, 2018; Benelli and Romano, 2019). Understanding how prior context experience affects the processing of sensory stimuli by an asymmetrical nervous system (e.g. to generate complex actions such as anti-predator behaviours) can represent an important goal in computational neuroscience. Indeed, it would add significant information to formulate advanced mathematical models on visuomotor neuro-machinery involving sensory processing, brain specialization, cognition and learning. Furthermore, it can provide important insights on predator–prey interaction dynamics.

The suborder Caelifera (Orthoptera) includes remarkable biological models to assess these issues, since the brain neuro-architecture is well described in these insects (Rind, 2002; Santer et al., 2006, 2008; Kurylas et al., 2008; Fotowat et al., 2011). In particular, in Caelifera – such as locusts – each optic lobe (included in the protocerebrum) contains a neuron [the lobula giant movement detector (LGMD)] at the level of the third neuropil that responds specifically to looming stimuli (e.g. an approaching predator) (Santer et al., 2006, 2008; Fotowat et al., 2011) by producing trains of spikes transmitted to the descending contralateral movement detector (DCMD) that conveys visual information to motor centres (O'shea et al., 1974; Simmons, 1980; Fotowat et al., 2011). However, sky compass orientation (Homberg, 2004) and, as reported in flies, right–left motor coordination (Strauss and Heisenberg, 1993; Strauss, 2002), as well as visual memory (Liu et al., 2006), are regulated by the central complex,

52 which is another area included in the protocerebrum (Fotowat et al., 2011). Locusts have been reported
53 to be lateralized at an individual level and, interestingly, show refined motor control in the preferred
54 forelimb (Bell and Niven, 2014, 2016). Additionally, locusts were found to be lateralized at an individual
55 level during escape from a predator, and were lateralized at a population level during predator
56 surveillance (Romano et al., 2017a).
57 An important issue concerns the basic knowledge on the network connecting sensory signals with
58 asymmetrical motor outputs/orientations, and how they are modulated by experience. To manipulate a
59 predator–prey interaction, we took a bio-hybrid approach, which involved developing a robotic
60 apparatus that actuates an artificial agent mimicking a predator of locusts, the leopard
61 gecko *Eublepharis macularius* (Blyth 1854) (Squamata: Eublepharidae) (Thorogood and Whimsterf,
62 1979; Cooper and Williams, 2014).
63 In highly unstructured natural environments, many harmless objects move into these scenarios (e.g.
64 leaves and twigs moved by the wind). Thus, it looks conceivable that prey can identify some selected
65 cues to recognize a threat (Karplus and Algom, 1981; Honma et al., 2006) to escape only if necessary and
66 reduce the cost of the escape (Ydenberg and Dill, 1986). A number of researches relied on biomimetic
67 dummies resembling real predators to study lateralization of the escape responses (Facchin et al.,
68 1999; Lippolis et al., 2002, 2005), staging a more likely predator–prey interaction. Many other studies
69 have successfully elicited escapes with looming disks in several animal species, such as frogs and
70 insects (e.g. Ingle, 1973; Schlotterer, 1977; Santer et al., 2005; Peron and Gabbiani, 2009; Rodriguez-
71 Romaguera and Stuber, 2018).
72 However, the emerging scientific field of ethorobotics offers new paradigms of experimental
73 manipulations of intraspecific and interspecific interactions (Todd, 1993; Webb, 1995, 2000; Halloy et al.,
74 2007; Partan et al., 2009; Krause et al., 2011; Romano et al., 2017a). Therefore, ethorobotics enables the
75 achievement of highly standardized and controllable testing in animal behavioural research, by avoiding
76 mutual influences and/or feedbacks between multiple animals (Herbert-Read et al., 2012; Jolles et al.,
77 2017; Harcourt et al., 2009).
78 Furthermore, biologically inspired robots exhibit 3-dimensional life-like cues that can be perceived more
79 realistically by animals (Polverino et al., 2012; Halloy et al., 2013; Romano et al., 2017b, 2018b; Bonnet et al.,
80 2018; Kim et al., 2018; Bierbach et al., 2018), compared with other synthetic approaches (Tinbergen,
81 1951; Lippolis et al., 2002; Woo and Rieucau, 2011). Conversely, the study of living organisms with this bio-
82 hybrid strategy can leverage robot design and improve the development of advanced bioinspired
83 artifacts that efficiently perceive the environment and behave within it (Ijspeert et al., 2005; Wood, 2008; Li
84 et al., 2012; Stefanini et al., 2012; Bonsignori et al., 2013; El Daou et al., 2014; Daler et al., 2015).
85 Herein, gregarious individuals of *Locusta migratoria* (Linnaeus 1758) (Orthoptera: Acrididae) were
86 trained by using a robotic leopard gecko in order to observe directional-biased predator approaches.
87 Since antipredator behaviour importantly contributes to survival and fitness boosting in animals, we
88 investigated the hypothesis that we could 'programme' the locust jumping escape direction as well as
89 surveillance orientation as an adaptive consequence of prior exposure to the robotic predator in
90 lateralized training sessions. Furthermore, the jumping escape lateralization in locusts is age-related,
91 while surveillance asymmetry is equally exhibited among different instars (Romano et al., 2017a). Based
92 on this, herein we evaluated whether the developmental stage has a sensible role in the modulation of
93 antipredator responses in this species.

MATERIALS AND METHODS

Ethic statements

96 This research adheres to the guidelines for the treatment of animals in behavioural research and
97 teaching (ASAB/ABS, 2014) as well as the Italian and EU laws (D.M. 116192 and European Commission,
98 2007, respectively). All experiments are behavioural observations, and no specific permits are required
99 in the country where the experiments were conducted.

Insect rearing and general observations

101 Locusts were fed *ad libitum* with wheat, vegetables and water (Bell and Niven, 2016), and maintained at
102 25±1°C, 55±5% relative humidity (R.H.) with a 16 h:8 h light:dark photoperiod. Second-instar, fourth-

103 instar and adult *L. migratoria* individuals of both sexes were tested. Experiments were conducted in the
104 laboratory under the same experimental conditions described above. Light intensity around the test
105 arena was about 1000 lux. The behaviour of locusts was directly recorded by an observer during the
106 experiments (Benelli et al., 2015d; Romano et al., 2017a). A white wall of filter paper (Whatman)
107 surrounded the arena and the observer was dressed in a white coat to minimize his impact on *L.*
108 *migratoria* behaviour (Romano et al., 2016b, 2017a).

Robotic leopard gecko and experimental apparatus

110 A gecko replica of *E. macularius* was designed in SolidWorks (Dassault Systemes, Vélizy-Villacoublay,
111 France) and fabricated by rapid prototyping in acrylonitrile butadiene styrene (ABS). The biomimetic
112 morphology of the *E. macularius* replica includes the head with the mouth, two eyes and nostrils, main
113 body and the two forelegs with feet. The leopard gecko replica has a total length of 107 mm and a total
114 width of 44 mm, reasonably corresponding to the size of *E. macularius* (Kratovíl and Frynta, 2002), as it
115 reproduces only a portion of the body of the authentic lizard.
116 To produce a similar colour pattern and the rough skin texture of a real *E. macularius*, the artifact was
117 endowed with a skin-like coating, obtained by mixing a transparent liquid silicone rubber (Dragon Skin,
118 Smooth-On, Pennsylvania, USA) with nontoxic pigments and used to cover the leopard gecko replica
119 by turning (rotational) moulding (Romano et al., 2017a). The leopard gecko replica was connected with a
120 DC gearmotor (model: 212-103), forming a robotic arm.
121 In order to integrate the robotic platform, the leopard gecko replica was inserted in a dedicated slot in
122 the middle of one of the shorter sides of a support. The support includes a flat top surface (150×220
123 mm), and four cylindrical legs (120 mm, diameter 20 mm) in polyoxymethylene (POM), fabricated using
124 a computer numerical control (CNC) machine.

125 When the DC gearmotor is activated, the leopard gecko replica can be vertically rotated from the
126 bottom of the test bench to the horizontal plane on the top surface. Even if one can argue that this
127 movement did not carbon-copy an attack by a living gecko, the predatory event displayed by the robotic
128 leopard gecko was perfectly symmetric in appearance and movement to avoid any lateral bias in
129 locusts during the experiments. Fig. 1A and B show the robotic leopard gecko lying on the flat top
130 surface with a locust frontally placed, as well as the jumping escape of a locust during the robotic
131 leopard gecko approach, respectively. The activation of the leopard gecko replica's attack was
132 performed via a microcontroller (Arduino, Mega 2560).
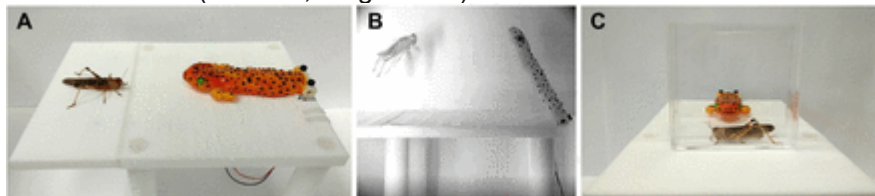
133
134 **Fig. 1.**
135 **The animal–robot interaction.** (A) The robotic leopard gecko and a locust (*Locusta migratoria*) on the top
136 surface of the experimental apparatus. (B) Snapshot of a bio-hybrid predator–prey interaction, showing
137 the approaching robotic leopard gecko and an escaping locust. (C) A locust in the transparent cage with
138 the right side exposed to the predator-mimicking robot during the training phase.

Training phase

140 Locusts were individually released in a transparent cage (80×15×80 mm for adults; 40×15×80 mm for
141 neanids and nymphs), wide enough to prevent the constraining of their natural posture but significantly
142 reducing insect locomotion. This enabled us to impose a given orientation during the simulated attacks
143 by the robotic leopard gecko. The transparent cage containing the locust was placed on the top surface
144 of the test bench, perpendicularly to the longitudinal body axis of the robotic leopard gecko at a
145 distance>70 mm from its mouth when it lies on the horizontal plane (see Fig. 1C). The posture of *L.*
146 *migratoria* was constantly monitored during the training phase to ensure correct orientation. The training
147 phase lasted 60 min, in which the robotic leopard gecko appeared from the bottom of the test bench
148 (not visible to the locust) to the horizontal plane of the top surface, and then returned, at intervals of 30
149 s. According to the side of the locust that was exposed to the simulated predator, we obtained left/right-
150 trained locusts.

151 It is well acknowledged that just the risk of predation can produce physiological stresses in a wide
152 number of species. These stresses have been reported to have long-lasting influences on prey escape
153 responses (Cannon, 1915; Selye, 1936; Hawlena et al., 2011). Thus, although the robotic leopard gecko did
154 not physically injure the locusts (this would not be acceptable from an ethical point of view), its
155 presence as a potential predator and its simulated attack would affect the locust escape response.
156 Before training a new locust, the whole setup was rotated manually 90 deg horizontally in a clockwise
157 direction, to randomize its orientation and to avoid positional effects (Canale et al., 2015). Therefore, at
158 the end of a training we removed the locust and rotated the setup. Soon after the rotation of the setup,
159 a new locust was introduced.
160 The control treatments were similarly conducted, with the exceptions that the robotic leopard gecko was
161 not visible to the locusts (i.e. hidden below the test bench), and no simulated attacks were displayed.
162 The interval between the training phase and the experiments described below was 120 min, during
163 which locusts were isolated from other subjects to avoid subsequent experiences affecting the effect of
164 the training.

Experiment 1: modulating the jumping escape lateralization

166 Here, the possibility to condition the direction of the jumping escape response by providing a symmetric
167 stimulus to previously trained locusts was evaluated. *Locusta migratoria* were trained by presentation of
168 the robotic stimulus to the left or right compound eye, and were placed individually [as were control
169 subjects (naïve)] on the top surface of the test bench, with their longitudinal body axis accurately
170 centred (±0.5 deg) with the longitudinal body axis of the robotic leopard gecko at a distance of ≃70 mm
171 from its mouth when it lies on the horizontal plane. The robotic apparatus was placed in the centre of a
172 rectangular white arena (800×600×600 mm), equidistant from the left and right side, to minimize
173 external cues affecting the locust's behaviour. At the beginning of the test, the robotic leopard gecko
174 was hidden below the test bench and thus not visible to the tested locust.
175 The jumping escape direction of the locust was recorded following the robotic leopard gecko rotation
176 from the bottom of the test bench to the horizontal plane on the top surface (ω=4.97 rad s^{-1}), emulating
177 a predator that comes out of a hiding place (see Fig. 2A). Locusts that were not accurately centred with
178 the robotic leopard gecko when approached by it were not considered for laterality observations.
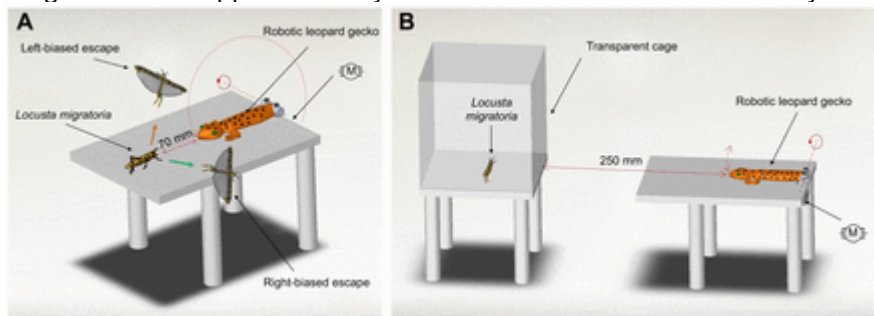
179
180 Fig. 2.
181 **Experimental setup.** Schematic illustrations of (A) experiment 1 and (B) experiment 2.
182 For each replicate, the whole setup was rotated manually as described in the 'Training phase'
183 paragraph, to avoid positional effects.

184 For each developmental stage of *L. migratoria* considered in our study (e.g. second instar, fourth instar
185 and adult), 25 naïve subjects, 25 left-trained subjects and 25 right-trained subjects, escaping after a
186 perfectly symmetric predator–prey interaction, were analyzed. The direction of 30 jumps, delayed 10
187 min from each other, was recorded for each insect (Romano et al., 2017a).

Experiment 2: modulating the surveillance lateralization

189 Here, the assumption to manipulate the eye use preference during surveillance in locusts by a prior bio-
190 robotic interaction was evaluated. A transparent cubic cage (150×150×150 mm) containing a locust was
191 placed in the middle of a rectangular white arena (1300×900×600 mm) at the same distance from the
192 right and left side of the arena. The cubic cage was positioned at 250 mm from the robotic leopard
193 gecko mouth when it lies on the horizontal plane. The floor of the cubic cage and the top surface of the
194 test bench were positioned on the same horizontal plane.

195 Before starting the test, the robotic leopard gecko was not visible to the tested locust since it was
196 hidden below the test bench. After 5 min from the introduction of the locust inside the transparent cage,
197 the test started, and the robotic leopard gecko rotated from the bottom of the test bench to the
198 horizontal plane on the top surface. Then, for the whole duration of this experiment, a still phase lasting
199 15 s, in which the robotic leopard gecko was motionless on the top surface, was alternated with a pitch
200 phase, lasting 5 s. During the pitch phase, the robotic leopard gecko rotated 30 deg from bottom to top
201 and then returned (5 Hz) (see Fig. 2B). The larger distance of the robotic agent from the locust,
202 compared with the experiment 1, and the short angle and duration of the pitch phases ensured to better
203 select cryptic and surveillance behaviours (Eterovick et al., 1997; Ruxton et al., 2004; Ruxton, 2006) and to
204 avoid an excessive threat triggering an escape response.
205 The test lasted 30 min, and the exposure duration of orientation of each side of the locust body to the
206 robotic leopard gecko was recorded for each animal by following the method by Romano et al. (2017a). In
207 particular, to ensure the monocular vision of the robot by the locust, only locusts with their steered body
208 axis forming an angle >45 deg with the initial orientation of their body axis, aligned with the stimulus,
209 were considered for laterality observations (Horridge, 1977; Kral and Poteser, 1997). For each replicate,
210 the whole setup was rotated manually as described in the 'Training phase' paragraph, to avoid
211 positional effects. A total of 25 naïve subjects, 25 left-trained subjects and 25 right-trained subjects
212 were tested for each *L. migratoria* developmental stage observed in this research (e.g. second instar,
213 fourth instar and adult).

214 **Statistical analysis**

215 To analyze the differences in the direction of jumping escape responses, as well as in the use of the
216 right and left eye during predator surveillance, a laterality index (LI) was calculated for each insect,
217 following the method by Frasnelli et al., (2012): $LI=(R–L/R+L)$.
218 For the predator escape behaviour, R and L indicate, respectively, the normalized mean number of
219 times in which each locust jumped to the right or to the left. A score of 1.0 indicated exclusive
220 preference to jump to the right, while a score of −1.0 indicated exclusive preference to jump to the left.
221 A score of 0 indicated equal numbers of right and left jumps during jumping escape acts.
222 For the predator surveillance behaviour, R and L indicate, respectively, the normalized mean value of
223 the duration in which each locust used the right eye or the left eye to oversee the robotic predator. A
224 score of 1.0 indicated exclusive use of the right eye, while a score of −1.0 indicated exclusive use of the
225 left eye. A score of 0 indicated equal duration in using the right and left eye during surveillance.
226 Furthermore, the absolute value of the laterality index (ABLI) was considered, to discriminate
227 individuals with a bilateral dominance from individuals with a lateral dominance, regardless of the left or
228 the right direction of the bias, and to evaluate the strength of lateralization (Bisazza et al., 2000; Seghier,
229 2008; Romano et al., 2017a).
230 Laterality differences among naïve, right-trained and left-trained locusts over different instars (second
231 young instars, fourth young instars and adults) displaying right- or left-biased jumping escapes, as well
232 as right- or left-biased eye use during surveillance, were analyzed by JMP 9 (SAS) using a general
233 linear model with two factors, i.e. the tested naïve/trained instar and laterality. $P<0.05$ was used to
234 assess the significance of differences between means. Moreover, for each treatment, the difference in
235 the number of locusts jumping to the right or left as well as using left or right eyes during the interaction
236 with the biomimetic gecko predator was analyzed using a χ^2 test with Yates' correction ($P<0.05$).

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237 **RESULTS**

238 **Experiment 1: modulating the jumping escape lateralization**

239 The direction of motor outputs towards left or right during the jumping escape from the robotic leopard
240 gecko was successfully manipulated in all locust instars following exposure in the training phase, thus
241 allowing to control the lateralization of the jumping escape at a population level. The population mean
242 value of the jumping escape's LI was significantly modulated by different contexts of the training phase
243 ($F_{8,224}=41.077$; $P<0.0001$). The LI of each left-trained locust instar shows the preference to jump to the
244 right. The LI of each naïve instar shows an equal preference of these locusts to jump to the right and to

245　the left. The LI of each right-trained locust instar shows the preference to jump to the left
246　(**Fig. 3**A, Table S1).
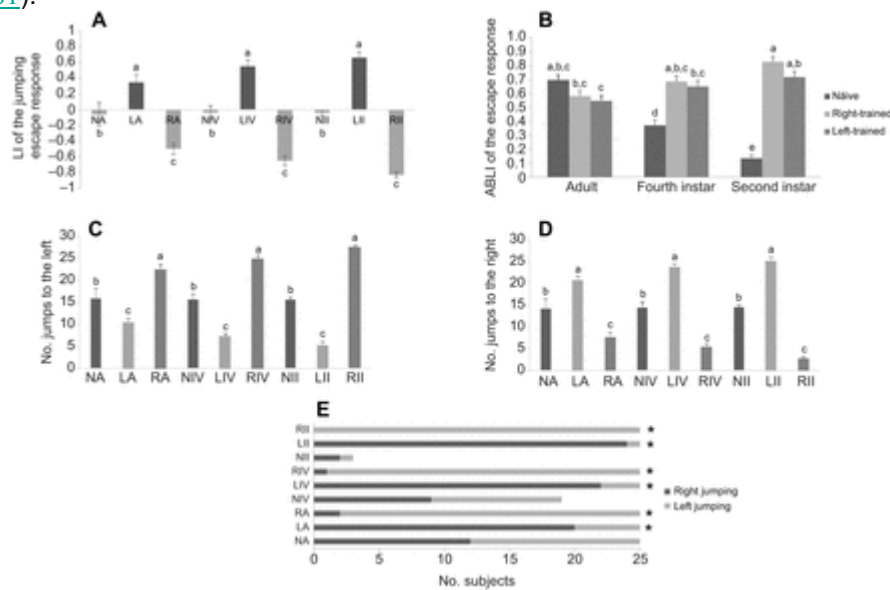
247
248　**Fig. 3.**
249　**Experiment 1: laterality index (LI), absolute value of the laterality index (ABLI) and direction bias in**
250　**jumping escape.** (A) LI, (B) ABLI, (C) left- and (D) right-biased jumps characterizing the jumping escape
251　response of different trained *L. migratoria* during the symmetric exposure to the robotic leopard gecko.
252　Lowercase letters near each bar indicate significant differences ($P<0.05$). T-bars represent s.e.m. (E)
253　Left- and right-biased *L. migratoria* showing a lateralized jumping escape during the symmetric
254　exposure to a robotic leopard gecko. Asterisks indicate significant differences between left- and right-
255　biased locusts ($P<0.05$). NA, naïve adult; LA, left-trained adult; RA, right-trained adult; NIV, naïve fourth
256　instar; LIV, left-trained fourth instar; RIV, right-trained fourth instar; NII, naïve second instar; LII, left-
257　trained second instar; RII, right-trained second instar.
258
259　The ABLI was significantly modulated by the training phase ($F_{8,224}=31.684$; $P<0.0001$). In adults, the
260　ABLI was marginally higher in naïve subjects compared with right-trained locusts, and significantly
261　higher compared with left-trained locusts. In fourth instar locusts, the ABLI of both right-trained and left-
262　trained subjects was significantly higher compared with the ABLI of naïve locusts. In second instar
263　locusts, the ABLI of right-trained subjects as well as left-trained insects was significantly higher
264　compared with the ABLI of naïve locusts (**Fig. 3**B, Table S1).
265　The number of jumps to the left was importantly affected by the training phase ($F_{8,224}=41.080$; $P<0.0001$).
266　For each locust instar considered, the number of left jumps was higher in right-trained insects
267　compared with naïve individuals. In addition, the number of left jumps was lower in left-trained insects
268　compared with naïve individuals (**Fig. 3**C, Table S1).
269　The number of jumps to the right was significantly affected by the training phase
270　($F_{8,224}=41.071$; $P<0.0001$). Regardless of the tested locust instars, the number of right jumps was higher
271　in left-trained individuals compared with naïve individuals, and the number of right jumps was lower in
272　right-trained locusts compared with naïve individuals (**Fig. 3**D, Table S1).
273　The lateralization of the jumping escape at population level was successfully determined in each locust
274　instar involved in a training context (**Fig. 3**E, Table S1). The number of naïve adult locusts that
275　preferentially jumped to the left did not significantly differ from the number of naïve adult locusts that
276　preferentially jumped to the right (left versus right: 13 versus 12; $\chi^2_1=0.001$; $P=0.99$). The same results
277　were observed testing naïve fourth instar locusts (left versus right: 10 versus 9; $\chi^2_1=0.001$; $P=0.99$), as
278　well as naïve second instar locusts (left versus right: 2 versus 1; $\chi^2_1=0.001$; $P=0.99$).
279　Right-trained locusts preferentially jumped to the left when approached by the robotic-borne
280　combination of cues, in each instar considered, including adult locusts (left versus right: 23 versus 2;
281　$\chi^2_1=16$; $P<0.0001$), fourth instar locusts (left versus right: 24 versus 1; $\chi^2_1=19.36$; $P<0.001$) and second
282　instar locusts (left versus right: 25 versus 0; $\chi^2_1=23.04$; $P<0.001$) (Table S1).
283　Left-trained locusts preferentially jumped to the right when approached by the robotic leopard gecko.
284　This was noted for adult locusts (left versus right: 5 versus 20; $\chi^2_1=7.84$; $P=0.0051$), fourth instar locusts
285　(left versus right: 3 versus 22; $\chi^2_1=12.96$; $P=0.0003$) and second instar locusts (left versus right: 1
286　versus 24; $\chi^2_1=19.36$; $P<0.0001$) (Table S1).

Experiment 2: modulating the surveillance lateralization

This experiment showed innate lateralization of *L. migratoria* using a preferential eye during surveillance. The poor plasticity of this feature to adapt to environmental perturbations (e.g. a predator-mimicking robotic stimulus) was also reported. The population mean value of the LI of eye use was not significantly influenced by different contexts of the training phase ($F_{8,224}$=0.936; P=0.486). Each training phase produced right-biased locusts, regardless of the instar of the insects (**Fig. 4**A, Table S2).
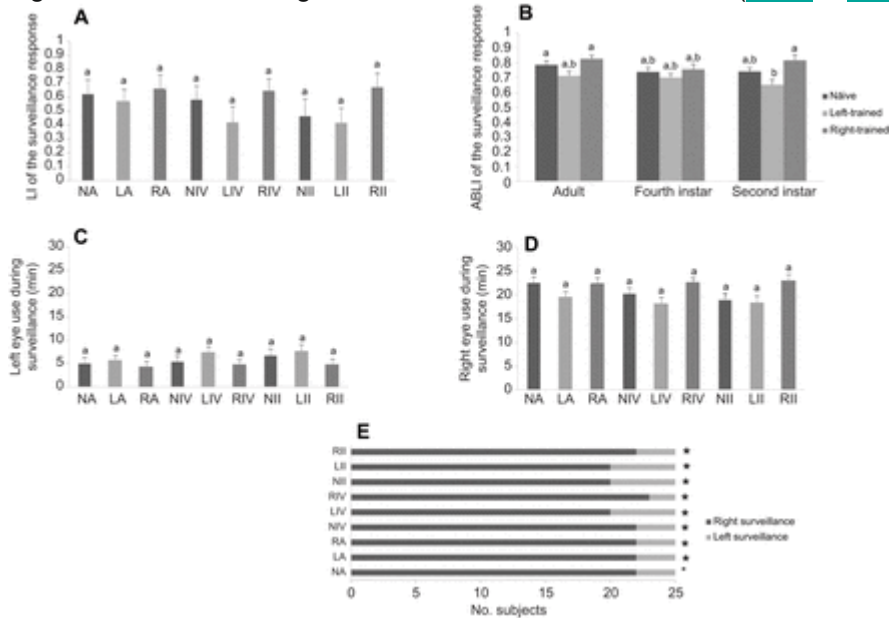
Fig. 4.

Experiment 2: LI, ABLI and direction bias in surveillance. (A) LI, (B) ABLI, (C) left- and (D) right-biased eye use characterizing surveillance of different trained *L. migratoria* during the symmetric exposure to the robotic leopard gecko. Lowercase letters near each bar indicate significant differences (P<0.05). T-bars represent s.e.m. (E) Left- and right-biased *L. migratoria* during the surveillance of a robotic leopard gecko. Asterisks indicate significant differences between left- and right-biased locusts (P<0.05). NA, naïve adult; LA, left-trained adult; RA, right-trained adult; NIV, naïve fourth instar; LIV, left-trained fourth instar; RIV, right-trained fourth instar; NII, naïve second instar; LII, left-trained second instar; RII, right-trained second instar.

The ABLI was marginally modulated by the training phase ($F_{8,224}$=3.585; P=0.0006). Compared with naïve subjects of each developmental stage, only left-trained adults, as well as left-trained and right-trained second instar locusts, had a marginally different ABLI (**Fig. 4**B, Table S2).

The duration of time intervals in which each locust used the left eye to oversee the robotic predator was not importantly affected by the training phase ($F_{8,224}$=0.919; P=0.501). For each locust instar, the duration of the left eye use to oversee the robotic leopard gecko was similar for naïve, left-trained and right-trained subjects (**Fig. 4**C, Table S2).

The duration of time intervals in which each locust used the right eye to oversee the robotic predator was marginally affected by the training phase ($F_{8,224}$=1.899; P=0.061). Naïve, left-trained and right-trained locusts performed the surveillance with the right eye with a similar duration in each locust instar considered (**Fig. 4**D, Table S2).

Surveillance lateralization at population level was not affected by training (**Fig. 4**E, Table S2). The number of naïve adults (left versus right: 3 versus 22; χ^2_1=12.96; P=0.00031), left-trained adults (left versus right: 3 versus 22; χ^2_1=12.96; P=0.00031) and right-trained adults (left versus right: 3 versus 22; χ^2_1=12.96; P=0.00031) that preferentially used the right eye to oversee the robotic leopard gecko was significantly higher compared with naïve, left-trained and right-trained adults that preferentially used the left eye during surveillance (Table S2). Also, naïve fourth instar locusts (left versus right: 3 versus 22; χ^2_1=12.96; P=0.0003), left-trained fourth instar locusts (left versus right: 5 versus 20; χ^2_1=7.84; P=0.0051) and right-trained fourth instar locusts (left versus right: 2 versus 23; χ^2_1=16; P<0.0001), as well as naïve second instar locusts (left versus right: 5 versus 20; χ^2_1=7.84; P=0.0051), left-trained second instar locusts (left versus right: 5 versus 20; χ^2_1=7.84; P=0.0051) and right-trained second instar locusts (left versus right: 3 versus 22; χ^2_1=12.96; P=0.00031), preferentially used the right eye to oversee the robotic leopard gecko (Table S2).

DISCUSSION

How earlier lateral sensory stimuli, for example generated by an approaching artifact mimicking a predator, are processed by the nervous system, and how they produce a lateralized behaviour in the subsequent presence of symmetric stimuli, represents a novel and important area of investigation in relation to the evolutionary neuro-behavioural ecology of a species. Here, we used *L. migratoria* as a model organism, since the locust nervous system has been deeply investigated (Rind, 2002; Santer et al., 2006; 2008; Kurylas et al., 2008; Fotowat et al., 2011), reporting these insects as innately lateralized in several behaviours (Bell and Niven, 2014, 2016; Romano et al., 2017a). To maximize the standardization and accuracy of the experimental conditions in such delicate testing contexts, a remarkable role is played by robotic agents, which enable researchers to establish bio-hybrid interactions with animals (Todd, 1993; Webb, 1995, 2000; Halloy et al., 2007; Partan et al., 2009; Krause et al., 2011; Polverino et al., 2012; Romano et al., 2017a,b; Bonnet et al., 2018; Kim et al., 2018; Bierbach et al., 2018). However, further efforts are needed to provide an in-depth analysis comparing the effect of different strategies used to provide stimuli evoking antipredator behaviours (Dill, 1974).

The results reported in this study offer new and fascinating insights on how a lateralized brain reacts and adapts to dynamic events that are crucial for survival, such as predator–prey interactions (Bonsall and Hassell, 2007; Millon and Bretagnolle, 2008; Dessborn et al., 2009). The most interesting fact is that the same visual cues (e.g. robotic-borne stimuli), laterally perceived during the training phase, were able to influence the jumping direction of subsequent escape responses to symmetric stimuli, but they did not affect the preferential eye use for surveillance (**Figs 3** and **4**). This indicates a high plasticity of those escape motor outputs that are occurring almost in real time with the perceived stimuli, making them greatly adaptable and compliant to environmental changes in order to be effective and reliable. In particular, we observed that locusts can adapt the direction of the jumping escape to external asymmetric events repeatedly occurring, and to maintain this preference when no stimuli or symmetric stimuli are presented.

We found that the main factor conditioning training is represented by vision, directing sensory information to motor centres and likely producing motor learning at the level of the prothoracic ganglion of locusts (Horridge, 1962; Rowell, 1961, 1964). In fact, motor action was not involved, because of external constraints that prevented jumping escapes. Although insects could make limited movements inside the cage during the training phase, they could not display a real jumping escape.

It has been reported that several species are right-biased during escape responses, while others are left-biased (Cantalupo et al., 1995; Lippolis et al., 2009; Bonati et al., 2010). In populations with a high risk of predation, prey prefer to observe the predator with a given eye over the other (Brown et al., 2004). Several vertebrate species exhibit a higher reactivity when they are approached by predators from their left visual field (controlled by their right hemisphere) (Lippolis et al., 2002, 2005; Austin and Rogers, 2007), if compared to the right visual field. These studies confirm that, in vertebrates, the right hemisphere is specialized for the control of fear and escape responses (Lippolis et al., 2005), potentially representing a phylogenetic ancient trait (Lippolis et al., 2002). Interestingly, our results are consistent with this right-hemisphere specialization, although the biological model here was an invertebrate. Indeed, in insects, each compound eye is connected with the ipsilateral optic lobe included in the protocerebrum (Strausfeld, 2005). Therefore, since locusts preferentially used the right compound eye for surveillance, we can assume that also in insects the right hemisphere of the cerebrum controls fear and escape functions.

In addition, surveillance lateralization has been found to have a strict programme to adhere to. This is probably part of a higher level of brain organization that includes other functions to be carried out in parallel (Vallortigara and Rogers, 2005; Frasnelli, 2013; Romano et al., 2017a). Furthermore, surveillance lateralization is a population-level feature innately occurring in *L. migratoria* aggregations, as reported by earlier research (Romano et al., 2017a): its low forgeability by environmental factors would avoid disorganization at swarm level and would improve swarm coordination during group tasks, as also confirmed by findings on other gregarious species (Chivers et al., 2016).

The highly lateralised response of young instars after the training with a leopard-gecko-mimicking robot is particularly interesting, although naïve young individuals do not exhibit innately lateralized jumping escapes (Romano et al., 2017a). This is in contrast with recent evidence in rats (Kurzina et al., 2018), where lateralized motor behaviours in young subjects were little affected by previous learning compared to adults. A possible explanation is consistent with the hypothesis that young individuals belonging to several mammalian species are largely depending on parental care. These mammal species first

develop cortical functions such as perception, language and cognition (Merzenich, 2001; Cornelissen et al., 2004; Shtyrov et al., 2010); consequently, motor learning is developed slower. Although young locusts do not present innately lateralized traits in the jumping escape, they have shown an impressive reactive motor learning system producing lateralized adaptive behaviours. This is probably due to their greater vulnerability to predation compared with adults (Gillett and Gonta, 1978), an aspect that is shared with many other prey species (Geist, 1971; Fitzgibbon, 1990).

The successful induction of lateralized jumping escape in young locusts, besides adults, can be related to an antipredator tactic based on early motor learning to environmental factors. Further efforts are needed to understand how motor learning is affected by lateralized stimuli in young insects belonging to species exhibiting parental care. Our findings add novel insights to different hypothesis on lateralization in vertebrates and invertebrates that would be determined by a common ancestor or by convergent evolution (Ghirlanda and Vallortigara, 2004; Frasnelli, 2013).

FOOTNOTES

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