To flee or to freeze in front of a predator? Young preys do not need to learn the best strategy

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ABSTRACT

Using appropriate anti-predatory responses is crucial for survival. While slowing down reduces the chances of being detected from distant predators, fleeing away is advantageous in front of an approaching predator. Whether appropriate responses depend on experience with moving objects is still an open question. To clarify whether adopting appropriate fleeing or freezing responses requires previous experience, we investigated responses of movement-naive chicks. When exposed to the moving cues mimicking an approaching predator (a rapidly expanding, looming stimulus), chicks displayed a fast escape response. In contrast, when presented with a distal threat (a small stimulus sweeping overhead) they decreased their speed, a maneuver useful to avoid detection. The fast expansion of the stimulus toward the subject, rather than its size *per se* or change in luminance, triggered the escape response. These results show that young animals, in the absence of previous experience, can use motion cues to select the appropriate responses to different threats. The adaptive needs of young preys are thus matched by spontaneous defensive mechanisms that do not require learning.

KEYWORDS: Anti-predatory behaviors, motion cues, threat detection, chicks, defense strategies, naive animals.

INTRODUCTION

Appropriate reactions to predators are fundamental for survival: primary defenses prevent detection by predators, while secondary defenses delay, inhibit or elude an approaching predator (1). This dichotomy, and the evidence that anti-predatory responses are commensurate with the perceived risk (2), show that preys can use predator-related cues to identify threats and respond accordingly. Visual cues of motion are particularly effective in triggering anti-predatory behaviors (3–9). For instance, mice rapidly detect overhead motion and assess the threat level posed by various stimuli, fleeing from displays mimicking an on-going attack (a looming stimulus), and freezing to the displays of a more distal threat (a small stimulus smoothly moving overhead (3). Whether these responses are spontaneous or mediated by learning is, however, an old debated question (10). Only scarce (if any) convincing empirical evidence supports the widespread idea that the choice of appropriate anti-predatory responses is innate, and that preys require no learning to use visual cues to adopt context-appropriate defensive behaviors (4, 7). It remains to clarify whether young preys are able to produce appropriate anti-predatory responses to different type of threats in the absence of learning.

Among highly predated animals, chicks are a good model system to address this issue. Chicks have a relatively mature sensory and motor system soon after hatching (11, 12) and enact anti-predatory/avoidance behaviors at the beginning of life (5, 7). Chickens possess a highly specialized vision, characterized by a large visual field (11) and lower-field myopia, enabling them to focus on the ground and at the same time to scan overhead (13). Galliformes are subjected to a high predation rate, both from terrestrial and aerial predators, and strongly react to both (14, 15). Chickens respond to a sweeping raptor model that moved overhead by displaying anti-predatory responses (6). The optimal response is observed for stimuli larger than 4° of visual angle, moving faster than 7.5 length/s. Interestingly, in front of such a stimulus 8-day old chicks exhibit defensive behaviors, ranging from peeping to running away (5). These precocial animals can easily be raised in a controlled environment (16). We thus tested the spontaneous, unlearned responses of chicks to moving stimuli presented overhead. We first determined whether chicks that had no experience with moving stimuli would modulate their responses to different overhead motion stimuli (Exp. 1). Then, we characterized the properties that triggered fleeing defensive responses [Exp. 2-3; (8, 9)].

RESULTS

Inexperienced chicks produce appropriate responses to different threatening stimuli

In Exp. 1, we examined whether chicks reared without experience with overhead movement react to different types of threat with appropriate responses. The immediate threat stimulus was a looming stimulus, whereas the distal threat stimulus was a sweeping stimulus (Fig. 1B).

During the presentations, chicks were faster in response to looming compared to sweeping stimuli (U=198, $r=0.526$, $p<0.001$; Fig. 1C). In response to rapidly expanding (looming) stimuli, that mimicked an immediate predator attack, chicks increased their speed (W=268, $r=0.472$, $p<0.01$; Fig. 1C; Movie S1). In response to a far sweeping stimulus, similar to the movement of a cruising raptor, chicks slowed down (W=-349, $r=-0.543$, $p<0.01$; Fig. 1C; Movie S2). Similar results were obtained for the speed 1s after the offset (U=132, $r=0.638$, $p<0.001$; *Looming:* W=228, $r=0.401$, $p<0.05$; *Sweeping*: W=-463, $r=-0.721$, $p<0.001$; Fig. 1D). The effects were long-lasting, since chicks presented with sweeping stimuli were still less active than chicks exposed to looming during the 30 s following stimuli offset (U=294, $r=0.365$, $p<0.01$; Fig. 1E).

A rapid expansion towards the subject triggers fast escape

We analyzed the features inducing the fast avoidance response to looming stimuli. In Exp. 2, we tested whether the direction of the movement (i.e. expansion), rather than a fast change or large angular size, was sufficient to elicit a rapid escape. Comparing chicks exposed to looming and receding stimuli (Fig. 1F), we observed that both during and after the presentations chicks exposed to looming stimuli were faster than chicks exposed to receding stimuli (*During*: U=259, $r=0.327$, $p<0.05$; *After*: U=290, $r=0.263$, $p<0.05$; Fig. 1G,H). A clear difference in the temporal dynamics of movements appeared: while no clear pattern of speed change was observed during the receding stimulus (W=-128, $r=-0.225$, $p>0.05$; Movie S3), the speed of the chicks exposed to the looming displays increased during the displays and came back to baseline after the offset (*During*: W=194, $r=0.448$, $p<0.05$; *After*: W=78, $r=0.18$, $p>0.05$; Fig. 1G,H). In contrast, a slight speed reduction was detected during the 1s period directly following the offset of the receding stimuli (W=-210, $r=-0.37$, $p<0.05$; Fig. 1H). This effect was transient though (distance travelled during the 30 s; U=406, $r=-0.026$, $p>0.05$; Fig. 1I).

In Exp. 3, we tested whether a change in luminance, a feature accompanying the expansion of the dark looming stimulus, is sufficient to trigger a fast escape response by comparing responses to dimming *vs.* looming stimuli (Fig. 1J). Both during and after the display, reactions of the chicks exposed to the looming and dimming stimuli differed (*During*: U=169, $r=-0.525$, $p<0.001$; *After*:

U=198, *r*=-0.467, *p*<0.001; Fig. 1K,L). While the fast increase in speed triggered by the looming stimulus disappeared after the offset (*During*: W=203, *r*=0.382, *p*<0.05; *After:* W=1, *r*=0.002, *p*>0.05; Fig. 1K,L), the dimming stimulus induced a strong decrease in speed both during and immediately after its display (*During*: W=-345, *r*=-0.693, *p*<0.001; *After*: W=-371, *r*=-0.745, *p*<0.001; Fig. 1K,L; Movie S4). However, this effect quickly faded (distance travelled during the 30 s; U=359, *r*=-0.15, *p*>0.05; Fig. 1M).

The results of Exp. 2-3 showed that the rapid expansion of the stimulus is responsible for the escape response from the looming stimulus. Further analysis revealed that this fast escape was initiated, if not earlier, 0.520 ms after the stimulus onset (Exp. 1-3; n=88. stimulus size: ± 24°; One-sample: W=1048, *r*=0.232, *p*<0.05).

DISCUSSION

Producing appropriate anti-predatory responses has a high adaptive value, and in different taxa preys exhibit differential responses to immediate and background threats (3, 17). For this reason, it is expected that evolutionary pressures have equipped preys with mechanisms to counteract predators in different situations. Lorenz and Tinbergen suggested that avian species spontaneously exhibit stronger anti-predatory reactions to short-neck (predator birds) *vs.* long-neck (non-predator birds) dummies, in line with their idea of innate releasing mechanisms (18, 19). Their report on greater anti-predatory responses, though, has been contradicted multiple times [see (10)]. Tinbergen himself shifted his view to an experience-dependent explanation (selective habituation hypothesis). Based on the little and contradictory evidence available (4, 7, 10), the question is still open.

To clarify whether motion sensitivity and anti-predatory related mechanisms depend on specific experience, we tested young chicks raised in isolation and assessed their responses to looming vs. sweeping visual stimuli. We showed that inexperienced chicks are able to selectively react to different type of overhead moving stimuli on the basis of their threat level, fleeing from rapidly approaching objects, and slowing down in response to sweeping objects. Furthermore, we observed that a rapid expansion toward the subject, exceeding an angular size of ± 24°, is responsible for the initiation of an escape response to an immediate looming threat, similarly to other taxa (8, 9), but earlier than previously assumed in chicks (7). These results show that young animals, in the absence of relevant experience, differently react to motion cues mimicking various predation risks. Interestingly, the responses we observed in controlled laboratory experiments parallel field studies showing that movement rate and vigilance of ungulate prey species are affected by the perceived risk of predation (17, 20). Solving the long-standing issue of the evolutionary origins of anti-predatory behaviors, these findings suggest that the adaptive needs of young preys are matched by *spontaneous* threat recognition and use of appropriate defensive mechanisms that do not require learning.

MATERIALS AND METHODS

Chicks (218, *Gallus gallus*) were used. Chicks were hatched in darkness and housed individually with an artificial imprinting object hang at the eye level, thus experiencing no overhead movement before the test.

After previous habituations to the testing apparatus, chicks were individually tested on the 4th day of life in a rectangular black arena virtually divided in a Departure zone (where chicks were initially located) and a Stimulus delivery zone (Fig. 1A). When the chick entered this area, the first stimulus was displayed on the overhead monitor (MG248Q, Asus, 120 Hz). Subsequent displays of the same stimulus (up to 6) were played when the chicks were moving for 2 s in this zone, with a minimum inter-stimulus interval of 120 s to prevent habituation (21). Each chick was presented with one type of stimulus only. The test session lasted no longer than 32 minutes. Chicks' behavior was monitored using an infrared camera located below a semi-transparent floor and coupled with a tracking system (Ethovision, Noldus). Only chicks that left the Departure zone were included in the analysis (181 chicks).

We displayed four types of stimuli: looming, sweeping, receding and dimming (Fig. 1B,F,J). The looming stimulus (Exp. 1-3) was a black disc expanding from 1° to 45° of visual angle (0.56 to 26.3 cm) in 1 second. The sweeping stimulus (Exp. 1) was a black disc (4°) moving at a constant speed of 7.1 length/second (6), and crossing the entire screen in 3.5 s. The receding stimulus (Exp. 2) had opposite dynamics than looming (shrinkage from 45° to 1° in 1 second) and was used to assess the importance of the direction of movement. The dimming stimulus, designed to assess the role of change in luminosity, consisted in a series of displays of the 45° circle, whose grey level changed over time to match the overall luminosity of the looming images. All the stimuli were prepared with 120 fps (22). Size and speed were calculated based on (6), assuming a distance of 32 cm between the eyes and the screen (13). All the movies used a white background that illuminated the apparatus.

To determine whether the stimuli elicited flight or freezing responses, we measured the speed of chicks during and after (1 s) their presentation. We analyzed the speed changes compared to the second preceding the onset of the stimulus [Speed during (%); Speed after (%)]. The distance traveled during the 30 s directly following the offset was also examined. Values related to each presentation (up to 6) were averaged to obtain a single value per chick for each variable of interest. The influence of the stimulus type was investigated using Mann-Whitney tests (U). Significant departure from baseline level (mu=100%) was also examined for the average speed change values, using one-sample Wilcoxon signed rank test (W). An alpha level was set to 0.05. All tests were two-tailed. The 95% confidence intervals (CI) are shown. The effect sizes were assessed through *r* values.

All the experiments adhered to the Italian and EU directives on animal research, license n° 161/2018-PR by the Ministero della Salute. Data are available on Zenodo (doi: 10.5281/zenodo.3461083).

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FIGURE LEGENDS

Figure 1. Naïve chicks use motion cues to assess risks and to exhibit appropriate anti-predatory responses. (A) Apparatus. **(B-E)** Exp. 1: Looming (n=31) *vs.* sweeping (n=33). **(F-I)** Exp. 2: Looming (n=27) *vs.* receding (n=31). **(J-M)** Exp. 3: Looming (n=30) *vs.* dimming (n=29). Visual stimuli (B,F,J). Speed change during the displays (C,G,K) and 1 second after the offset (D,H,L). Distance travelled during the 30 seconds following the offset (E,I,M). Graphs show median and 95% confidence interval. Dashed lines represent the baseline speed level (100%). *: one-sample Wilcoxon signed-rank test (mu=100). ¤: Mann-Whitney test. *Dep.: Departure; Exp.: Experiment; IR: Infrared.*

AUTHOR CONTRIBUTIONS

M.H., E.V. and G.V. designed the experiments. M.H. carried out the experiments, analyzed the data and conducted the statistical analyses. M.H. wrote the first version of the manuscript. M.H., E.V. and G.V. revised and edited the manuscript.

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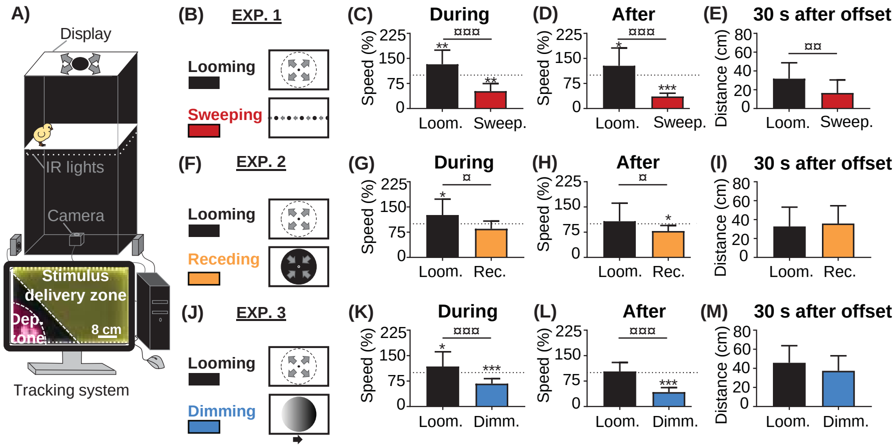
SUPPLEMENTARY VIDEO LEGENDS

Movie S1: Typical fleeing responses in reaction to the display of the looming stimulus (Exp. 1-3). Centre-point speed (in cm s-1) recorded every 80 ms. The 1 s period of stimulus display is highlighted (white background and red line). The red dot corresponds to the chick's center-point.

Movie S2: Typical long-lasting immobilization in reaction to the display of the sweeping stimulus (Exp. 1). Centre-point speed (in cm s-1) recorded every 80 ms. The 3.5 s period of stimulus display is highlighted (white background and red line). The red dot corresponds to the chick's center-point.

Movie S3: Absence of clear response during the display of the receding stimulus (Exp. 2). Centre-point speed (in cm s-1) recorded every 80 ms. The 1s period of stimulus display is highlighted (white background and red line). The red dot corresponds to the chick's center-point.

Movie S4: Transient speed reduction during the display of the dimming stimulus (Exp. 3). Centre-point speed (in cm s-1) recorded every 80 ms. The 1s period of stimulus display is highlighted (white background and red line). The red dot corresponds to the chick's center-point.


A)
Display
IR lights
Camera
Stimulus delivery zone
Dep. zone
8 cm
Tracking system
(B)
EXP. 1
Looming
Sweeping
(F)
EXP. 2
Looming
Receding
(J)
EXP. 3
Looming
Dimming
(C)
During
Speed (%)
225
150
75
0
¤¤¤
**
**
Loom.
Sweep.
(D)
After
Speed (%)
225
150
75
0
¤¤¤
*
***
Loom.
Sweep.
(E)
30 s after offset
Distance (cm)
80
60
40
20
0
¤¤
Loom.
Sweep.
(G)
During
Speed (%)
225
150
75
0
¤
*
Loom.
Rec.
(H)
After
Speed (%)
225
150
75
0
¤
*
Loom.
Rec.
(I)
30 s after offset
Distance (cm)
80
60
40
20
0
Loom.
Rec.
(K)
During
Speed (%)
225
150
75
0
¤¤¤
*
***
Loom.
Dimm.
(L)
After
Speed (%)
225
150
75
0
¤¤¤
***
Loom.
Dimm.
(M)
30 s after offset
Distance (cm)
80
60
40
20
0
Loom.
Dimm.