



Inexperienced preys know when to flee or to freeze in front of a threat

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Using appropriate antipredatory 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 chicks naive to movement. 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.

antipredatory behaviors | motion cues | threat detection | defense strategies | naive animals

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 antipredatory 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 antipredatory behaviors (3–9). For instance, mice rapidly detect overhead motion and assess the threat level posed by various stimuli, fleeing from displays mimicking an ongoing 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 antipredatory 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 antipredatory 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 antipredatory/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 antipredatory 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-d-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 (experiment [exp.] 1). Then, we characterized the properties that triggered fleeing defensive responses (exp. 2 and 3; refs. 8 and 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, which mimicked an immediate predator attack, chicks increased their speed ($W = 268$, $r = 0.472$, $P < 0.01$; Fig. 1C and 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 and Movie S2). Similar results were obtained for the speed 1 s 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 toward 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: $U = 290$,

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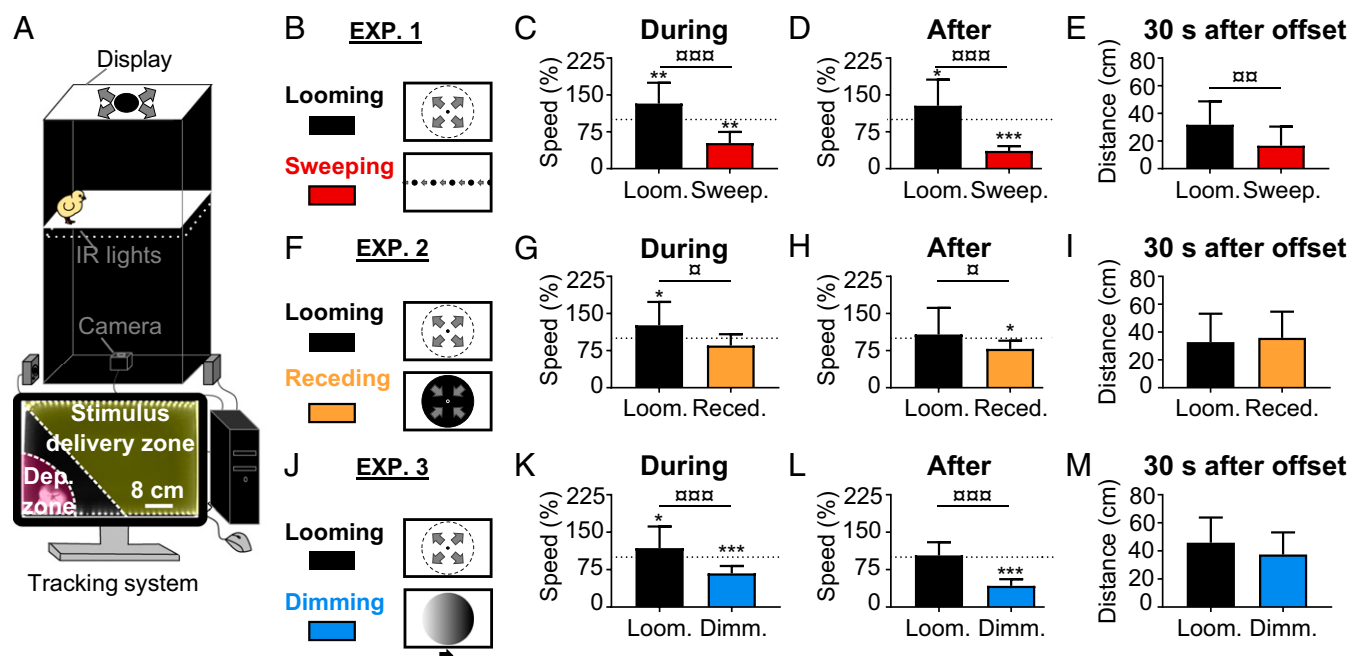


Fig. 1. Naive chicks use motion cues to assess risks and to exhibit appropriate antipredatory responses. (A) Apparatus. (B–E) Exp. 1: looming ($n = 31$) vs. sweeping ($n = 33$). (F–I) Exp. 2: looming ($n = 27$) vs. receding ($n = 31$). (J–M) Exp. 3: looming ($n = 30$) vs. dimming ($n = 29$). Visual stimuli (B, F, and J). Speed change during the displays (C, G, and K) and 1 s after the offset (D, H, and L). Distance traveled during the 30 s following the offset (E, I, and M). The graphs show median and 95% confidence interval. The 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.

$r = 0.263$, $P < 0.05$; Fig. 1 G and 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. 1 G and H). In contrast, a slight speed reduction was detected during the 1-s period directly following the offset of the receding stimuli ($W = -210$, $r = -0.37$, $P < 0.05$; Fig. 1H). This effect was transient, however (distance traveled 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. 1 K and 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. 1 K and 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. 1 K and L and Movie S4). However, this effect quickly faded (distance traveled 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, $\pm 24^\circ$; one-sample: $W = 1048$, $r = 0.232$, $P < 0.05$).

Discussion

Producing appropriate antipredatory 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 antipredatory reactions to short-neck (predator birds) vs. long-neck (nonpredator birds) dummies, in line with their idea of innate releasing mechanisms (18, 19). Their report on greater antipredatory responses, however, has been contradicted multiple times (see ref. 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 antipredatory-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 types 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 $\pm 24^\circ$, 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 antipredatory 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 hanging 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 fourth 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 interstimulus 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 min. Chicks' behavior was monitored using an infrared camera located below a semitransparent 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 4 types of stimuli: looming, sweeping, receding, and dimming (Fig. 1 B, F, and J). The looming stimulus (exp. 1–3) was a black disk expanding from 1° to 45° of visual angle (0.56 to 26.3 cm) in 1 s. The sweeping stimulus (exp. 1) was a black disk (4°) moving at a constant speed of 7.1 length/s (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 s) 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 gray level changed over time to match the overall luminosity of the looming images. All of the stimuli were prepared

with 120 fps (22). Size and speed were calculated based on ref. 6, assuming a distance of 32 cm between the eyes and the screen (13). All of 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 (percentage); speed after (percentage)]. 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 α level was set to 0.05. All tests were two-tailed. The 95% confidence intervals are shown. The effect sizes were assessed through *r* values.

All of the experiments adhered to the Italian and European Union directives on animal research, License 161/2018-PR by the Ministero della Salute. Data are available on Zenodo (DOI: [10.5281/zenodo.3461083](https://doi.org/10.5281/zenodo.3461083)); ref. 23.

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