Who is looking? Eyespot cues as a tool to assess affective state in the red junglefowl

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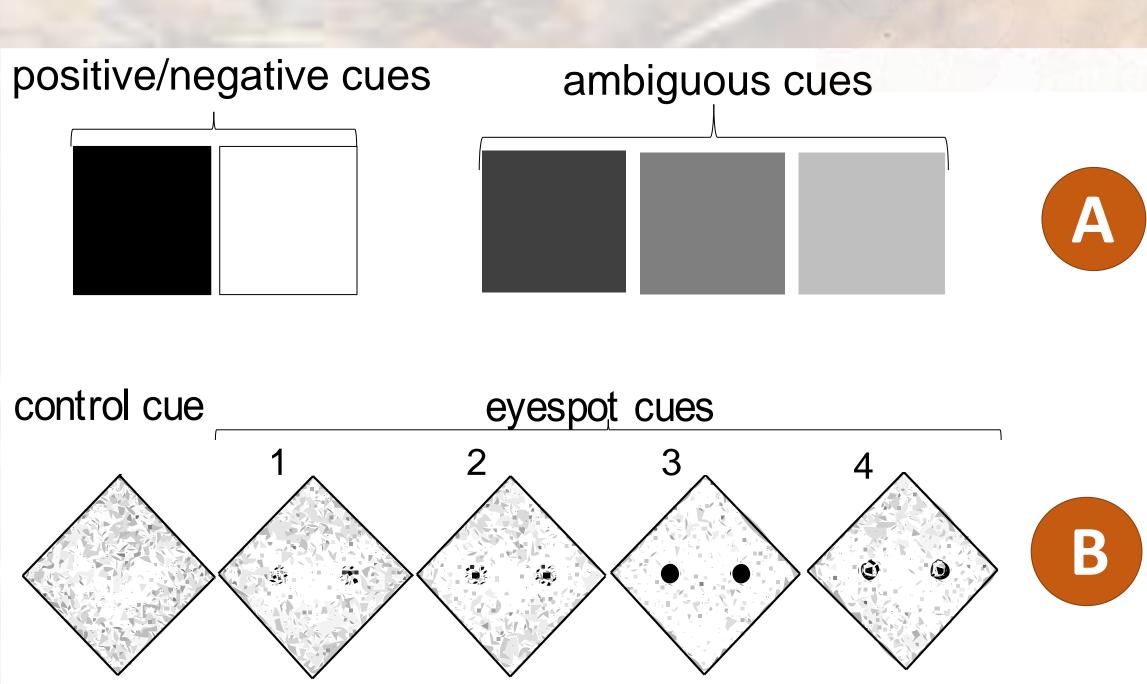
Background

To provide good animal welfare requires accurate interpretation of affective state. The most common affective state test (cognitive judgement bias test), needs extensive training of animals. Most birds show innate response of avoidance towards eyespot patterns and birds showing weaker reactions could be in a better state. Moreover, training prior to testing is not needed.



To validate a simpler alternative to the judgement bias test, we tested if red junglefowl chicks' responses to ambiguous cues from the judgement bias test correlated with responses to eyespot patterns used as cues in a here designed "eyespot test".

Figure 1: cues used for A) the judgement bias test B) the eyespot test; cue 1 and 2: ambiguous eyespot cues; cue 3 and 4: full eyespot cues. The ambiguous middle grey cue and the full eyespot cue 3 are the cues whose responses correlated the most in the original tests. The same cues were the only cues used for the simplified tests.



Methods

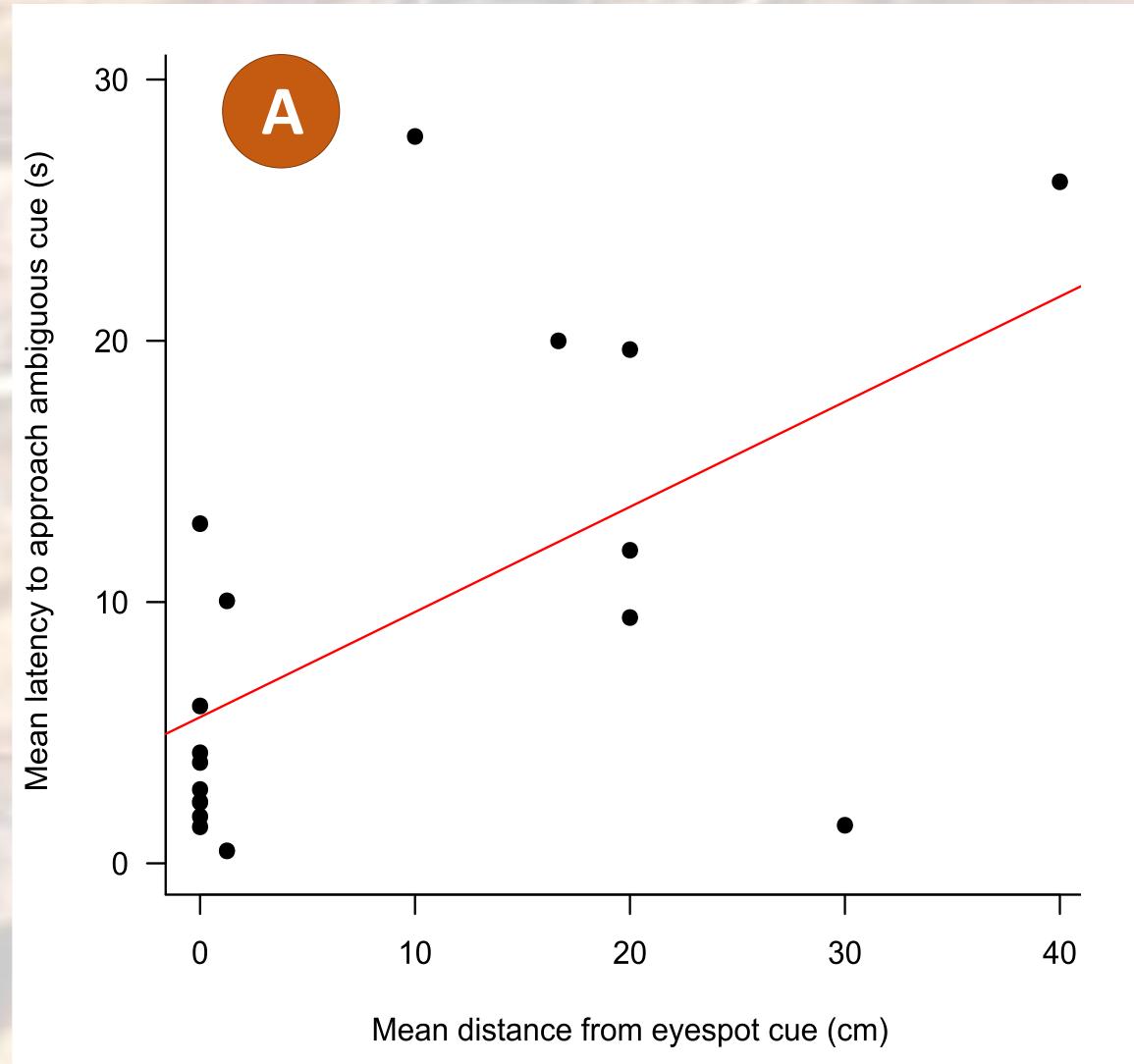
We tested red junglefowl (*Gallus gallus*) chicks from two cohorts with both judgement bias test and eyespot test in two versions differing in the number of test cues: **original** and **simplified** version.

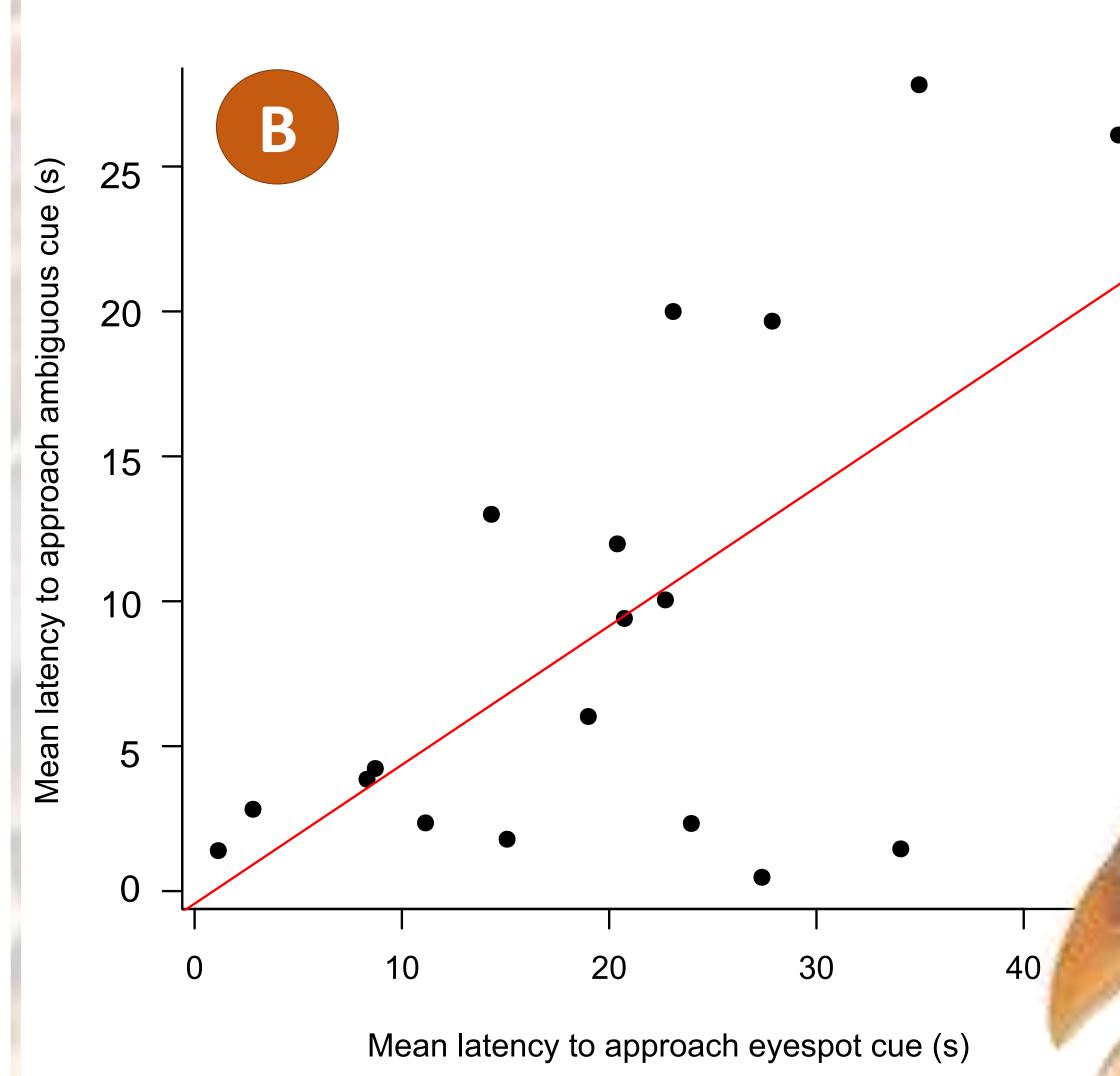
In the judgement bias test, after training chicks to discriminate a positive and a negative cue, we measured the latency to approach ambiguous cues and compared it to responses to learnt cues. A response closer to responses to the positive cue putatively indicated a better state. In the eyespot test, after chicks learnt to associate a position (control cue) with a reward, we measured the latency to approach and distance from

a full or ambiguous eyespot cue in the same position and compared them to responses to the control cue. A response closer to responses to control cue putatively indicated a better state.

Results

Chicks **learnt** that the **ambiguous cue** was **unrewarded** after several exposures with the simplified tests. **Age** and **sex** affected the **strength of responses**. **Females** tested at **older** age showed a moderately strong positive relationship between **latency to approach** the middle grey ambiguous cue in the judgement bias test and both **distance approached** and **latency to approach** the full eyespot cue 3 in the original tests.





Conclusions

The original eyespot test using one of the full eyespot cues is supported as simpler alternative to the judgement bias test for female chicks tested at older age.

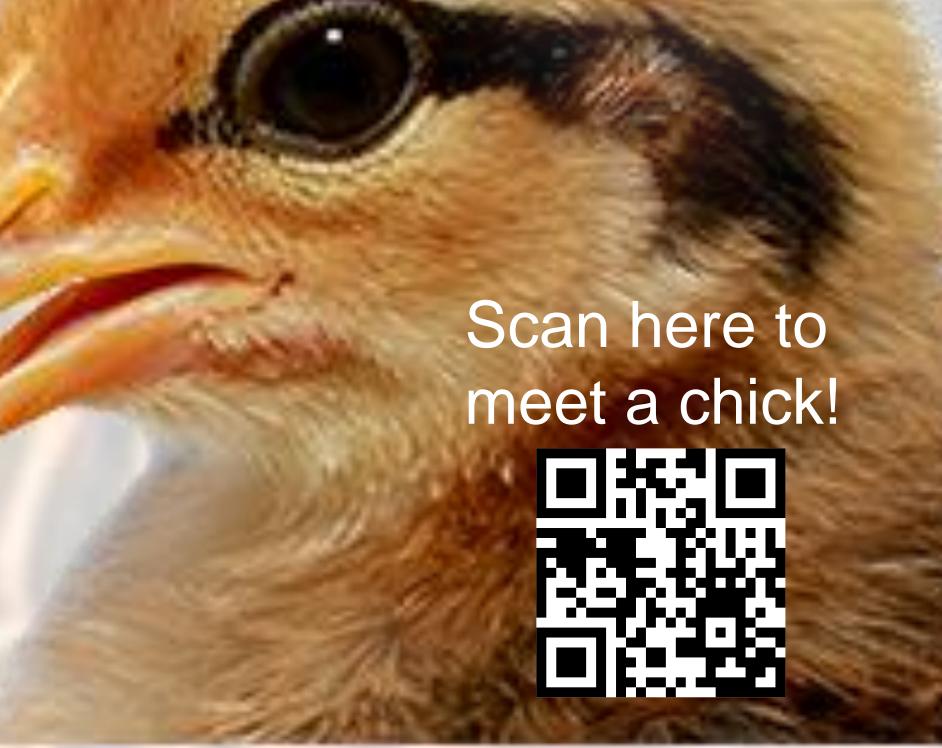


Figure 2: the comparison of behavioural responses of females to the middle grey cue from the original judgement bias test at 2 weeks and to the full eyespot cue 3 from the original eyespot test at 8 weeks shows a moderately strong positive relationship between A) latency and distance ($R_S = 0.45$, n = 18, p = 0.06) and B) latencies ($R_S = 0.39$, n = 18, p = 0.11). Results from Spearman's test when not accounting for loss of ambiguity. Correlation line in red.