Habituation of the alertness response in crayfish is modulated by stimuli with socially relevant information

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Habituation consists of a decreased response to repeated stimulation and not involving fatigue or sensory adaptation [1], and is common to many living entities, from single cells to the most complex organisms [2-3]. It has a considerable evolutionary value, since it allows an organism to disregard irrelevant repetitive stimuli.

Here, we tested whether two different stimuli, with possible different social values, may have a different impact on habituation in individuals of crayfish *Procambarus clarkii*, a highly aggressive crustacean often fighting with conspecifics for the control of shelter and resources [4].

The stimuli consisted in a red upright Y-shaped figure, resembling a crayfish displaying its aggressive posture (high intensity stimulus condition), and a red downright Y-shaped figure, identical to the other but rotated of 180° and resembling the animals' relaxed posture (low intensity stimulus condition). A habituation paradigm was used, in which two groups of 24 animals were presented with nine consecutive looming stimulations, thus creating the illusion of an approaching stimulus. Furthermore, a tenth looming presentation of a completely different stimulus (a circular blue moebius) was used to test for a spontaneous recovery of the response. As dependent measure, we recorded the duration of the alert reaction to the approaching shape (measured in video frames, recorded at 29 fps).

Crayfish showed habituation in both conditions (p=0.002). The animals tested in the low intensity condition abruptly habituated to the repeating stimulus, and then showed a strong response recovery to the new stimulus (p=0.001). By contrast, crayfish in the high intensity condition maintained a consistently higher alertness during the administration of the repeating upright Y-shaped figure (M=11.61 ± 1.10) as compared to those in the low intensity condition (M=8.72 ± 1.21). Moreover, crayfish in the high intensity condition showed no spontaneous recovery to the approaching stimulus when it was changed in shape and colour (p=0.120).

Our data confirm previous findings on looming in crayfish [5-6] and indicate that they responded as if they faced real opponents [7]. Indeed, the more sustained pattern of response evoked by the upright stimulus seems to be due to a different social valence between the two schematic stimuli, with one stimulus being perceived as more threatening than the other. Our results show that *P. clarkii* is sensitive to the abstract visual representation of conspecifics.

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