

Comment

Nemo through the looking-glass: a commentary on Desjardins & Fernald

Mirror-elicited aggression has been widely used in animal behaviour research. Recently, Desjardins & Fernald [1] reported that males of the cichlid fish *Astatotilapia burtoni* fighting a mirror had higher expression of immediate early genes in brain areas homologous to the amygdala and the hippocampus than males fighting a real opponent, despite the fact that the behavioural and androgen responses did not differ between the two conditions. Surprisingly, Desjardins & Fernald [1] omitted two recent papers (Oliveira *et al.* [2] in another cichlid fish, *Oreochromis mossambicus*, and Hirschenhauser *et al.* [3] in quail, *Coturnix japonica*), which also found a dissociation between the behavioural and the physiological responses towards mirror images. In these two studies, the mirror image elicited an aggressive response but failed to trigger the androgen response present when fighting a real opponent, suggesting that the physiological response to the fight depends on the cognitive appraisal that the individuals make of the situation [4]. This omission is even more surprising as the two divergent results support contrasting adaptive roles for socially driven changes in androgen levels (aka ‘challenge hypothesis’, [5]): the lack of hormonal response to a mirror fight suggests the need for asymmetric feedback to adjust androgen production, which is therefore only functional for subsequent interactions; the presence of an androgen response triggered by a mirror fight suggests a role for androgens in the modulation of aggressive behaviour in the ongoing fight. Both the *O. mossambicus* and quail studies support the first hypothesis, while the *A. burtoni* study is the first evidence for the latter.

Three different kinds of factors may contribute to the divergent androgen responses.

- *Phylogenetic bias.* Phylogeny is an unlikely source of variation in this case since two of the species showing divergent responses are closely related cichlid fishes, one of which shares the same pattern of response with the phylogenetically relatively distant quail.
- *Differences in the behavioural tasks.* In the real opponent treatment, Desjardins & Fernald [1] staged fights between males previously placed in separate compartments. Therefore, the fish interacting across the transparent partition were mimicking a territorial border fight between two territorial males, with no initial

asymmetry in resource-holding potential and no clear outcome. In contrast, in the other two studies (Hirschenhauser *et al.* [6], was used as a real opponent control for the mirror fights in Oliveira *et al.* [2]) a resident/intruder paradigm was used. This could explain why Desjardins & Fernald [1] did not find differences both in aggressive behaviour and in androgen levels between mirror and real fights, but it does not explain why mirror fights elicited a significant androgen response when compared with control males who had not been in an aggressive encounter.

- *Differences in hormone assays.* Androgen levels reported by Desjardins & Fernald [1] are much lower (ca. 2000 times) than those published for other cichlids, including those previously reported for the same species by the same laboratory [7]. Since Desjardins & Fernald [1] appear to have used blood plasma directly in the assay, and not the extracts of fluids (as done in the other studies, i.e. Oliveira *et al.* [2], Parikh *et al.* [7] and Hirschenhauser *et al.* [3]), the presence of androgen-binding globulins could have the effect of reducing apparent levels of androgens in unpredictable and not necessarily proportional ways. The biological meaning of changes at such a low level (less than 150 pg ml⁻¹) is questionable, but assuming a hypothetical proportional distribution of the putative error among the three experimental groups of Desjardins & Fernald [1], the divergent results cannot be explained.

The fact that Desjardins & Fernald [1] assayed hormones from plasma, and in the other studies hormones were assayed from excreted metabolites in urine [2], or from droppings [3], raises the possibility that the delayed time for the excretion of hormones could be generating these differences. However, the sampling time used in both non-invasive studies has taken into account the known excretion time for these metabolites [3,8], and should reflect the circulating androgen levels at the end of the fights. Moreover, both studies did detect a response in the presence of real opponents using the same time course, and in cichlid fish, a significant positive correlation was detected between plasma and urine levels [9].

Finally, it should be noted here that in the target paper, the expression of the immediate early gene *egr-1* in the pre-optic area (POA) exhibited a non-significant trend (owing to high intra-treatment variation) to be higher in fish fighting real opponents than their mirror image. Since the expression of *egr-1* in response to social stimuli is highly co-localized with GnRH-1 neurons in the POA ($R = 0.93$, see Burmeister *et al.* [10]), these data suggest a higher activity of GnRH-1 neurons in fish fighting real opponents than in mirror fighters, which should translate into higher androgen levels in the former ones. Therefore, the *egr-1* expression data in the POA is in agreement with androgen levels reported in previous studies and contrasts with the ones reported in the target paper.

In summary, the divergent results between Desjardins & Fernald [1] and previously published

studies could have a biological or artefactual basis and should not be ignored. The standardization of behavioural paradigms and hormone assay methods and the integration of brain activation measures in future studies need to be considered in order to promote the integration of data and the establishment of general principles in the field, namely the adaptive function of socially driven changes in androgen levels.

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