Journal of Neuroendocrinology

Journal of Neuroendocrinology, 2013, **25**, 644–654

ORIGINAL ARTICLE

Neuroendocrine Mechanisms Underlying Sensory Integration of Social Signals

L. A. O'Connell*†[1,2], M. M. Rigney*†[2], D. W. Dykstra‡ and H. A. Hofmann*†§

*Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, TX, USA.

†Section of Integrative Biology, University of Texas at Austin, Austin, TX, USA.

‡Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, TX, USA.

§Institute for Neuroscience, University of Texas at Austin, Austin, TX, USA..

Journal of Neuroendocrinology

Correspondence to:
Dr Hans A. Hofmann, Section of Integrative Biology, The University of Texas at Austin, 2415 Speedway – C0990, Austin, TX 78712, USA (hans@mail.utexas.edu).

[1] *Present address: FAS Center for Systems Biology, Harvard University, Cambridge MA, USA*

[2] *These authors contributed equally to this study.*

Individuals integrate information about their environment into adaptive behavioural responses, yet how different sensory modalities contribute to these decisions and where in the brain this integration occurs is not well understood. We presented male cichlid fish (*Astatotilapia burtoni*) with sensory information in three social contexts: intruder challenge, reproductive opportunity and a socially neutral situation. We then measured behavioural and hormonal responses along with induction of the immediate early gene c-Fos in candidate forebrain regions. In the intruder challenge context, males were exposed to either a visual stimulus of a dominant male, the putative male pheromone androstenedione, or both. We found that, compared to the neutral context, a visual stimulus was necessary and sufficient for an aggressive response, whereas both chemical and visual stimuli were needed for an androgen response. In the reproductive opportunity context, males were exposed to either a visual stimulus of a receptive female, a progesterone metabolite (female pheromone) only, or both. We further found that the visual stimulus is necessary and sufficient for an androgen response in the reproductive opportunity context. In the brain, we observed c-Fos induction in response to a visual challenge stimulus specifically in dopaminergic neurones of area Vc (the central region of the ventral telencephalon), a putative striatal homologue, whereas presentation of a chemical stimulus did not induce c-Fos induction in the intruder challenge context. Our results suggest that different sensory cues are processed in a social context-specific manner as part of adaptive decision-making processes.

Key words: c-Fos, challenge response, androstenedione, social behaviour, dopamine, androgens

doi: 10.1111/jne.12045

Individuals integrate sensory information with internal physiology into context-appropriate behaviour that ultimately promotes fitness. Despite an astonishing diversity of ecological adaptations and sensory specialisations (1), animals display similar behavioural responses to social challenges (male–male competition) and opportunities (sexual behaviour) (2). Yet how the brain integrates different sensory modalities in these social contexts remains unclear.

Steroid hormones respond acutely to social stimuli and modulate subsequent behaviour. The 'challenge hypothesis' was proposed to explain the dynamic androgen responses to social challenges in some songbirds (3) and similar responses have been demonstrated across vertebrates (4), although this pattern does not apply in all species (5). Although less studied, males exposed to females also exhibit androgen responses (6). Thus, acute endocrine responses are important in both challenge and opportunity contexts and may facilitate behavioural responses by modulating relevant neural circuits in a context-specific manner. Despite these advances, how different sensory cues denoting various social contexts are processed through conserved neural circuits remains unclear.

To delineate the contributions of different sensory stimuli to context-appropriate behavioural responses, we used the cichlid fish, *Astatotilapia burtoni*, a model system in social neuroscience (7). Male social interactions can be distinctly separated into an aggressive challenge context (male–male competition) or a reproductive opportunity context. Dominant males aggressively defend territories where they mate with females. Distinct colour patterns and behavioural displays serve as visual signals that attract females and

stimulate aggression in other males (8,9). Across teleosts, pheromonal cues, such as androgen metabolites, are released into the water and can act as chemical signals of social and/or reproductive status (10). Females also release metabolites, such as progestins, into the water that act as pheromones (11–13). *Astatotilapia burtoni* males discriminate between male and female odours (14) and they court more when exposed to chemical cues of a receptive female (15). Although the sensory cues that promote context-specific behaviour are relatively well understood in teleosts, which brain regions and cell types integrate these signals in the brain to promote context-appropriate behavioural responses is unknown.

We presented dominant male *A. burtoni* with visual and chemical cues of either intruder males or reproductive females and investigated how hormonal and neural responses contribute to context-appropriate behaviour. We quantified immediate early gene (IEG) induction in response to various social stimuli as a marker of neuronal activity (16) in brain regions known to receive visual and/or olfactory information (17), as well as the preoptic area, a neuroendocrine integration centre important for male sexual behaviour and aggression (18). Furthermore, because evaluation of the salience of (social) stimuli is mediated in part by the dopaminergic reward system (19), we quantified c-Fos induction in several forebrain dopaminergic cell groups (18).

Materials and methods

Animals

Astatotilapia burtoni descended from a wild-caught stock population were kept in aquaria under conditions mimicking their natural environment (20). The day light period for all experiments was 08.00–20.00 h with 10 min of dim light before and after to simulate dawn and dusk. Every care was taken to minimise the pain or discomfort of the animals, and all work was approved by the Institutional Animal Care and Use Committee at the University of Texas at Austin.

Behaviour trials

We presented socially dominant, territorial *A. burtoni* males (mean ± SD; body mass: 4.28 ± 0.64 g; standard length : 5.31 ± 0.29 cm) with either visual or chemical or both visual and chemical (simultaneously) sensory stimuli to examine the behavioural, hormonal and neural responses to sensory modality-dependent information in different socially relevant contexts: intruder challenge, reproductive opportunity and neutral (n = 56; n = 8 per group; Fig. 1). After males had been dominant for at least 1 week in a naturalistic community, they were placed into the testing paradigm. First, to ensure focal males were dominant, three similarly-sized males were each placed into one of three compartments of a 110-l tank, separated by perforated transparent acrylic dividers. Each compartment contained one male and three females. All males in these separate compartments were of dominant status because they were aggressively defending a territory within the tank, courting females and displaying bright colour and the characteristic eye bar. Then, on the day before testing (between 16.00 and 18.00 h), focal males were transferred overnight into the experimental tanks to allow for acclimation to the new environment. The experimental set-up consisted of two separate clear 38-l tanks that each contained a terracotta bower (facing the opposite tank) and a shelter (facing away from the opposite tank). Opaque acrylic dividers visually isolated the tanks until the stimulus period began the next morning. Finally, at 09.00 h on the day of the test, males were exposed to a sensory stimulus for 1 h in a neutral, challenge, or reproductive opportunity context (Fig. 1). Both the focal and stimulus tanks were observed with a digital video surveillance system (VideoInsight, Houston, TX, USA) for the first hour of exposure. We quantified the behavioural interactions throughout the 1-h stimulus period in 10-min segments for a randomly chosen subset of experimental animals and found that the most robust responses to chemical and/or visual stimuli occurred 20–30 min into the stimulus hour. Therefore, social behaviour, including aggressive displays and courtship displays, of each focal male was quantified during this time period by a single observer who was blinded to treatment group. Displays of aggressive (sum of charges, bites, threats, border disputes) and courtship (sum of quivers and leads) behaviour were quantified as described previously (21).

Social challenge paradigm

In the challenge context, focal males were presented with a dominant male (visual stimulus), waterborne androstenedione (AD) (chemical stimulus) (22), or both simultaneously (n = 8 males each). Stimulus dominant males were confirmed to be dominant for at least 1 week before the test, as described above, and transferred into the experimental tank on the day before testing. At the beginning of the stimulus hour, the opaque divider separating the focal male and stimulus male was removed and the focal male was exposed to the visual stimulus of a dominant male for 1 h. For the chemical stimulus, 0.033 mg of 4-androstene-3,17-dione (AD; catalogue number 46033; Sigma, St Louis, MO, USA) dissolved in ethanol was added to the top centre of the focal male tank 1 min before the opaque divider was removed and the stimulus period began. The stimulus tank contained ten juveniles as a neutral visual stimulus. Given that *A. burtoni* is a highly social species, we assumed that a group of sexually immature juveniles would constitute a more appropriate control stimulus than isolation (i.e. pilot studies showed that this results in a stress response and complete lack of behavioural displays). Sixty juveniles (lacking any body colouration, and less than 2 cm in length) were used and each focal male was exposed to a randomly selected subgroup of ten such individuals. In a third sensory stimulus situation, males were exposed to both a dominant male and waterborne AD simultaneously. Each stimulus male was only used once.

Reproductive opportunity paradigm

In the reproductive opportunity context, focal males were presented with a receptive female (visual stimulus), an exogenously administered progesterone metabolite (chemical stimulus) (13,23,24), or both simultaneously (n = 8 males each). The stimulus females were prepared for the experiment as follows. After spawning in a community setting, females were stripped of their brood and returned to their community tanks. Three days later, females were transferred and acclimated overnight to an isolated experimental tank. On the day of the experiment, stimulus females were injected i.p. with 0.1 mg/g body weight of prostaglandin F2α (PGF2; catalogue number P0424; Sigma) 15 min before visual exposure to the male to induce receptive behaviour (24,25). At the beginning of the stimulus hour, the opaque divider separating the focal male and stimulus tank was removed and the male was exposed to the visual stimulus of a receptive female for 1 h. For the chemical stimulus, 0.033 mg of 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-P; catalogue number P6285; Sigma) dissolved in ethanol was added to the top centre of the focal male tank 1 min before the opaque divider was removed and the stimulus period began. This dose corresponds to a concentration approximately three-fold higher than the amount of 17α,20β-P released into the water by a gravid female over the course of 1 h (26) and has previously been shown to elicit both sperm motility and courtship behaviour in male cichlids (12,13). The stimulus tank contained ten juveniles as a neutral visual stimulus. Each female was used only once as the opportunity visual

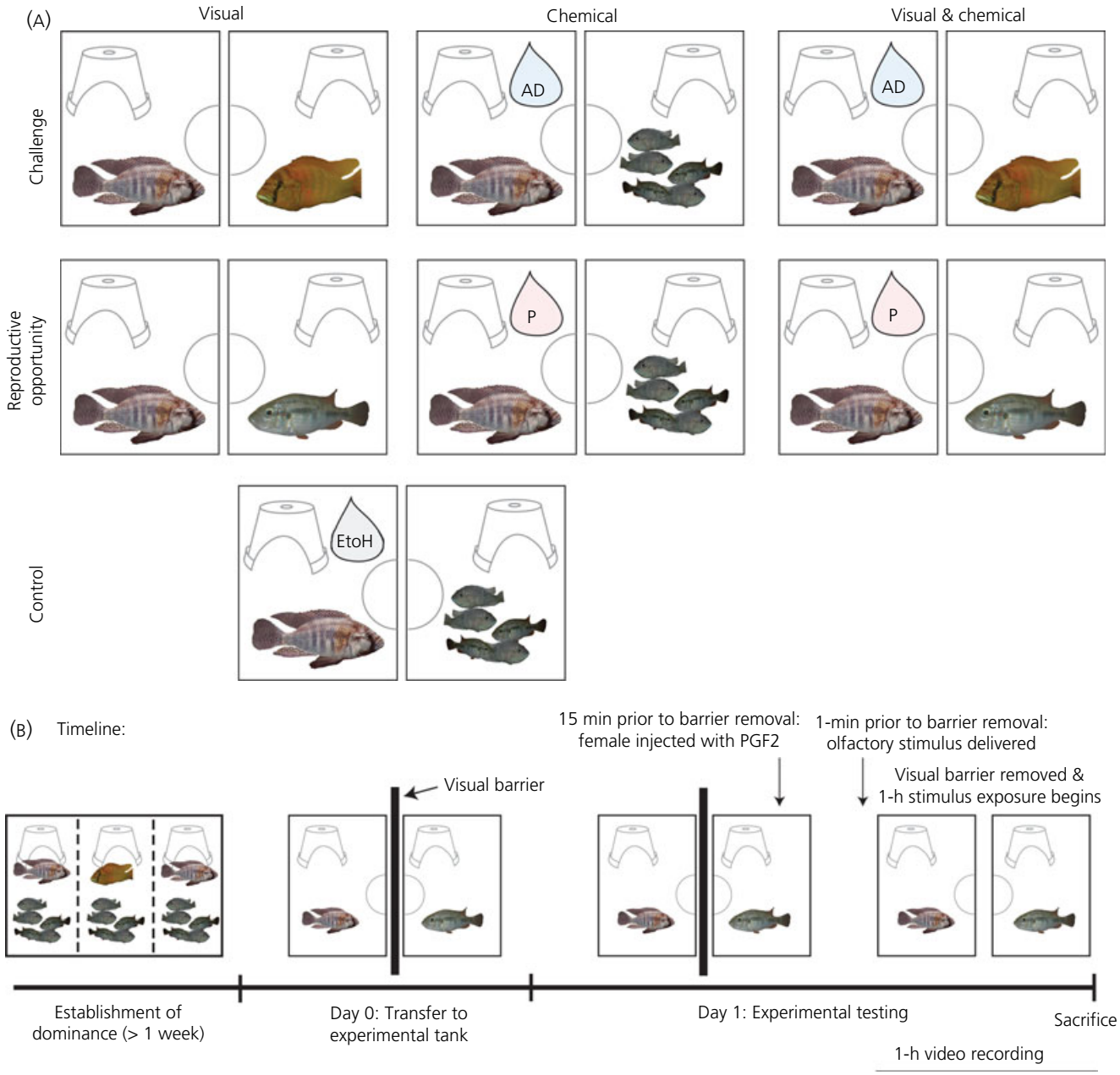

Fig. 1. Experimental paradigm. (A) Dominant *Astatotilapia burtoni* males were presented with either visual (dominant male or proceptive female), or chemical (androstenedione, AD, putative male pheromone; 17α-20β-dihydroprogesterone, P, putative female pheromone) stimuli, or both visual and chemical cues simultaneously in either intruder challenge (top row) or reproductive opportunity (middle row) contexts. When receiving the chemical cue only, a neutral social stimulus (juveniles) was present in the stimulus tank. The control situation included males presented with a neutral social stimulus and ethanol (EtOH) as the chemical cue vehicle control. Each tank contained a shelter (terracotta pot) and a terracotta dish (semicircles) that served as a bower. (B) Timeline of experimental paradigm. The reproductive opportunity paradigm is depicted, although intruder challenge and social control paradigms are identical, with the exception of the stimulus presented. PGF2, prostaglandin F2α.

stimulus. In a third sensory stimulus situation, males were exposed simultaneously to both a receptive female and exogenous 17α,20β-P.

Socially neutral paradigm (control)

In the neutral (control) context, focal males (n = 8) were presented with a group of ten juveniles as a neutral visual stimulus in the opposite tank. Additionally, 33 μl of 100% ethanol (vehicle for AD and 17α,20β-P treatments) was added to the top centre of the focal male's tank 1 min before the visual barriers being removed. The juveniles mostly displayed shoaling behaviour and did not show any adult-typical behaviour, such as aggressive or reproductive displays. Because males only rarely direct any reproductive or aggressive displays towards juveniles, this context can be considered socially neutral and is thus preferred to social isolation, which is stressful in this species. Both the neutral visual stimulus and the neutral olfactory stimulus were administered in the manner described above.

Tissue collection

At the end of the stimulus hour, focal males were removed from the experimental tanks, measured and weighed, and then blood was collected through the dorsal aorta using heparinised 26-G butterfly infusion sets (Becton Dickson, Franklin Lakes, NJ, USA). Plasma was stored at −80 °C for hormone analysis. Focal males were then killed and brains rapidly dissected. One hour is an appropriate time course for measuring neuronal activity by quantification of c-Fos protein in teleosts (27).

Hormone assays

An enzyme-linked immunosorbent assay was used to determine free circulating levels of testosterone, 17β-oestradiol (Enzo Life Sciences, Farmingdale, NY, USA) and 11-ketotestosterone (11-KT; Caymen Chemicals, Ann Arbor, MI, USA) as described by Kidd *et al.* (28) and in accordance with the manufacturer's instructions. Two assay plates were analysed for each hormone. The average intra- and interplate variation was 7.95% and 9.51% for testosterone, 1.19% and 1.87% for 11-KT, and 2.44% and 6.77% for 17β-oestradiol, respectively.

Immunohistochemistry (IHC)

After being fixed in 4% paraformaldehyde at 4 °C overnight, brains were washed in 1 × phosphate-buffered saline (PBS) and cryoprotected in 30% sucrose overnight at 4 °C before embedding in O.C.T. (Tissue-Tek; Fisher Scientific Co., Pittsburgh, PA, USA) and storing at −80 °C. Brains were then sectioned on a cryostat at 30 μm and thaw-mounted onto Super-Frost Plus slides (Fisher Scientific) in four series that were stored at −80 °C for 1–6 weeks until processing for IHC.

Brightfield detection of c-Fos

Sections were removed from −80 °C and air-dried before processing for immunohistochemical detection of c-Fos, as described previously (29), using 1 : 500 rabbit anti-c-Fos primary antibody (catalogue number sc-253; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). For control sections, all procedures were the same, except that primary antibody was omitted. Specificity of the c-Fos antibody was analysed by western blotting (see below).

Fluorescent detection of c-Fos and tyrosine hydroxylase (TH)

To quantify IEG induction in dopaminergic neurones, we colocalised c-Fos and TH, the rate-limiting enzyme in catecholamine synthesis and a robust neurochemical marker for dopaminergic cell populations, in the teleost fore- and midbrain (30). An alternate series was used for c-Fos and TH fluorescent double-labelling immunohistochemistry, as described previously (29), using a mix of 1 : 500 mouse anti-TH (catalogue number MAB318; Millipore, Billerica, MA, USA) and 1 : 500 rabbit anti-c-Fos. After incubation overnight in primary antibody, slides were washed twice in PBS and then incubated in a mix of 1 : 200 goat anti-rabbit Cy3 (Jackson Immunoresearch, West Grove, PA, USA) and 1 : 200 goat anti-mouse Alexa Fluor 488 (Life Technologies, Grand Island, NY, USA). Slides were rinsed twice in PBS and cover-slipped with 4',6-diamidino-2-phenylindole (DAPI) hardset fluorescent mounting media (Vector Laboratories, Burlingame, CA, USA). Specificity of the TH antibody was analysed by western blotting.

Western blotting

To determine whether the c-Fos and TH antibodies bind specifically to their respective *A. burtoni* antigens, we extracted protein from *A. burtoni* whole brain using a Mammalian Cell Lysis kit (Sigma) in accordance with the manufacturer's instructions. Whole brain protein extract was subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis and then transferred onto a nitrocellulose membrane overnight. The membrane was then blocked in 5% dry milk in wash buffer [0.5% TritonX-100, 0.1% Tween-20 in 1 × Tris-buffered saline (TBS)] for 30 min and then incubated in primary antibody (1 : 5000 rabbit anti-c-Fos or 1 : 5000 mouse anti-TH in 1 × TBS and 2% NaN3) for 1 h. The membrane was washed five times for 3 min each in wash buffer, and then incubated in goat-anti-rabbit or goat-anti-mouse HRP-conjugated antibody (Santa Cruz Biotechnology) in blocking solution for 30 min. After washing five times for 3 min each with wash buffer, the membrane was exposed to HRP substrate (Immobilon Western, Millipore, Billerica, MA, USA) and exposed to film for 10 min. Using the c-Fos antibody (see Supporting information, Fig. S1), two bands were visualised at the predicted size of 52 and 68 kDa, putatively representing two c-Fos paralogues, as previously characterised in another cichlid fish species (29) and zebrafish (31). Using the TH antibody, one band was visualised at the predicted size of 64 kDa, representing *A. burtoni* TH (30).

Cell counting

Brightfield c-Fos quantification

Cells labelled by c-Fos IHC were counted using the Fractionator routine of the STEREO INVESTIGATOR software package (Microbrightfield, Williston, VT, USA) as described previously (29). We focused on brain regions that putatively receive visual and/or olfactory projections (17): central part of the dorsal telencephalon (Dc, receives visual and olfactory inputs), ventral zone of the ventral region of the lateral part of the dorsal telencephalon (Dlvv receives visual and olfactory inputs), the posterior part of the dorsal telencephalon (Dp, receives olfactory input), the central part of the ventral telencephalon (Vc, receives visual and olfactory input) and the ventral part of the ventral telencephalon (Vv, receives olfactory input). We also measured IEG induction in the parvocellular and magnocellular subnuclei of the preoptic area (pPOA and mPOA, respectively), which differ in their neurochemical profiles and function in relation to social status (32). Briefly, a region of interest was defined using *A. burtoni* neuroanatomical maps (20,30) under low power, and then, under higher magnification, positive cells were counted that fell within 75-μm square counting frames. The average number of randomly placed counting frames in each section analysed was 12 for the pPOA, mPOA, Vv, and Vc and 25 for Dc, Dp, and Dlvv. The software placed counting frames systematically within the region, after a randomly chosen start-site. Cell nuclei containing c-Fos protein were clearly marked by dark brown staining and were counted using a × 20 objective. For each brain region in each section, the estimated number of c-Fos-immunoreactive nuclei (based on random sampling) was divided by the area of the target brain region. For each individual, c-Fos induction for each brain region in this analysis was counted in three to four sections, which were then averaged within each individual for each brain region. Slides were coded and processed by an observer who was blind to treatment.

Quantification of c-Fos and TH colocalisation

A fluorescence signal was detected using a AxioImager.A1 AX10 microscope (Zeiss, Oberkochen, Germany) equipped with DAPI, fluorescein isothiocyanate (FITC) and tetramethyl rhodamine isothiocyanate (TRITC) filters to allow visualisation of the DAPI counter-stain, immunoreactivity of TH with the FITC channel, and immunoreactivity of c-Fos with the TRITC channel. Images were taken with a digital camera (AxioCam MRc; Zeiss) using the AXIOVISION (Zeiss) image acquisition and processing software. Colocalisation quantification was conducted as described previously (29). Briefly, the total number of TH-positive neurones in each section was determined in select dopaminergic brain regions (pPOA, mPOA, giantocellular POA, the rostral periventricular

pretectal nucleus, the periventricular part of the posterior tuberculum (TPp), and area Vc (18). These cell groups are all dopaminergic because there are no noradrenergic/adrenergic neurones in the teleost forebrain (33). The number of neurones exhibiting colocalisation of both TH and c-Fos was quantified by superimposing images generated by FITC and TRITC light filters. The number of neurones positive for TH, and the number of TH and c-Fos colocalised neurones for each brain region was determined by averaging two to three sections per individual. Data are presented as average ratios of neurones co-expressing TH and c-Fos to the total number of TH neurones. Slides were coded and processed by an observer who was blind to treatment.

Statistical analysis

All analyses were conducted in SPSS (SPSS Inc., Chicago, IL, USA) with a significance threshold of $P < 0.05$. For behaviour data, threat displays and bites directed towards the stimulus tank were summed into an aggression index, and lateral displays, quivers and leads were summed into a courtship index. For behavioural as well as testosterone and 17β-oestradiol measures, data were not normally distributed and therefore were log-transformed, which resulted in normally distributed data. An ANOVA was conducted separately for each social context (comparing challenge and neutral groups and comparing reproductive opportunity and neutral groups separately), with sensory stimulus (visual only, chemical only, both visual and chemical, and neutral) as the independent variables and behaviour, hormone levels, cell counts quantifying c-Fos induction or the colocalisation of TH and c-Fos as the dependent variable. For post-hoc analyses, Tukey's honestly significant difference (HSD) test was used. We also conducted Pearson correlation analyses between behaviour and c-Fos data. To account for multiple hypothesis testing using multiple ANOVA as post-hoc analyses and multiple Pearson correlations, a Benjamini–Hochberg false discovery rate correction was applied (34). To determine whether there were any differences in the total number of TH-immunoreactive cells between groups, we used an ANOVA with the total number of TH-positive neurones as the dependent variable, treatment group as the independent variable and focal male length as a covariate.

Results

Context-appropriate behavioural responses to different sensory cues

Aggression levels displayed by focal males depended on the modality of sensory information in the challenge context (ANOVA: $F_{3,28} = 8.747$, $P = 0.0003$) but not in the reproductive opportunity context, where males did not differ from the social control group (ANOVA: $F_{3,28} = 1.869$, $P = 0.158$; Fig. 2A). In the challenge context, the visual stimulus of a dominant male was necessary and sufficient to elicit an aggressive response from the intruder male (visual only and visual + chemical compared to the social control group: $P < 0.014$), whereas aggression of males that only received the chemical stimulus in the challenge context did not differ from the control group ($P = 0.786$).

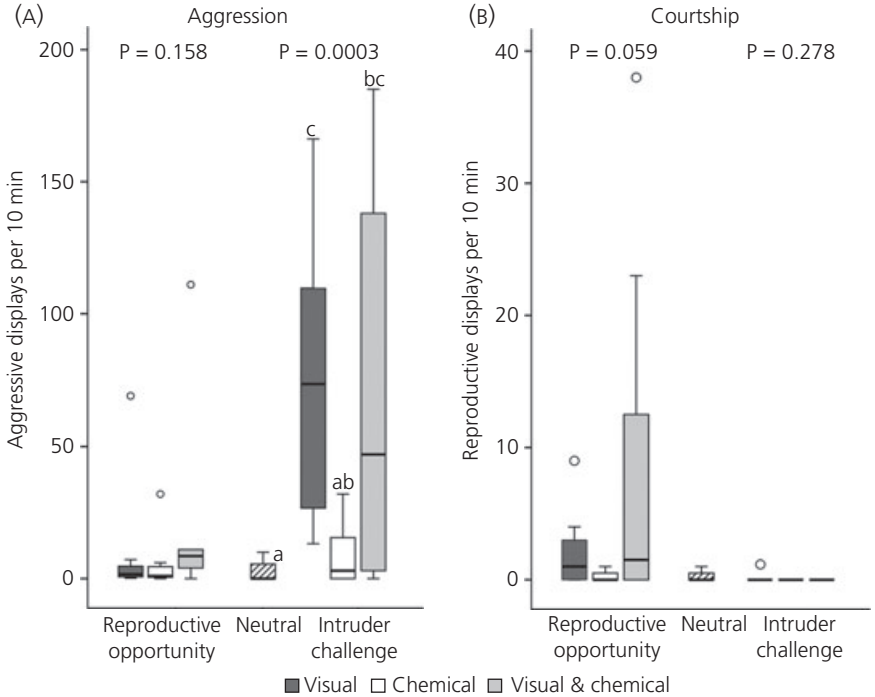

Fig. 2. Behavioural responses to an assortment of sensory cues in different social contexts. Aggression (A) and sexual behaviour (B) were recorded when dominant *Astatotilapia burtoni* males were presented with visual (dark grey), chemical (white), or both visual and chemical cues simultaneously (light grey) in either reproductive opportunity (left part of each graph) or intruder challenge (right part of each graph) contexts; the neutral context is in the middle of each graph (hatched); n = 8 per group. Data are presented as box plots where the top and bottom of the box represent the third and first quartile, respectively, the line represents the median value, the whiskers mark the maximum and minimum values, and the circles represent outliers. Statistics from ANOVA are presented at the top of each graph where males in the reproductive opportunity and intruder challenge contexts were compared separately with the control context. Boxes not joined by the same letter are significantly different according to post-hoc Tukey's honestly significant difference tests.

Courtship behaviour (Fig. 2B) was only displayed in the reproductive opportunity context, although, compared with the social control group, this result was only marginally significant overall (ANOVA: $F_{3,28} = 2.791$, $P = 0.059$), and sensory modality had no significant effect according to post-hoc tests. The courtship behaviour of males exposed to an intruder challenge did not differ from the social control group (ANOVA: $F_{3,28} = 1.351$, $P = 0.278$).

Hormonal responses to social context depends on sensory information

To better understand how sensory cues in different contexts contribute to acute endocrine responses, we measured bioavailable circulating testosterone, 11-KT, and 17β-oestradiol 1 h after stimulus presentation.

Compared to the social control group, testosterone significantly varied in both the intruder challenge (ANOVA: $F_{3,28} = 14.272$, $P = 0.000008$) and reproductive opportunity contexts (ANOVA: $F_{3,28} = 3.407$, $P = 0.031$) (Fig. 3A). In the reproductive opportunity context, a visual stimulus of a proceptive female was sufficient to elicit an androgen response compared to the control group ($P = 0.047$), whereas the chemical stimulus failed to elicit a robust response ($P = 1.000$) compared to the control situation. In the intruder challenge context, however, both visual and chemical information were sufficient to elicit an androgen response compared to the control group (visual versus control: $P = 0.032$; chemical versus control: $P = 0.00005$; visual and chemical versus control: $P = 0.00002$), the presentation of both simultaneously elicited an even higher response than visual information alone ($P = 0.039$).

We also measured circulating levels of 11-KT, an androgen specific to teleosts and some other fish (35), and found a significant effect in the males exposed to the intruder challenge context (ANOVA: $F_{3,28} = 3.841$, $P = 0.020$) and reproductive opportunity (ANOVA: $F_{3,28} = 3.402$, $P = 0.031$) compared to the control group (Fig. 3B). Specifically, both visual and chemical cues were necessary for an 11-KT response in the intruder challenge context ($P = 0.018$). However, post-hoc comparisons between reproductive opportunity and the control group were not significant after correcting for multiple hypothesis testing (34).

Because 17β-oestradiol regulates aggression in *A. burtoni* males (36), we also measured the acute responses of 17β-oestradiol to an assortment of sensory information in different social contexts. We did not find significant differences in males exposed to either reproductive opportunity contexts (ANOVA: $F_{3,26} = 2.169$, $P = 0.116$)

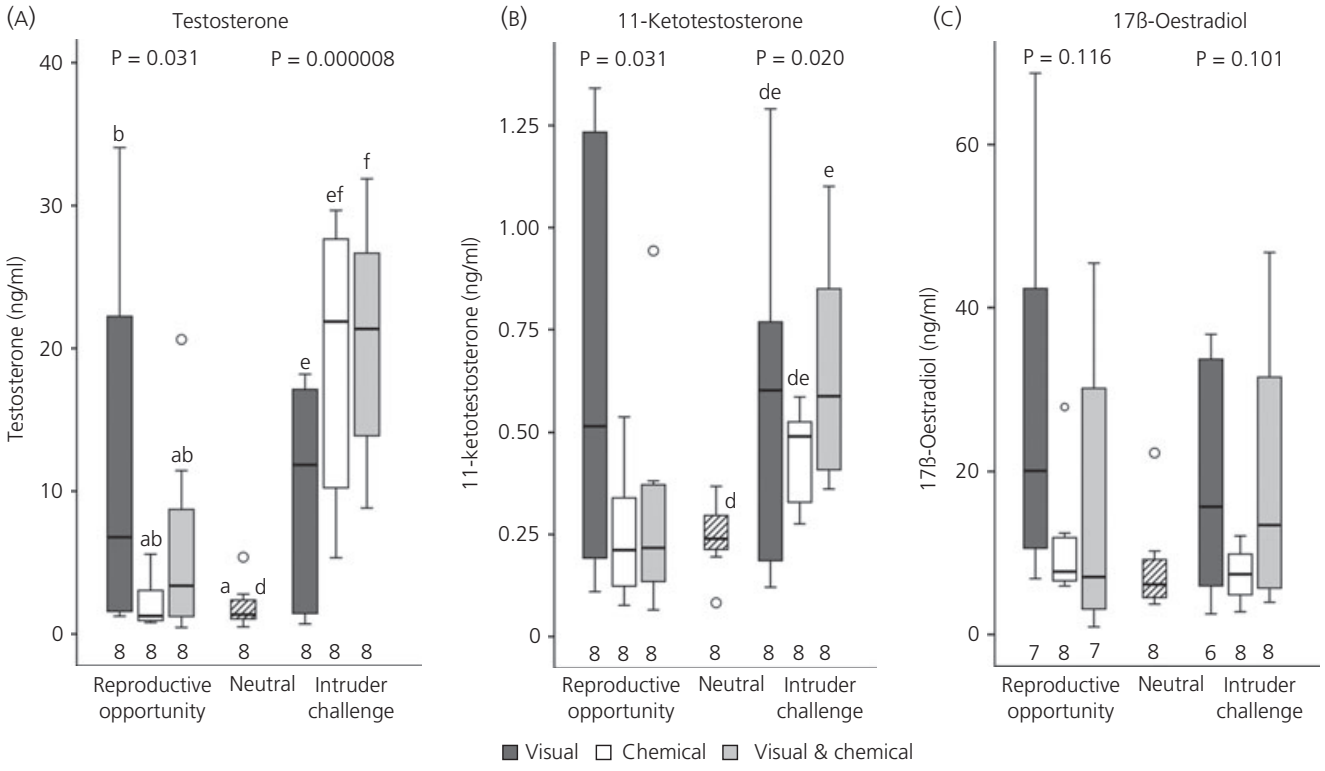

Fig. 3. Hormonal responses to stimuli in different social contexts depend on the sensory cue. Circulating testosterone (A), 11-ketotestosterone (B), and 17β-oestradiol (C) were recorded 1 h after dominant *Astatotilapia burtoni* males were presented with either visual (dark grey), chemical (white), or both visual and chemical cues simultaneously (light grey) in either reproductive opportunity (left part of each graph) or intruder challenge (right part of each graph) contexts; the neutral context is in the middle of each graph (hatched); number of samples in each group are at the bottom of each graph. ANOVA statistics are presented at the top of each graph, where males in the reproductive opportunity and intruder challenge contexts were compared separately with the control context. Boxes not joined by the same letter are significantly different according to post-hoc Tukey's honestly significant difference tests.

or intruder challenge (ANOVA: $F_{3,26} = 2.299$, $P = 0.101$) compared to the control group (Fig. 3C).

Neural integration of sensory information in different social contexts

To investigate which brain regions may be involved in the integration of context-dependent sensory information, we measured c-Fos induction in candidate brain regions that receive either visual or olfactory sensory input and are involved in mediating social behaviour (17,18). We did not find any significant differences in c-Fos induction between the social control situation and any of the experimental groups after correcting for multiple hypothesis testing. However, in some brain regions, there were significant differences of c-Fos induction between the reproductive opportunity sensory paradigms (see Supporting information, Fig. S2 and Table S1).

Induction of specific dopaminergic neurones depends on sensory information

Because evaluation of the salience of (social) stimuli is generally considered to be mediated by the dopaminergic reward system (19), we investigated whether different dopaminergic cell populations varied in IEG induction in different contexts with different sensory information. There were no differences in the number of dopamine cells between treatment groups in most of the dopaminergic cell populations analysed (for detailed statistics, see Supporting information, Table S2), except the TPp (i.e. putative homologue of the mammalian midbrain dopamine neurones) varied in the reproductive opportunity context (ANOVA: $F_{3,23} = 4.010$, $P = 0.020$), although pairwise post-hoc comparisons did not indicate any significant differences between groups (Tukey's HSD, $P > 0.083$) (see Supporting information, Fig. S3).

We next quantified c-Fos induction in forebrain dopaminergic cell populations. Induction of c-Fos in area Vc dopamine neurones significantly varied in both the reproductive opportunity context (ANOVA: $F_{3,28} = 3.723$, $P = 0.023$) and the intruder challenge context (ANOVA: $F_{3,28} = 5.205$, $P = 0.006$) compared to the control group (Fig. 4A,B). In the reproductive opportunity context, the simultaneous presentation of both visual and chemical cues increased c-Fos induction in Vc dopaminergic neurones compared to the control group ($P = 0.020$). In the intruder challenge context, males exposed to the visual stimulus of a dominant male had increased c-Fos induction in Vc dopaminergic neurones compared to the control group ($P = 0.013$). Because c-Fos induction of Vc neurones occurred in groups that displayed elevated aggression in behavioural trials, we next examined correlations of c-Fos induction in Vc dopamine neurones with behaviour. Importantly, within males exposed to the stimuli in the intruder challenge context, c-Fos induction in Vc dopaminergic neurones correlated significantly with aggression ($r = 0.455$, $n = 24$, $P = 0.026$) (Fig. 4C), but not overall locomotor activity ($r = -0.340$, $n = 24$, $P = 0.104$) (data not shown). There were no differences in c-Fos induction in other dopaminergic cell groups quantified (Table 1; see also Supporting information, Fig. S4).

Discussion

Behavioural and hormonal responses to sensory cues differ with social context

Male *A. burtoni* responded in a context-appropriate manner in both social contexts. In the intruder situation, a visual stimulus was necessary and sufficient for aggression, whereas male visual and chemical cues are both important for an androgen response to occur. This androgen or 'challenge' response has been extensively studied across vertebrates (3–5,37,38). We found that both visual and chemical cues together elicit a more robust androgen response than visual cues alone, and this response was similar for both testosterone and 11-KT. This important role for chemical cues is consistent with a previous study reporting that dominant *A. burtoni* males increase their urination frequency during aggressive encounters (39).

AD functions as a male pheromone in multiple teleost species (22,40–42). However, even though we have shown in the present study that waterborne AD induces a testosterone response, physiological evidence for cichlids detecting free (unconjugated) AD is still lacking (43,44). Note that AD (a testosterone precursor) could also be readily absorbed via the gill epithelium (45), although it takes several hours to metabolise such exogenous steroids; thus, the androgen response that we have observed in the present study in response to waterborne AD is most likely olfactory in nature.

The female pheromone stimulus (17α-20β-P) by itself was not sufficient to elicit courtship behaviour or a hormonal response in *A. burtoni* males in this experimental paradigm. This is in contrast to work conducted in other teleosts suggesting that 17α-20β-P increases courtship displays by males in the presence of a female (12,24). Similarly, Crapon de Caprona (14) reported that the smell of a female elicited more of a behavioural response than the presentation of a (visual) dummy female. We found the visual stimulus of a female was sufficient to stimulate an increase in androgen levels. It is unclear whether the rise in testosterone produces courtship displays or is itself a consequence of the behaviour (or both). The androgen receptor mediates courtship displays (but not aggressive behaviour) in male *A. burtoni* (36), suggesting this testosterone response may indeed facilitate courtship behaviour. Although this result was not significant, both chemical and visual cues appear to elicit sexual behaviour more than either cue alone. This is consistent with reports that *A. burtoni* males court more when they have full sensory information rather than visual information only (39). A more robust response may have been elicited in the reproductive opportunity context if very gravid females had been used. Although PGF2α induces proceptive behaviour in females, the distended abdomen of a gravid female, along with her overall size, may be a more salient cue than female behaviour alone (46).

Circulating levels of 17β-oestradiol did not change in response to challenge or opportunity contexts. We have previously demonstrated that 17β-oestradiol via oestrogen receptors mediate aggression in *A. burtoni* males (36). Much of the 17β-oestradiol is likely synthesised by aromatase, and so circulating 17β-oestradiol levels may not show acute changes because the synthesis of more aro-

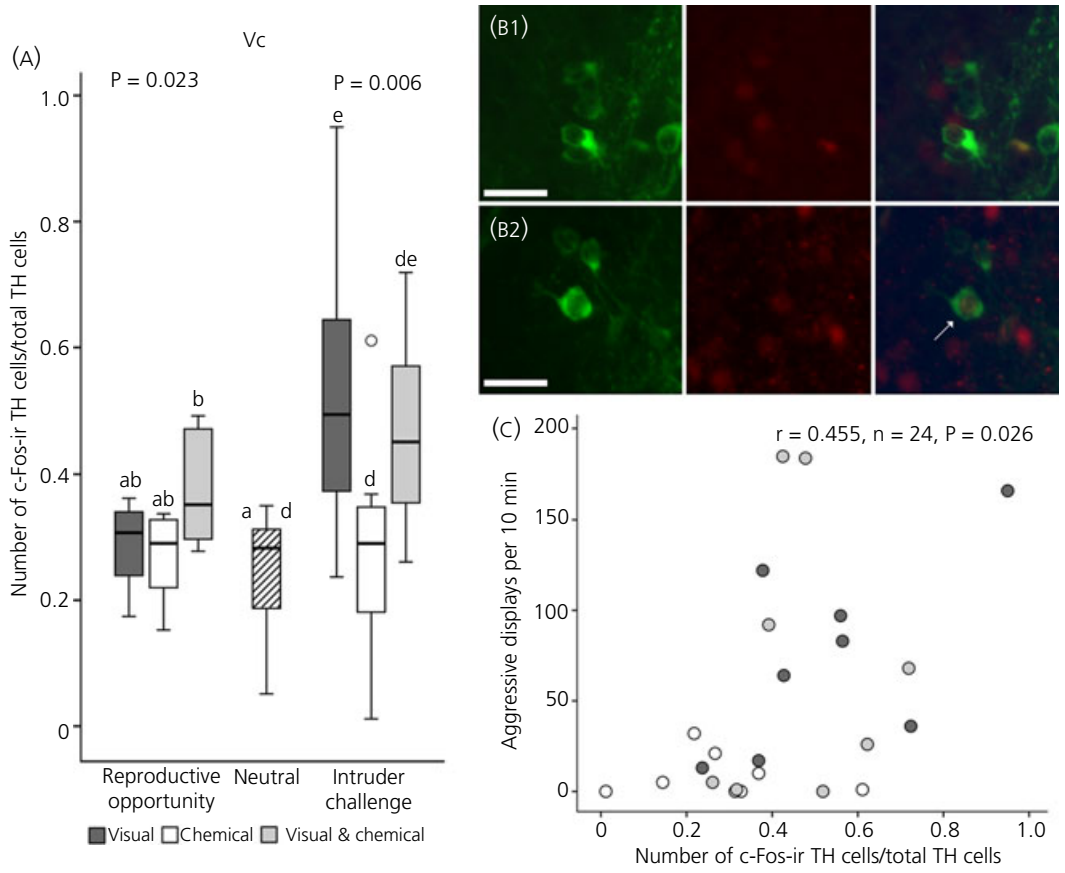

Fig. 4. Visual cues are necessary and sufficient for the induction of c-Fos in ventral telencephalon (Vc) dopaminergic neurones in the intruder challenge context. (A) Induction of c-Fos in Vc dopaminergic cells was quantified after a 1-h exposure to either visual (dark grey), chemical (white), or both visual and chemical cues simultaneously (light grey) in either reproductive opportunity (left part of each graph) or intruder challenge (right part of each graph) contexts; the neutral context is in the middle of each graph (hatched); n = 8 per group. ANOVA statistics are presented at the top of the graph, where males in the reproductive opportunity and intruder challenge contexts were compared separately with the control context. Boxes not joined by the same letter are significantly different according to Tukey's honestly significant difference test. (B) Representative micrographs of c-Fos induction in Vc dopaminergic neurones from a male exposed to the neutral control context (B1) or the visual stimulus of a male intruder (B2). First panel (green) shows dopaminergic neurones identified by tyrosine hydroxylase (TH) immunoreactivity, the second panel (red) shows c-Fos immunoreactivity, and the third panel shows the merged image, where the white arrow indicates TH and c-Fos colocalisation. Scale bars = 20 μm. (C) Induction of c-Fos dopaminergic neurones in Vc is correlated with aggression in the challenge context groups (visual intruder cue only, dark grey circles; chemical male cue, white circles; visual intruder and chemical male cue simultaneously, light grey circles). Fos-ir, Fos-immunoreactive.

Table 1. Detailed Statistics for c-Fos Induction in Dopaminergic Cell Populations in Various Brain Regions.

	ANOVA	
Brain region	Intruder challenge	Reproductive opportunity
Vc	$F_{3,28}$ = 5.205, P = 0.006	$F_{3,28}$ = 3.723, P = 0.023
pPOA	$F_{3,27}$ = 0.261, P = 0.853	$F_{3,28}$ = 0.332, P = 0.803
mPOA	$F_{3,28}$ = 1.559, P = 0.221	$F_{3,28}$ = 1.136, P = 0.351
PPr	$F_{3,27}$ = 0.715, P = 0.552	$F_{3,27}$ = 0.849, P = 0.479
TPp	$F_{3,25}$ = 0.395, P = 0.758	$F_{3,24}$ = 1.488, P = 0.243

mPOA, magnocellular part of the preoptic area; pPOA, parvocellular part of the preoptic area; PPr, rostral periventricular pretectal nucleus; TPp, periventricular part of the posterior tuberculum; Vc, central part of the ventral telencephalon.

matase protein may be required. The results of the present study suggest that 17β-oestradiol and its receptors may be involved in the long-term regulation of behaviour rather than acting in real time during territorial disputes. Alternatively, there may be a more acute response of 17β-oestradiol that peaks within less than 1 h after the onset of stimulus presentation and thus would have been missed in the present study. Moreover, it is possible that 17β-oestradiol levels in the brain could change as a result of local synthesis from circulating testosterone, which may not be reflected in plasma 17β-oestradiol measurements (47,48).

In summary, we have demonstrated not only an interaction of sensory cues that differs in challenge and opportunity contexts, but also a decoupling between the sensory cues that trigger hormonal and behavioural responses. What might be the adaptive value of dissociating endocrine responses in a manner not only dependent

on social context, but also on sensory modality? Because *A. burtoni* often find themselves in turbid environments (49), a potential intruder's most salient signal over a larger distance is likely chemical in nature. An androgen response to the odours of another nearby male is thus likely adaptive in that it anticipates territorial defence in the immediate future. Gravid female odours, on the other hand, are always present in natural communities of this continuously breeding species; thus, an androgen response may not be beneficial unless a male can (visually) court a female with some likelihood of mating success. Clearly, future studies will need to test these hypotheses.

Induction of c-Fos in dopaminergic neurones is sensory and context-dependent

The mesolimbic dopamine system is central for evaluating the salience of social stimuli (19). Co-localisation of IEGs with dopaminergic cell markers has provided insights into how this system processes different social contexts or sensory information. For example, in birds that perform courtship or sexual behaviour, c-Fos expression is induced in dopaminergic neurones of the ventral tegmental area and the periaqueductal grey [Japanese quail: (50); zebra finch: (51,52)]. Unexpectedly, we did not find significant effects of social context or sensory stimulus on c-Fos induction in preoptic or diencephalic dopamine neurones. This may be a result of the duration of stimulus presentation because zebra finches were exposed to stimuli for 90 min before brain collection (51,52). The lack of differential c-Fos induction in TPp dopaminergic neurones between different social contexts can be interpreted in several ways. First, given that *A. burtoni* is a highly social species, exposure to juveniles, which we considered to be a socially neutral control stimulus, may in fact constitute an appetitive stimulus in and of itself. If this was indeed the case, then the absence of significant between-group variation in c-Fos induction in dopaminergic neurones of the TPp may simply indicate that the three different social contexts did not differ in overall salience. A pre-exposure control could help to address this issue in future comparisons of c-Fos induction. Alternatively, the teleost TPp may not be functionally equivalent to the mammalian and avian ventral tegmental area as far as the integration of socially motivating stimuli is concerned.

We found significant IEG induction in dopaminergic neurones only in area Vc in response to different social cues or sensory stimuli, where a visual stimulus of a male intruder was necessary and sufficient for IEG induction of Vc dopaminergic cells. Moreover, c-Fos induction in these dopaminergic neurones was correlated with aggression but not with overall locomotion, suggesting this IEG induction was not the result of increased motor activity. To our knowledge, the function of these neurones is unknown in teleosts, although research conducted in mammals provides some interesting parallels. The teleost area Vc is putatively homologous to the mammalian striatum (18), which, at least in primates, contains many dopaminergic cells compared to other mammals (53). In humans, ventral striatum lesions impair the ability to recognise aggression in others (54), which is dependent on dopamine D_2 receptors (55). The present study suggests that, in teleosts, these dopaminergic cells in this striatum-like region may similarly serve to assess the valence of a visual social challenge.

Although our results present interesting and novel implications for specific cell types integrating social information in a sensory dependent manner, there are important caveats that must be considered with respect to any IEG study. First, it is important to note that the fact a significant difference in IEG induction in a particular brain region was not found does not mean that the target brain region plays no role in the process under study. It is possible that c-Fos induction in various brain regions is limited to particular cell types, as indeed was observed for area Vc in the present study. Alternatively, social stimulation may induce the expression of IEGs other than c-Fos and with different temporal dynamics. Additionally, we measured the number of c-Fos-immunoreactive cells in defined brain regions, which is not necessarily equivalent to intracellular mRNA or protein levels. Finally, we cannot rule out the possibility that the socially neutral control, a group of juveniles, constituted an appetitive stimulus that might have generated c-Fos responses to social situations in general. However, we chose this control condition to avoid a stress response as a result of social isolation in our focal animals. Clearly, further functional and colocalisation studies are required to determine temporal dynamics of IEG induction as well as the roles (if any) that these brain regions might play in integrating sensory information and orchestrating an adaptive behavioural response.

Neuroendocrine mechanisms of social decision-making

We report an integrative analysis combining behavioural, hormonal and neural responses to various sensory signals in different social contexts. Across vertebrates, brain regions that receive olfactory, visual and other sensory stimuli are well described (56–58), although how sensory stimuli that carry social information are integrated in the brain to produce context-appropriate behavioural responses is not well understood. The present study represents a significant step towards addressing this question. We previously proposed that examining the behavioural, hormonal and neural contributions to adaptive behaviour within challenge and reproductive opportunity contexts will establish a foundation for determining to what extent the neural bases of these adaptive behaviours are conserved across vertebrates (59). In the present study, we report the first step towards this goal by using IEG induction to detect dopaminergic cell groups involved in integrating information within different social contexts. Expanding this work to teleost species other than cichlids, as well as other vertebrate classes, will greatly advance our understanding of how the mechanisms underlying social behaviour have evolved.

Acknowledgements

We thank Matthew Fuxjager and Karen Maruska for their helpful comments on earlier versions of this manuscript, as well as Norm Stacey for discussions. This work was supported by an NSF DDIG 1011253 to L.A.O., a University Co-Op Undergraduate Research Fellowship to M.M.M., an NSF grant 0843712, and the Alfred P. Sloan Foundation, as well as a Dwight W. and Blanche Faye Reeder Centennial Fellowship in Systematic and Evolutionary

Biology and an Institute for Cellular and Molecular Biology Fellowship to H.A.H.

Received 25 November 2012,
revised 21 April 2013,
accepted 26 April 2013

References

1 Krebs JR, Davies MB. *An Introduction to Behavioral Ecology*. Oxford: Blackwell, 1993.
2 O'Connell LA, Hofmann HA. Genes, hormones, and circuits: an integrative approach to study the evolution of social behavior. *Front Neuroendocrinol* 2011; **32**: 320–335.
3 Wingfield JC, Hegner RE, Dufty AM Jr, Ball GF. The 'challenge hypothesis': theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am Nat* 1990; **136**: 829–846.
4 Hirschenhauser K, Oliveira RF. Social modulation of androgens in male vertebrates: meta-analyses of the 'challenge hypothesis'. *Anim Behav* 2006; **71**: 265–277.
5 Goymann W, Landys MM, Wingfield JC. Distinguishing seasonal androgen responses from male–male androgen responsiveness-revisiting the challenge hypothesis. *Horm Behav* 2007; **51**: 463–476.
6 Amstislavskaya TG, Popova NK. Female-induced sexual arousal in male mice and rats: behavioral and testosterone response. *Horm Behav* 2004; **46**: 544–550.
7 Robinson GE, Fernald RD, Clayton DF. Genes and social behavior. *Science* 2008; **322**: 896–900.
8 Leong CY. The quantitative effect of releasers on the attack readiness of the fish *Haplochromis burtoni*. *Z Vergl Physiol* 1969; **65**: 29–50.
9 Chen CC, Fernald RD. Visual information alone changes behavior and physiology during social interactions in a cichlid fish (*Astatotilapia burtoni*). *PLoS ONE* 2011; **6**: e20313.
10 Sorensen PW, Hara TJ, Stacey NE, Dulka JG. Extreme olfactory specificity of male goldfish to the preovulatory steroidal pheromone 17α-20β-dihydroxy-4- pregnen-3-one. *J Comp Physiol A* 1990; **166**: 373–383.
11 Sorensen PW, Scott AP, Stacey NE, Bowdin L. Sulfated 17a,20b-dihydroxy-4-pregnen-3-one functions as a potent and specific olfactory stimulant with pheromonal actions in the goldfish. *Gen Comp Endocrinol* 1995; **100**: 128–142.
12 Souza SMG, Lucion AB, Wassermann GF. Influence of 17a,20b-dihydroxy-4-pregnen-3-one injected into a postovulatory female on the reproductive behavior of male Nile tilapia (*Oreochromis niloticus*). *Comp Biochem Physiol A* 1998; **119**: 759–763.
13 Pinheiro MFM, Souza SMG, Barcellos LJG. Exposure to 17a,20b-dihydroxy-4-pregnen-3-one changes seminal characteristics in Nile tilapia, *Oreochromis niloticus*. *Aquacult Res* 2003; **34**: 1047–1052.
14 Crapon de Caprona MD. Olfactory communication in a Cichlid fish, *Haplochromis burtoni*. *Z Tierpsychol* 1980; **52**: 113–134.
15 Crapon de Caprona MD. The effect of chemical stimuli from conspecifics on the behavior of *Haplochromis burtoni* (Cichlidae, Pisces). *Experientia* 1974; **30**: 1394–1395.
16 Hoffman GE, Smith MS, Verbalis JG. c-Fos and related immediate early gene products as markers of activity in neuroendocrine systems. *Front Neuroendocrinol* 1993; **14**: 173–213.
17 Wullimann MF, Mueller T. Teleostean and mammalian forebrains contrasted: evidence from genes to behavior. *J Comp Neurol* 2004; **475**: 143–162.
18 O'Connell LA, Hofmann HA. The vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. *J Comp Neurol* 2011; **519**: 3599–3639.
19 Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Brain Res Rev* 1998; **28**: 309–369.
20 Munchrath LA, Hofmann HA. Distribution of sex steroid hormone receptors in the brain of an African cichlid fish, *Astatotilapia burtoni*. *J Comp Neurol* 2010; **518**: 3302–3326.
21 Fernald RD. The effect of testosterone on the behavior and coloration of adult male cichlid fish (*H. burtoni*, Günther). *Horm Res* 1976; **7**: 172–178.
22 Sorensen PW, Pinillos M, Scott AP. Sexually mature male goldfish release large quantities of androstenedione into the water where it functions as a pheromone. *Gen Comp Endocrinol* 2005; **140**: 164–175.
23 Stacey NE, Sorensen PW. 17α,20β-Dihydroxy-4-pregnen-3-one: a steroidal primer pheromone which increases milt volume in the goldfish, *Carassius auratus*. *Can J Zoolog* 1986; **64**: 2412–2417.
24 Kidd MR, Dijkstra PD, Alcott C, Lavee D, Ma J, O'Connell LA, Hofmann HA. Prostaglandin F2α facilitates female mating behavior based on male performance. *Behav Ecol Sociobiol* 2013; in press.
25 Stacey NE, Peter RE. Central action of prostaglandins in spawning behaviour of female goldfish. *Physiol Behav* 1979; **22**: 1191–1196.
26 Kidd MR, O'Connell LA, Kidd CE, Chen CW, Fontenot MR, Williams SJ, Hofmann HA. Female preference for males depends on reproductive physiology in the African cichlid fish *Astatotilapia burtoni*. *Gen Comp Endocrinol* 2013; **180**: 56–63.
27 Okuyama T, Suehiro Y, Imada H, Shimada A, Naruse K, Takeda H, Kubo T, Takeuchi H. Induction of c-fos transcription in the medaka brain (*Oryzias latipes*) in response to mating stimuli. *Biochem Biophys Res Commun* 2011; **404**: 453–457.
28 Kidd CE, Kidd MR, Hofmann HA. Measuring multiple hormones from a single water sample using enzyme immunoassays. *Gen Comp Endocrinol* 2010; **165**: 277–285.
29 O'Connell LA, Matthews BJ, Hofmann HA. Isotocin regulates paternal care in a monogamous cichlid fish. *Horm Behav* 2012; **61**: 725–733.
30 O'Connell LA, Fontenot MR, Hofmann HA. Characterization of the dopaminergic system in the brain of an African cichlid fish, *Astatotilapia burtoni*. *J Comp Neurol* 2011; **519**: 75–92.
31 Hirayama J, Cardone L, Doi M, Sassone-Corsi P. Common pathways in circadian and cell cycle clocks: light-dependent activation of Fos/AP-1 in zebrafish controls CRY-1a and WEE-1. *Proc Natl Acad Sci USA* 2005; **102**: 10194–10199.
32 Greenwood AK, Wark AR, Fernald RD, Hofmann HA. Expression of arginine vasotocin in distinct preoptic regions is associated with dominant and subordinate behaviour in an African cichlid fish. *Proc Biol Sci* 2008; **275**: 2393–2402.
33 Ma PM. Catecholaminergic systems in the zebrafish. I. Number, morphology, and histochemical characteristics of neurons in the locus coeruleus. *J Comp Neurol* 1994; **344**: 242–255.
34 Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B Methodol* 1995; **57**: 289–300.
35 Borg N. Androgens in teleost fishes. *Comp Biochem Phys C* 1994; **109**: 219–245.
36 O'Connell LA, Hofmann HA. Social status predicts how sex steroid receptors regulate complex behavior across levels of biological organization. *Endocrinology* 2012; **153**: 1341–1351.
37 Oliveira RF, Lopes M, Carneiro LA, Canário AV. Watching fights raises fish hormone levels. *Nature* 2001; **409**: 475.
38 Gleason ED, Fuxjager MJ, Oyegbile TO, Marler CA. Testosterone release and social context: when it occurs and why. *Front Neuroendocrinol* 2009; **30**: 460–469.
39 Maruska KP, Fernald RD. Contextual chemosensory urine signaling in an African cichlid fish. *J Exp Biol* 2012; **215**: 68–74.

40 Irvine IAS, Sorensen PW. Acute olfactory sensitivity of wild common carp, *Cyprinus carpio*, to goldfish sex pheromones is influenced by gonadal maturity. *Can J Zoolog* 1993; **71**: 2199–2210.
41 Pinillos ML, Guijarro AI, Delgado MJ, Hubbard PC, Canario AVM, Scott AP. Production, release and olfactory detection of sex steroids by the tench (*Tinca tinca L*). *Fish Physiol Biochem* 2002; **26**: 197–210.
42 Corkum LD, Meunier B, Moscicki M, Zielinski BS, Scott AP. Behavioural responses of female round gobies (*Neogobius melanostomus*) to putative steroidal pheromones. *Behaviour* 2008; **145**: 1347–1365.
43 Cole T, Stacey NE. Olfactory responses to steroids in an African mouthbrooding cichlid, *H. burtoni* (Gunther). *J Fish Biol* 2006; **68**: 661–680.
44 Stacey NE. Chapter 9: hormonally derived sex pheromones in fishes. In: Norris DE, Lopez KH, eds. *Hormones and Reproduction of Vertebrates: Fishes*. New York, NY: Elsevier, 2011: 169–192.
45 Scott AP, Pinillos ML, Huertas M. The rate of uptake of sex steroids from water by *Tinca tinca* is influenced by their affinity for sex steroid binding protein in plasma. *J Fish Biol* 2005; **67**: 182–200.
46 Werner NY, Lotem A. Choosy males in a haplochromine cichlid: first experimental evidence for male mate choice in a lekking species. *Anim Behav* 2003; **66**: 293–298.
47 Schlinger BA, Remage-Healey L. Neurosteroidogenesis: insights from studies of songbirds. *J Neuroendocrinol* 2012; **24**: 16–21.
48 Diotel N, Do Rego JL, Anglade I, Vaillant C, Pellegrini E, Gueguen MM, Mironov S, Vaudry H, Kah O. Activity and expression of steroidogenic enzymes in the brain of adult zebrafish. *Eur J Neurosci* 2011; **34**: 45–56.
49 Fernald RD, Hirata NR. Field study of *H. burtoni*: quantitative behavioural observations. *Anim Behav* 1977; **25**: 964–975.
50 Charlier TD, Ball GF, Balthazart J. Sexual behavior activates the expression of the immediate early genes c-fos and Zenk (egr-1) in catecholaminergic neurons of male Japanese quail. *Neuroscience* 2005; **131**: 13–30.
51 Bharati IS, Goodson JL. Fos responses of dopamine neurons to sociosexual stimuli in male zebra finches. *Neuroscience* 2006; **143**: 661–670.
52 Goodson JL, Kabelik D, Kelly AM, Rinaldi J, Klatt JD. Midbrain dopamine neurons reflect affiliation phenotypes in finches and are tightly coupled to courtship. *Proc Natl Acad Sci USA* 2009; **106**: 8737–8742.
53 Betarbet R, Turner R, Chockkan V, DeLong MR, Allers KA, Walters J, Levey AI, Greenamyre JT. Dopaminergic neurons intrinsic to the primate striatum. *J Neurosci* 1997; **17**: 6761–6768.
54 Calder AJ, Keane J, Lawrence AD, Manes F. Impaired recognition of anger following damage to the ventral striatum. *Brain* 2004; **127**: 1958–1969.
55 Lawrence AD, Calder AJ, McGowan SW, Grasby PM. Selective disruption of the recognition of facial expressions of anger. *Neuroreport* 2002; **13**: 881–884.
56 Halasz N. *The Vertebrate Olfactory System: Chemical Neuroanatomy, Function, and Development*. Budapest: Akadémiai Kiadó, 1990.
57 Buck LB. Information coding in the vertebrate olfactory system. *Annu Rev Neurosci* 1996; **19**: 517–544.
58 Butler AB, Hodos W. *Comparative Vertebrate Neuroanatomy: Evolution and Adaptation*. New York, NY: Wiley, 1996.
59 O'Connell LA, Hofmann HA. Evolution of a vertebrate social decision-making network. *Science* 2012; **336**: 1154–1157.

Supporting Information

The following supplementary material is available:

Fig. S1. Specificity of commercial antibodies for *Astatotilapia burtoni* antigens.

Fig. S2. Induction of c-Fos in the reproductive opportunity context.

Fig. S3. Number of tyrosine hydroxylase-positive neurones in the posterior tuberculum.

Fig. S4. Induction of c-Fos in dopaminergic cells in the preoptic area and posterior tuberculum.

Table S1. Detailed statistics for c-Fos induction in candidate forebrain regions.

Table S2. Detailed statistics for the number of tyrosine hydroxylase-immunoreactive cells.