

Neuromolecular correlates of cooperation and conflict during territory defense in a cichlid fish



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ABSTRACT

Cooperative behavior is widespread among animals, yet the neural mechanisms have not been studied in detail. We examined cooperative territory defense behavior and associated neural activity in candidate forebrain regions in the cichlid fish, *Astatotilapia burtoni*. We find that a territorial male neighbor will engage in territory defense dependent on the perceived threat of the intruder. The resident male, on the other hand, engages in defense based on the size and behavior of his partner, the neighbor. In the neighbor, we find that an index of engagement correlates with neural activity in the putative homolog of the mammalian basolateral amygdala and in the preoptic area, as well as in preoptic dopaminergic neurons. In the resident, neighbor behavior is correlated with neural activity in the homolog of the mammalian hippocampus. Overall, we find distinct neural activity patterns between the neighbor and the resident, suggesting that an individual perceives and processes an intruder challenge differently during cooperative territory defense depending on its own behavioral role.

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1. Introduction

Cooperation both within and between species has evolved repeatedly in numerous lineages of vertebrates and invertebrates (Dugatkin, 1997; Sachs et al., 2004). Cooperation is the ‘acting together’ of two or more individuals to achieve an end situation which could not be achieved individually (Taborsky, 2007; Brosnan and de Waal, 2002). A cooperater pays a cost for another individual to receive a benefit, often resulting in an average increase in direct fitness (Brosnan and Bshary, 2010; Nowak, 2006). A defector, however, pays no cost and does not provide benefits (Nowak, 2006). Knowledge about and awareness of the actions of social partners are often key features of cooperative behavior. Importantly, without examining how the brain is processing and responding to social information our understanding of cooperative behavior remains quite limited (de Waal and Ferrari, 2010). To address this gap in our knowledge of cooperation, in this study, we examine neural correlates of cooperation and conflict in the form of cooperative territory defense.

Competition over resources can serve as a selective force for the evolution of cooperation (Díaz-Muñoz et al., 2014). Familiar neighboring territorial males of many species exhibit restraint in aggression toward

each other despite being in direct competition for mates, food, and shelter, though they will ferociously attack trespassing strangers (Fischer, 1954). It can be less costly to cooperate than to renegotiate boundaries with a new neighbor, which is why under certain conditions a territorial male may cooperate with other males by defending a neighboring territory from an intruder (Getty, 1987). Although this form of cooperation is predicted to be widespread in nature, it has been convincingly demonstrated in very few species. For example, in fiddler crabs (Backwell and Jennions, 2004) and possibly chipping sparrows (Goodwin and Podos, 2014) (but see Akçay and Beecher, 2015), neighboring males will cooperate and come to the aid of a neighbor depending on the perceived degree of threat posed by an intruder. Interestingly, only the behavior of the neighbor has been examined in these cases. It is unknown how the recipient responds to receiving help. Here, we study both the cooperater and the recipient to gain an understanding of how an individual's behavioral role during territory defense may affect both behavior and the underlying neural processes involved.

The brain regions and gene networks that mediate decision-making in the context of cooperation are as of yet unknown (Taborsky and Taborsky, 2015), although strategic social cooperation very likely involves the integration of spatial and affective memory with reward processing and aggression regulation. We therefore hypothesized that the social decision-making (SDM) network, a deeply conserved neural circuit involved in the integration of salient stimuli and reward processing across vertebrates (O'Connell and Hofmann, 2011, 2012) plays a

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central role in these processes. Given our current knowledge, SDM network nodes such as the hippocampus, amygdala, striatum, and preoptic area appear to be particularly good targets of investigation in this context. Briefly, across vertebrates, the amygdala is critical for encoding and responding to the affective valence of social (and other) signals (Adolphs, 2010; McGaugh, 2004). The striatum, a central region in the reward system, regulates motivated behavior (Balleine et al., 2007; Báez-Mendoza et al., 2013). The hippocampus is well known for organizing spatial memory and plays a critical role in social recognition (Kogan et al., 2000; Eichenbaum, 2004). The preoptic area is a neuroendocrine relay center essential for sexual and aggressive behavior (Hull and Dominguez, 2007). Finally, excitatory and inhibitory projections between these and other SDM network regions are essential to mediating complex behavior (Felix-Ortiz and Tye, 2014; van der Meer et al., 2014).

Numerous neurochemical pathways regulate social behavior across vertebrates. For example, the glucocorticoid cortisol (Cort) is typically associated with the physiological response to highly salient – e.g., social (Summers et al., 2005) – stimuli, often (though not necessarily) considered stressors. Androgens, such as testosterone (T), are also well-known to mediate social context and are typically released in response to social challenge (Wingfield et al., 1990). It has been proposed that T surges reinforce learning associated with aggressive challenges in a manner dependent on the location where the agonistic encounters take place (Gleason et al., 2009). Importantly, T interacts with the dopaminergic reward system to induce conditioned place preference (Schroeder and Packard, 2000). Dopamine (DA) plays several important roles in the regulation of behavior; it encodes the salience and rewarding properties of social stimuli, and it modulates motivated, goal-directed behavior (Riters, 2012; Trainor, 2011). It is then maybe not surprising that the DA system has been implicated in cooperative cleaning behavior of the Indo-Pacific bluestreak cleaner wrasse, *Labroides dimidiatus*. Specifically, treatment with a dopamine D1 receptor antagonist increased the number of cleaning interactions, as well as tactile stimulation toward clients, though it is unclear if cooperation was affected per se (Messias et al., 2016). Taken together, these findings suggest that glucocorticoids and androgens, possibly in conjunction with the mesolimbic DA system, could play central roles in regulating cooperative decision-making in general and cooperative territory defense more specifically.

The African cichlid fish, *Astatotilapia burtoni*, displays extraordinary context-dependent social behavior. As a result, this species has become a powerful model system in social neuroscience for investigating the neural bases of complex social behavior (Hofmann, 2003). Dominant *A. burtoni* males aggressively defend spawning territories (Parikh et al., 2006). Border conflict behavior occurs between dominant males in neighboring territories along their shared boundary (Fernald and Hirata, 1977). *A. burtoni* males modulate their behavior based on visually acquired social information (Grosenick et al., 2007; Desjardins et al., 2012; Alcazar et al., 2014; Chen and Fernald, 2011). They are capable of transitive inference and can deduce the dominance hierarchy by observing pairwise interactions between opponents (Grosenick et al., 2007). In addition, males perceive and respond to very small size differences between competitors (± 3 mm, $<8\%$) with an advantage to the larger of two opponents (Alcazar et al., 2014). Importantly, both the SDM network and the dopaminergic system have been described in detail in this species (O'Connell and Hofmann, 2011; O'Connell et al., 2011, 2013a).

To begin to examine the neural basis of cooperation, we investigated whether and if *A. burtoni* males display cooperative defense behavior in a series of four experiments. In Experiment 1 (habituation), we examined whether and how neighboring males socially habituate to each other (by exhibiting decreasing aggression) and how steroid hormone levels change in response. We hypothesized that neighbor familiarity would lead to a decrease in aggression and steroid hormone levels. In Experiment 2 (cooperative defense), we used male body size differences to assess the extent and attributes of cooperative defense in response to an intruder. We hypothesized that neighbors would engage

in cooperative territory defense in a size-dependent manner by aggressing the intruder more when the resident is smaller than the intruder, as smaller residents would benefit more in such a case. We predicted resident males would be aggressive to an intruder independent of size, given the immediate perceived threat, but may modulate behavior based on help received from their neighbor. To gain a better understanding of the interaction between partners and the significance of neighbor assistance, in Experiment 3 (neighbor defection), we simulated neighbor defection during a territorial intrusion and examined the behavioral response of the resident male. We hypothesized that neighbor defection would result in increased aggressive defense from the resident. Finally, in Experiment 4 (neural activity in cooperation and conflict), we analyzed immediate-early gene (IEG) induction to determine how variation in cooperation from neighbors and residents varied with neural activation in SDM network nodes such as the hippocampus, amygdala, striatum, and POA. Immediate-early genes, such as c-Fos, are widely used to assess region and cell type-specific responses to various stimuli (Morgan and Curran, 1991). Further, to gain insight into the function of activated cells, we examined the role of DA in a spatially explicit manner by co-labeling c-Fos with tyrosine hydroxylase (TH), which is often used as a marker of dopaminergic cells. We hypothesized that cooperation would lead to increased activity in reward-related regions and that the specific role an individual plays in a cooperative context might be associated with distinct neural activity patterns across key nodes of the social decision-making network.

2. Materials and methods

2.1. Animals

A. burtoni descended from a wild caught stock population were maintained in naturalistic communities, as described previously (Munchrath and Hofmann, 2010), until transfer to the experimental paradigm. All work was done in compliance with the Institutional Animal Care and Use Committee at the University of Texas at Austin.

2.2. Behavior

Paired adjacent 38 l aquarium tanks were established with one territorial *A. burtoni* male and two females taken from stable community tanks, as well as two juveniles from grow out tanks. Females and juveniles were included to encourage naturalistic male behavior. We selected brooding females and stripped them of their broods immediately prior to placement in the experimental tanks in order to control for reproductive state. Males that were to be paired (including the intruders) were selected from different community tanks to ensure they had not previously encountered one another. Each tank contained a halved terra-cotta pot to serve as a bower, and terra-cotta shards and plastic plants to serve as refuge for the juveniles and females. Opaque acrylic dividers visually isolated adjacent tanks for nine days in order to allow the animals to acclimate to the set-up. At the start of the experiments, the opaque divider between adjacent tanks was removed for 1 h each at 10:00 h and 15:00 h for four continuous days (Fig. 1). For Experiments 2 and 3, following habituation, a territorial intruder contained within a perforated transparent plastic cylinder was placed in the tank of the resident male at 10:00 h on Day 5 and the opaque divider was removed immediately (Fig. 1). The cylinder was placed with a 2 cm gap from the wall of the resident's tank, nearest the neighbor.

Behavior was video recorded during the first exposure on Day 1 through 4 for Experiment 1, and on Day 1, 4, and 5 for Experiments 2 and 3. Video scoring was done blind to day during the habituation period. Although large size differences could be visually detected, video scoring when the intruder was present was kept as blind as possible by scoring all behavior before analysis. Behavior of the focal male was quantified using JWatcher V1.0 (Blumstein and Daniel, 2007) and was scored for a 10 min window 20 min after removal of the opaque divider.

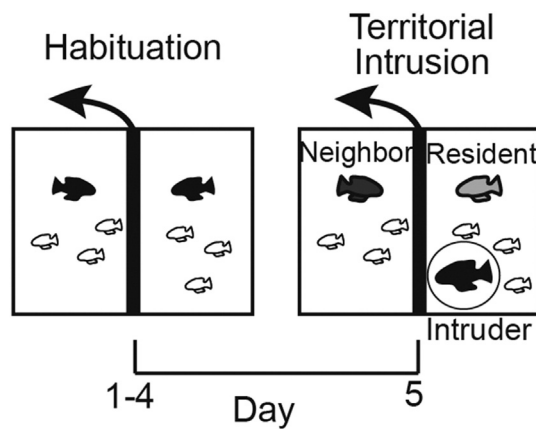


Fig. 1. Experimental design to examine effects of neighbor familiarity (Experiment 1; habituation period only) and the neural mechanisms of cooperation and conflict in defense coalitions (Experiments 2–4). On Days 1–4, the opaque divider was removed daily for 1 h at 1000 h and 1500 h. On Day 5 at 1000 h, an intruder was added to a randomly assigned tank. The male in whose tank the intruder was placed is referred to as the resident and the adjacent male as the neighbor.

This time window adequately captures variability in behavior as we have previously shown for a similar paradigm (O'Connell et al., 2013b). Behaviors recorded included forward displays to the partner, lateral displays to the partner, chases to the school, courtship displays (leads and quivers), and in the case of the Experiments 2 and 3, forward displays to the intruder and lateral displays to the intruder. For Experiment 1, the behavior of only the larger male was scored on Day 1 through 4. For Experiment 2, the behavior of only one focal male was scored for Day 1 and 4, but the behavior of both neighbor and resident was scored on Day 5. We chose to score the behavior of only one focal male during the habituation period as the aggressive displays are highly mirrored and dependent on the behavior of both males. In Experiment 3, the behavior of only the resident was scored on Day 5. We did not score the behavior of the intruder in any of the experiments. Typically (>85% of trials), the intruder did not show any aggressive behavior, likely as a consequence of experiencing the transfer into the new environment as stressful. Importantly, whether or not the intruder responded aggressively had no detectable impact on the behavior of the focal fish and hence presumably did not change the level of the perceived threat.

2.3. Experiment 1: habituation

We used a repeated measures design to collect waterborne hormone samples during the habituation period from the larger of the two males in a set ($n = 23$). Waterborne hormone sampling provides a non-invasive method to simultaneously sample multiple hormones from an individual (Scott et al., 2008; Kidd et al., 2010). Teleosts release free steroid hormones through their gills. These free steroids are representative of circulating levels as their release in water is a result of a concentration gradient between plasma and water (Scott et al., 2008; Scott and Ellis, 2007). 1 h after the first removal of the opaque divider on Day 1 through 4, the focal male was netted and placed in a beaker of 500 ml of fresh holding water for 1 h and then returned to its tank. The water was filtered to remove particulates and then frozen at -20°C until processing. Following the protocol from (Kidd et al., 2010), samples were thawed and drawn through activated C18 solid phase extraction columns (Waters Corp.) to bind steroid hormones and then frozen at -20°C . Columns from Day 1 and Day 4 were thawed and eluted with 100% EtOH split into 3 aliquots, then evaporated under nitrogen gas, and frozen at -20°C . Aliquots were thawed and resuspended in assay buffer and enzyme-linked immunosorbent assays (ELISA) were run for T (Day 1, $n = 21$; Day 4, $n = 23$), 11-KT (Day 1, $n = 11$; Day 4, $n = 11$) and Cort (Day 1, $n = 23$; Day 4, $n = 23$); T & Cort: Enzo Life Sciences, Farmingdale, NY, USA; 11-KT: Caymen Chemicals, Ann Arbor, MI, USA).

2.4. Experiment 2: cooperative defense

We staged trials such that neighbors, residents, and intruders varied in size (avg. 62.4 mm, range 43–73 mm; avg. 59.4, 44–71 mm, avg. 62.1, 45–75, respectively), and such that the size difference between males also varied (0–13 mm). 1 h after the introduction of the intruder, males were individually removed from the trial and measured for body mass and standard length. Blood was drawn from the dorsal aorta using heparinized 26G butterfly infusion sets (Becton Dickinson, Mountain View, CA) and plasma was stored at -80°C for hormone assays. Males were killed by rapid cervical transection and brains dissected and fixed overnight at 4°C in 4% paraformaldehyde. Plasma samples were diluted 1:30 and measured for T and Cort using ELISA (Enzo Life Sciences, Farmingdale, NY; Cayman Chemical, Ann Arbor, MI).

2.5. Experiment 3: simulated neighbor defection

We simulated neighbor defection by preventing the neighbor from participating in cooperative defense. For these trials, males were habituated as described above and then on Day 5, immediately prior to the introduction of the intruder, we inserted a transparent divider covered in one-way film (CollidEscape) between the tanks of the two males. With a light source opposite the neighbor's tank, the divider was used such that the resident could see the neighbor, but the neighbor could not view the resident or intruder. The neighbor was always larger than the resident (average body length (mm): Defection – N: 60.6 (54–65), R: 54.9 (47–60), I: 61.4 (58–67); Cooperation – N: 61.3 (49–67), R: 54.8 (44–62), I: 61.4 (45–70)). The average size difference between intruder and neighbor was 0.1 mm (-4 –3) for Cooperation and 0.8 mm (-5 –3) for Defection.

2.6. Experiment 4: neural activity in cooperation and conflict

To assess neural activity associated with cooperation and conflict in defense coalitions, we used the brains collected in Experiment 2. Tissue was harvested from 39 neighbors and 16 residents. Brain samples were processed from all resident males and a selected subset of 19 neighbor males.

2.7. Immunohistochemistry (IHC)

Brains were fixed overnight in 4% paraformaldehyde at 4°C , then washed with 1X phosphate-buffered saline (PBS) and cryoprotected overnight in 30% sucrose at 4°C , then finally embedded in O.C.T. compound (Tissue-Tek; Fisher Scientific Co., Pittsburgh, PA, USA) and stored at -80°C . A subset of brains were selected from individuals for IHC so as to span the variability in behavior and size differences between all three males ($n = 19$ for neighbors, $n = 16$ for residents). Selected brains were sectioned on a cryostat at $30\ \mu\text{m}$ and thaw-mounted onto Super-Frost Plus slides (Fisher Scientific) in four series that were stored at -80°C until IHC. For Brightfield detection of the immediate-early gene c-Fos, one series of sections was removed from -80°C , dried on a slide warmer, and processed for immunohistochemistry, as described previously (O'Connell et al., 2013b), using 1:500 rabbit anti-c-Fos primary antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA; catalogue # sc-253). To quantify activation of dopaminergic neurons, we co-localized c-Fos and tyrosine hydroxylase (TH), using an alternate slide series for fluorescent double-labeling IHC. TH catalyzes the rate limiting step in catecholamine synthesis and is a neurochemical marker for dopaminergic cell populations in the teleost fore- and midbrain (O'Connell et al., 2011). Slides were incubated overnight in a mix of 1:500 mouse anti-TH (catalogue # MAB318; Millipore, Billerica, MA, USA) and 1:500 rabbit anti-c-Fos. Slides were then washed in PBS and incubated for 2 h in a mix of 1:200 goat anti-mouse Alexa Fluor 488 (Life Technologies, Grand Island, NY, USA) and 1:200 goat anti-rabbit Alexa Fluor 594 (Life Technologies). Slides were then rinsed in PBS

and cover-slipped with Vectashield hardset mounting media with DAPI (Vector Laboratories, Burlingame, CA, USA).

2.8. Quantification of *c-Fos* induction

Slides were coded such that the experimenter was blind to treatment. Cell nuclei labeled by *c-Fos* IHC were clearly marked by dark brown staining and counted using the Optical Fractionator workflow of the StereoInvestigator software package (MicroBrightfield, Williston, VT, USA). We quantified *c-Fos* induction in the three subregions of the medial part of the dorsal telencephalon (Dm-1, Dm-2, Dm-3), which is considered homologous to the mammalian basolateral amygdala; one subregion of the supracommissural nucleus of the ventral pallidum (Vsm, a structure likely homologous to the medial amygdala/bed nucleus of the stria terminalis of mammals); the lateral subdivision of the lateral part of the dorsal telencephalon (Dlv, putative hippocampus homolog); and in the parvocellular (pPOA, homologous to the paraventricular nucleus) as well as magnocellular and gigantocellular cell groups (mPOA and gPOA, respectively; considered homologous to the supraoptic nucleus). Three sections of each brain region were quantified and averaged per individual. A region of interest was defined using a 2× objective, then positive cells were counted using a 20× objective. The counting frame and sampling grid parameters varied for each brain region to account for differences in cell density and area of each region (POA: 35 × 35 counting frame, 75 × 75 sampling grid; Vs: 30 × 30 counting frame, 100 × 100 sampling grid; Dm, Dlv: 50 × 50 counting frame, 150 × 150 sampling grid). For each brain region, data are presented as the estimated population of *c-Fos* immunoreactive nuclei using number weighted section thickness divided by the area of the region.

2.9. Quantification of fluorescent co-localization

Fluorescent signal was detected using a Zeiss AxioImager.A1 AX10 microscope equipped with DAPI, FITC, and TRITC filters. Photographs were taken under a 20× magnification with a digital camera (AxioCam MRc, Zeiss) using the AxioVision (Zeiss) image acquisition and processing software. The number of *c-Fos* and TH colocalized neurons was determined in select dopaminergic cell populations (pPOA, mPOA, the central part of the ventral telencephalon (Vc; homologous to the striatum (O'Connell and Hofmann, 2011))) by superimposing images generated by FITC and TRITC filters using the AxioVision software (Fig. 9). To correct for poor contrast in the TRITC channel, photographs were adjusted for brightness (−0.24) and contrast (2.15). The average number of photographs included for analysis varied between brain regions (Vc: $n = 6$; pPOA: $n = 3$; mPOA: $n = 3$). The resulting data are presented as the ratio of neurons co-expressing *c-Fos* and TH to the total number of TH expressing neurons counted.

2.10. Data analysis

All analyses were conducted in R v. 3.0.0. (R Core Team 2013). Forward and lateral aggressive displays were highly correlated and thus summed to 'Aggressive displays'. Courtship displays occurred infrequently and were excluded from further analyses. Behavior measures are reported as the number of displays per 10 min. Data were checked for normality by examining Q-Q plots and using the Shapiro-Wilk test. Nonparametric tests were used when necessary. We used Cohen's d to measure effect size when r values were not applicable.

For the behavior data in Experiment 1, we used a linear mixed model with 'Aggressive displays to the partner' as the dependent variable, day as the explanatory variable, and identity as a fixed effect. We used paired exact Wilcoxon sign-ranked tests to examine planned comparisons for changes in aggressive displays between

Day 1 and 2, Day 2 and 3, and Day 3 and 4, and for changes in hormone levels between Day 1 and 4 for T, 11-KT, and Cort. The relationships between waterborne hormone levels and aggressive displays were examined using linear regression analysis.

In Experiment 2, we asked which measure of male size or size relationship best predicted aggressive displays to the intruder. Neighbor and resident data were analyzed separately. With aggressive displays to the intruder as the dependent variable, we used multiple generalized linear regression analysis with a negative binomial model to account for over-dispersion of count data. We investigated measures of both absolute size and absolute size difference (i.e. larger – smaller) in our initial models. Employing the approach suggested by (Taylor and Elwood, 2003), we used the measure of size difference only if the results of the regression with the two focal male sizes were significant in equal and opposite directions.

In Experiment 3, we used Student's t -tests to compare differences in behavior when the neighbor cooperated or defected. We examined resident aggressive displays to intruder, total male directed aggressive displays, and total intruder received aggressive displays.

In Experiment 4, to gain specific insight into the mechanisms underlying cooperation, we calculated an "Engagement Index" (EI) for the neighbors by extracting the residuals from the regression between 'Displays to the Intruder' and 'Intruder length – Neighbor length' (based on results of experiment 2, see below). By removing the relationship between aggression and body size from the analysis this index thus measures how likely an individual is to engage in aggressive defense independent of its own size or the size of the intruder. Similarly, we calculated an EI for the resident by extracting the residuals from the regression between 'Displays to the Intruder' and 'Partner length' (based on results, see above). Using linear regression analysis, we examined correlations between these indices and measures of neural activation within seven brain regions/subregions (Dm-1, Dm-2, Dm-3, Dlv, Vs, pPOA, mPOA), as well as with measures of activity in dopaminergic cell populations in three regions (Vc, pPOA, mPOA). Additionally, as we found above that residents respond to the behavior of their neighbors, we also examined correlations between neural activation in the resident and 'Partner (i.e. Neighbor) displays to the Intruder.'

Finally, to visually demonstrate the relationships between behavioral and physiological measures and to generate hypotheses for future study, we created clustered correlation matrices for neighbor and resident males using the R package lattice. Physiological measures included overall *c-Fos* induction, the ratio of *c-Fos* expressing TH neurons, and T and Cort levels. Behavior data included 'Displays to Intruder,' 'Displays to Partner,' 'Partner displays to Intruder,' and 'Partner displays to Focal male.' In order to have a complete data matrix, missing values were imputed using the mean with R package Hmisc (Harrell, 2008). We used the R package pvclust to generate p -values for each cluster using multiscale bootstrap resampling. Clusters for which $p < 0.05$ are surrounded by a black square (Suzuki and Shimodaira, 2006). To examine similarity of the matrices for resident and neighbor, we used the Mantel test in R package ade4 to compute the correlation between the distance matrices.

3. Results

3.1. Experiment 1: aggressive behavior and hormone levels decreased with repeated social exposure

Upon repeated visual exposure to a territorial male (Fig. 1), the amount of aggressive displays decreased over time (LMER: $t = 11.315$, $p < 0.001$, Fig. 2). Aggressive displays decreased from Day 1 to Day 2 ($V = 171$, $p = 0.001$, $r = 0.47$), and from Day 2 to Day 3 ($V = 197.5$, $p < 0.001$, $r = 0.51$), but not from Day 3 to Day 4 ($V = 94$, $p = 0.98$, $r = 0.01$). Interestingly, small males displayed more frequently to their partner on Day 1 ($R^2 = 0.14$, $p = 0.002$) and

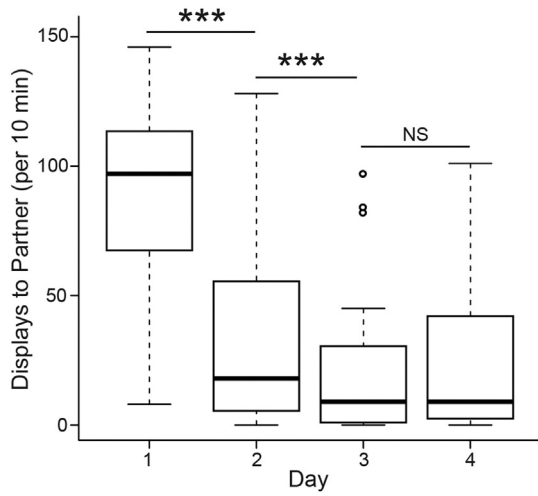


Fig. 2. Upon repeated visual exposure to a territorial male, the amount of aggressive displays decreased over time. Aggressive displays decreased from Day 1 to Day 2, and from Day 2 to Day 3, but not from Day 3 to Day 4.

Day 4 ($R^2 = 0.11$, $p = 0.006$). Using a non-invasive waterborne hormone sampling technique, we found that levels of testosterone (T), the teleost-specific androgen 11-ketotestosterone (11-KT), and cortisol (Cort) also decreased from Day 1 to Day 4 (T: $n = 23$, $V = 218$, $p < 0.001$, $r = 0.53$; 11-KT: $n = 11$, $V = 63$, $p = 0.005$, $r = 0.57$; Cort: $n = 23$, $V = 250$, $p < 0.001$, $r = 0.5$; Fig. 3a).

Aggressive displays to the partner on Day 1 and Day 4 were predicted by levels of T and 11-KT (Fig. 3b). Interestingly, T levels were more strongly correlated with aggressive displays to the partner on Day 4 ($R^2 = 0.33$, $p < 0.001$) than Day 1 ($R^2 = 0.22$, $p = 0.06$). In contrast, 11-KT correlated with aggressive displays to the partner on both Day 1 ($R^2 = 0.9$, $p < 0.001$) and Day 4 ($R^2 = 0.51$, $p = 0.01$). Finally, Cort

levels were not related to aggressive displays to the partner on either Day 1 ($R^2 = 0.07$, $p = 0.29$) or Day 4 ($R^2 = 0.04$, $p = 0.38$).

3.2. Experiment 2: males engage in territory defense

Once we established that male partners show reduced aggression over time as they become familiar with their neighbor, we investigated the conditions under which the neighbor and the resident would engage in territory defense against a conspecific male intruder. The assessment rules animals use in contest decisions are complex (Elwood and Arnott, 2012; Fawcett and Mowles, 2013), as individuals may assess their own and/or their opponent's size or some other measure of fighting ability (Arnott and Elwood, 2009). As such, we investigated measures of both absolute size and absolute size difference (i.e. larger – smaller). Neighbors displayed more frequent aggression to intruders when intruders were larger (i.e. Intruder – Neighbor length; $R^2 = 0.19$, $p < 0.001$; Fig. 4a). Resident males displayed more frequent aggression to intruders when their partner, the neighbor, was small ($R^2 = 0.15$, $p = 0.002$; Fig. 4b). Overall, resident males displayed more to the intruder than did neighbor males ($t = -3.04$, $p = 0.003$, $d = 0.59$).

3.3. Experiment 3: resident increases intruder-directed aggression when neighbor defects

To investigate the effects of neighbor behavior on the defense strategy of the resident, we performed an experiment that prevented the neighbor from displaying to the intruder by using a one-way transparent divider between adjacent tanks. This design allowed the resident male to observe the neighbor, but the neighbor was prevented from viewing the resident and intruder in the adjacent tank. When the neighbor did not aggress the intruder, the resident increased the frequency of aggressive displays to the intruder ($t = 3.0$, $p = 0.009$, $d = 1.41$; Fig. 5). Importantly, total male directed aggressive displays and total intruder

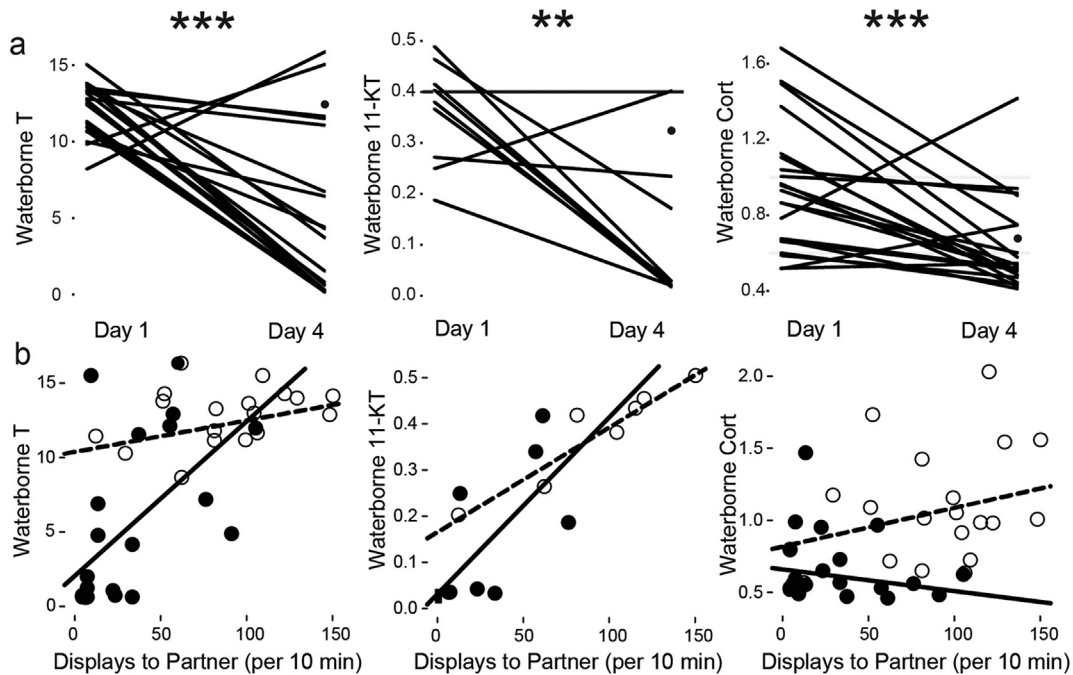


Fig. 3. Effects of social habituation on steroid hormone levels. a, Levels of T, 11-KT, and Cort decreased from Day 1 to Day 4. b, T was more strongly correlated with aggressive displays to the partner on Day 4 than Day 1. 11-KT correlated with aggressive displays to the partner on both Day 1 and Day 4. Cort was not related to aggressive displays to the partner on Day 1 or Day 4. Open circles, Day 1; closed circles, Day 4.

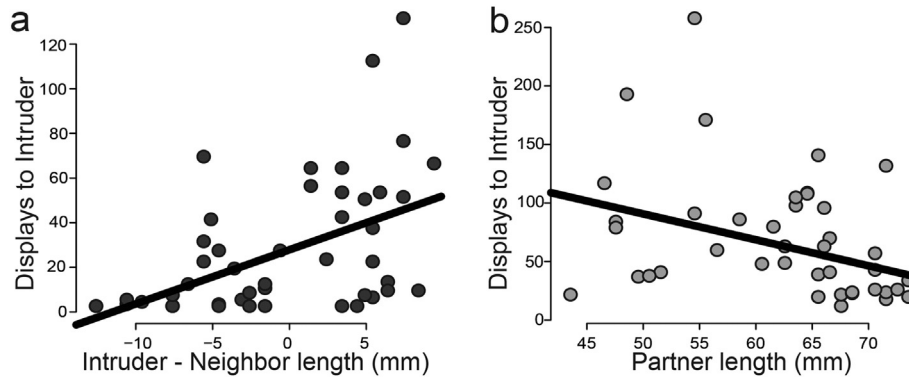


Fig. 4. a, Neighbors display more to relatively larger intruders. b, residents display more when their partner, the neighbor, is small.

received aggressive displays did not significantly differ between treatments ($t = 1.75$, $p = 0.10$, $d = 0.83$; $t = 1.46$, $p = 0.17$, $d = 0.69$).

3.4. Experiment 4: Engagement Index is associated with characteristic neural activity patterns

We investigated which brain regions may be involved in the control of cooperative territory defense behavior by performing c-Fos immunohistochemistry in seven select regions/subregions of the SDM network (14; Fig. 6a, b; Table S1). To examine how neural activity in central SDM network nodes relates to cooperative behavior per se, we calculated an Engagement Index (EI) for the neighbor. This index measures how likely an individual is to engage in cooperative defense independent of its own size or the size of the intruder (see [Materials and Methods](#)). Importantly, EI was weakly correlated with the neighbor's total male-directed aggression ($R^2 = 0.124$, $p = 0.015$) but highly correlated with intruder-directed aggression ($R^2 = 0.49$, $p < 0.001$). As EI increased, c-Fos induction in neighbors decreased in Dm-1 ($R^2 = 0.53$, $p = 0.003$; Fig. 7a) and increased in the mPOA ($R^2 = 0.29$, $p = 0.031$; Fig. 7b).

To gain insight into the role of the dopaminergic system in this context, we next co-localized c-Fos with tyrosine hydroxylase (a marker of dopaminergic cells (O'Connell et al., 2011 (Fig. 6c)) to examine the activation of dopaminergic neurons in the subregions of the POA and in area Vc. We found that in neighbors EI was positively correlated with c-Fos

induction in dopaminergic neurons of both pPOA ($R^2 = 0.53$, $p < 0.001$; Fig. 7c) and mPOA ($R^2 = 0.42$, $p = 0.005$; Fig. 7d). There was no correlation between EI and dopaminergic activity in area Vc ($R^2 = 0.001$, $p = 0.81$).

Similarly, we calculated an EI for the resident to examine how variation in resident behavior correlates with neural activity (Table S1). Interestingly, EI of residents did not correlate with activity in any of the brain regions we examined, even though total intruder directed aggression ($R^2 = 0.74$, $p < 0.001$) and total male directed aggression were highly correlated with resident EI ($R^2 = 0.616$, $p < 0.001$).

Given our findings in Experiment 3, which demonstrated that the resident modulates his aggression to the intruder based on the behavior of the neighbor, we also investigated the relationship between neural activity in the resident and his neighbor's displays to the intruder (Table S2). In the resident brain, we find a strong negative association between c-Fos induction in Dlv and the neighbor's intruder-directed aggression ($R^2 = 0.516$, $p = 0.0056$; Fig. 8), a relationship that is absent in the Dlv of neighbors ($R^2 = 0.01$, $p = 0.72$).

3.5. Co-variance in neural activity patterns in residents and neighbors

Finally, we hypothesized that the role an individual plays in a cooperative defense context would be associated with distinct neural activity patterns across key nodes of the social decision-making network. To

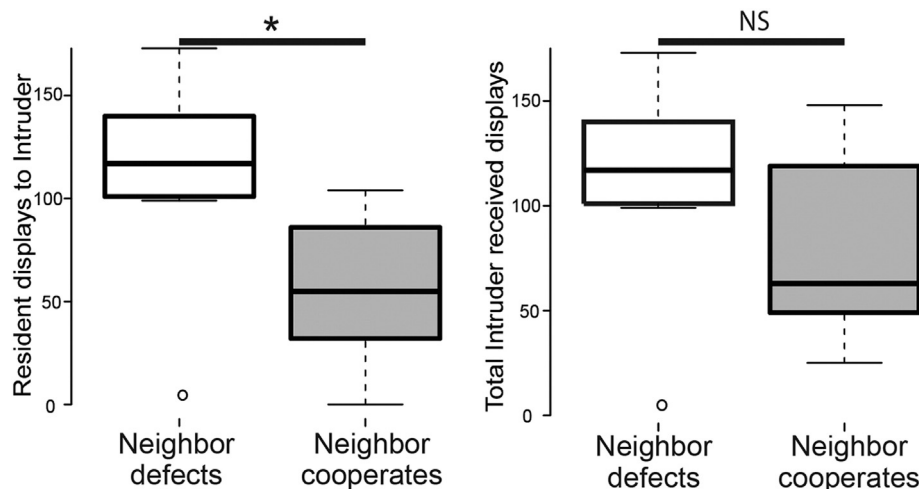


Fig. 5. When the neighbor was prevented from participating in joint territory defense, the resident displayed more frequently to the intruder. Total intruder received aggressive displays did not differ. ($n = 9$ per group).

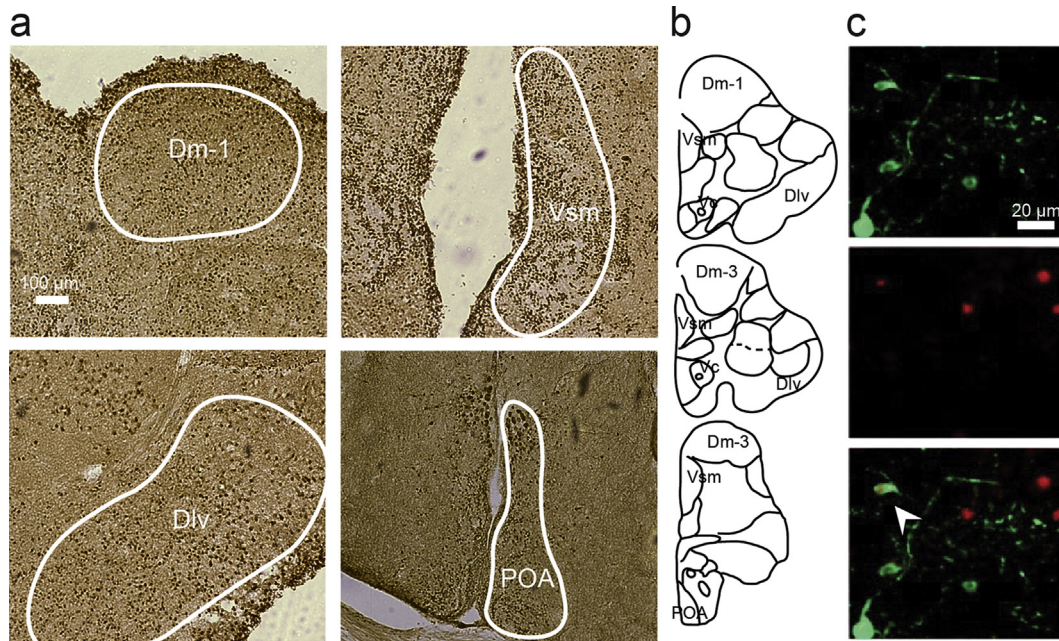


Fig. 6. Representative micrographs of brain regions of interest (a) and their spatial location to each other (b). c, The top panel shows TH+ cells labeled with green cytoplasm, the middle panel shows c-Fos+ cells labeled with red nuclei, and the bottom panel shows the merged image. Arrow indicates co-labeled neuron.

examine relationships between the behavioral and physiological levels we measured, we used hierarchical clustering on the correlation matrices of neural and behavioral measures for residents and neighbors (Fig. 9), with several clusters strongly supported by bootstrap resampling.

In residents (Fig. 9a), neural activity in subdivisions of area Dm is strongly associated with circulating levels of T and both EI and

aggressive displays to the intruder. Interestingly, while activity measures of area Div and both pPOA and mPOA are also strongly correlated, this relationship does not seem to be due to the activity of preoptic DA neurons. Note that Cort levels are not strongly correlated with any other measures. In strong contrast, in neighbors (Fig. 9b) dopaminergic activity in both pPOA and mPOA as well as area Vc is strongly associated with EI and aggressive displays to the intruder, along with circulating T

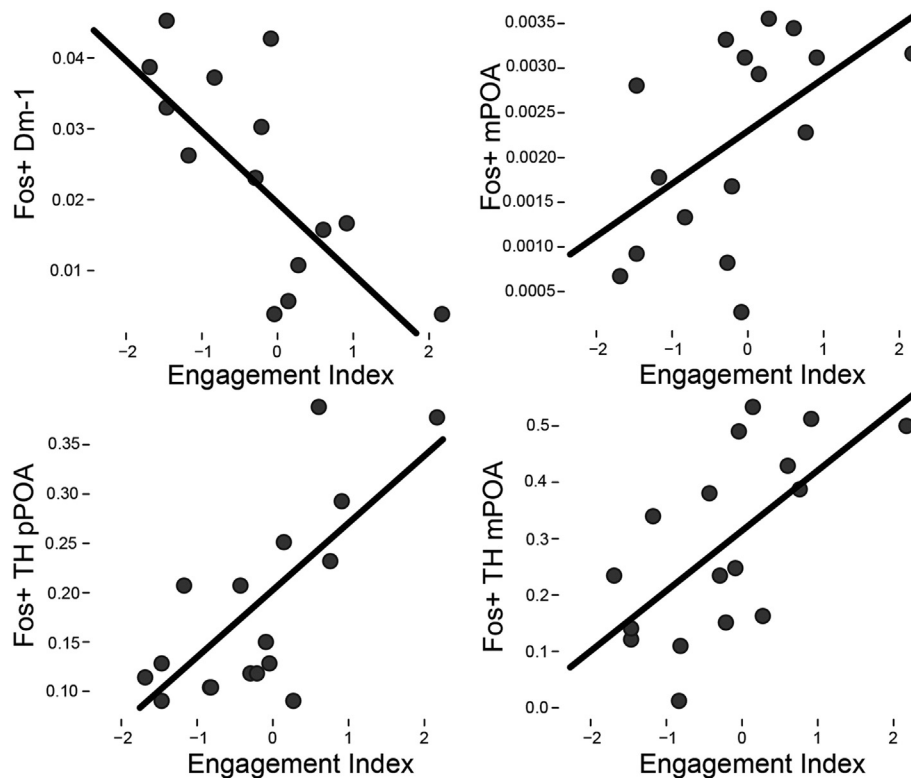


Fig. 7. Cooperation Index predicts neural activity measures in the Neighbor. a, Cooperative behavior in the Neighbor negatively correlated with neural activity in Dm-1 and positively correlated with activity in mPOA. b, Cooperative behavior in the Neighbor positively correlated with activity in dopaminergic cells in pPOA and mPOA.

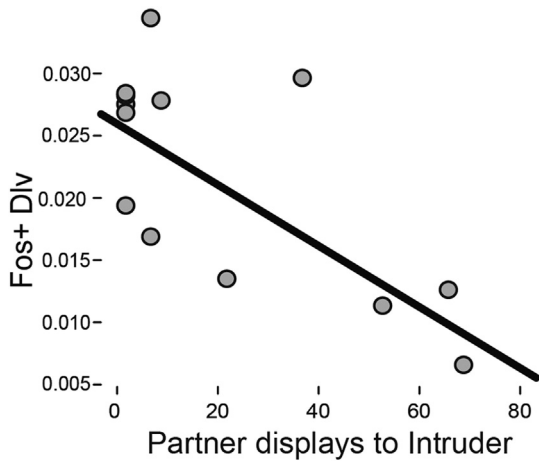


Fig. 8. Neural activity in the Div of the resident is negatively correlated with the neighbor's aggressive displays to the intruder.

and GSI. Overall activity in POA subregions as well as subdivisions of Dm and Div are more loosely associated with each other and with behavioral and Cort measures. Overall, the distance matrices for residents and neighbors were not significantly correlated using the Mantel test ($r = 0.130$, $p = 0.09$).

4. Discussion

We identified neural correlates of cooperation and conflict in the context of territory defense coalitions. A neighbor's decision to engage in territory defense is based on the perceived threat of the intruder. Interestingly, as the beneficiary of these actions, the resident modulates his defensive aggression toward an intruder based on the (perceived) level of cooperation by the neighbor. We find that the Engagement Index is associated with activity in the homologs of the mammalian basolateral amygdala and preoptic area, including dopamine neurons.

Importantly, there is no relationship between neural activity and the Engagement Index in residents, yet a strong association exists between activation in the hippocampus homolog and the partner's behavior. On a systems level, our results suggest a critical role of the putative hippocampus homolog Div in cooperative defense, along with preoptic and striatal dopamine neurons, possibly as mediators of the salience of the social context. In sum, our results reveal for the first time how the distinct behavioral roles of two interacting individuals, responding to the same stimulus, are reflected in disparate activity patterns in central nodes of the social decision-making network.

4.1. Steroid hormones vary with social habituation

For territorial males to engage in cooperative behavior, they first need to establish privileged relationships. As such, we examined whether *A. burtoni* males show social habituation by reducing aggression to familiar neighbors and how their hormone profiles change in response. This phenomenon, which has often been referred to as the 'Dear Enemy' effect in the behavioral literature, is common across taxa (Temeles, 1994), yet little is known of the physiological basis of this process. We find strong evidence of social habituation to territorial male neighbors in both behavior and hormone levels, suggesting a 'Dear Enemy' effect in *A. burtoni*. This phenomenon has previously been examined in several teleosts, including three cichlid species (Leiser and Itzkowitz, 1999; Frostman and Sherman, 2004; Aires et al., 2015). In concordance with our results, in resident Mozambique tilapia, *Oreochromis mossambicus*, novel males elicited more aggression and a higher 11-KT response compared to familiar neighbors (Aires et al., 2015). However, in *O. mossambicus*, there was no evidence of habituation in aggression to repeated intrusions (Aires et al., 2015), likely because in that study both familiar and novel males intruded into the territory of the focal male, whereas in our design males maintained their separate territories. If 'Dear Enemy' relationships are maintained by a Tit for Tat strategy, then we in fact expect that territory intrusion by a neighbor will disrupt the 'Dear Enemy' relationship between the familiar males and lead to retaliation against the defecting male (Akçay et al., 2009).

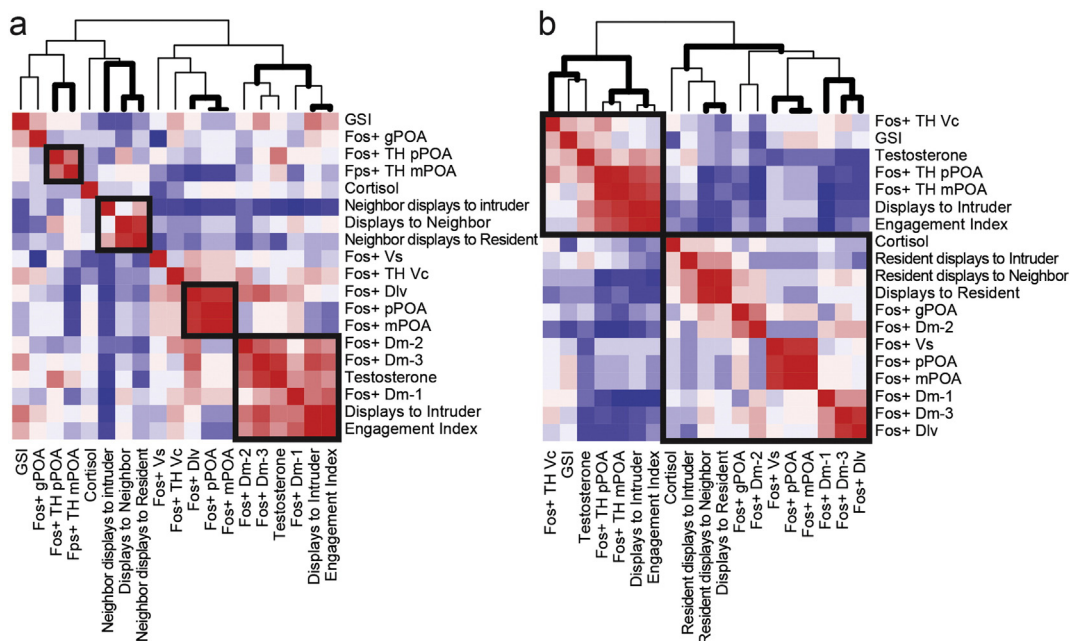


Fig. 9. Hierarchically clustered correlation matrices for resident (a) and neighbor (b). Bold lines indicate significant clusters as determined by bootstrap resampling. Red indicates positive correlations, blue indicates negative correlations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Transient increases in T following aggressive encounters between males have been widely documented (Wingfield, 2005), and our results add to this body of work. Interestingly, we find strong correlations between aggression and 11-KT levels on both Day 1 and 4, the two time points we tested, and also with T levels on Day 4. On Day 1, however, aggression and T levels are only weakly correlated, likely because during that first, highly aggressive, encounter T levels reach a physiological ceiling, as discussed in (Gleason et al., 2009). Importantly, T and DA may act together to induce a conditioned place preference, likely a fundamental aspect of territoriality (Packard et al., 1998). Furthermore, a surge in T may strengthen territoriality by increasing territorial behaviors without modulating aggression per se (Wingfield, 1994).

4.2. Males engage in territory defense in a context-dependent manner

For the first time for any vertebrate, our results provide experimental support that neighboring *A. burtoni* males engage in cooperative defense, and that they do so dependent on the absolute size difference between the neighbor and intruder, such that males are more aggressive to relatively larger intruders. As body length is a critical factor in determining fight outcomes in this species (Alcazar et al., 2014), a larger intruder is likely perceived as a greater threat to the neighbor. Negotiating territorial boundaries with a relatively larger male may increase the length and intensity of the fight, increase risk of injury, and it may also result in loss of all or part of a territory. There may be an additional cost imposed by sexual selection, as reproductive female *A. burtoni* prefer larger males when choosing a mate (Kidd et al., 2013).

Evidence for territorial defense coalitions in other species has thus far been limited to two bird species (*Anthus petrosus*: (Elfstrom, 1997); *Spizella passerine* (13, but see 66)), and two species of fiddler crabs (*Uca mjoebergi*: (Backwell and Jennions, 2004); *Uca annulipes* (Detto et al., 2010)). In *S. passerine*, the chipping sparrow, coalitions formed in response to trill rate, whereby coalition formation was most likely when the trill rate of the neighbor was greater than that of the resident, and the trill rate of the intruder exceeded both neighbor and resident (i.e., $I > N > R$; (Goodwin and Podos, 2014)). In *Uca annulipes*, the African fiddler crab, coalition formation was related to major claw length and was most likely to occur when the intruder was larger than the resident and the neighbor was larger than the intruder (i.e., $N > I > R$; (Detto et al., 2010)). We show that in *A. burtoni*, coalition formation was related to body size, and was most pronounced when the intruder was larger than the neighbor (i.e., $I > N$). The species differences in the conditions under which coalitions form is likely due to the differential costs of fighting behavior. For example, in fiddler crabs, where coalition formation is more likely when the neighbor is larger than the intruder, there are very high costs of fighting behavior, including claw loss and death (Milner et al., 2009). In contrast, in *A. burtoni* males, losing a fight may result in the loss of dominance, but severe injury or death are unlikely and dominance can be regained (Fox et al., 1997). Thus, the temporary costs of participating in defense may be less than the longer term costs of negotiating territorial boundaries with a new, larger neighbor. Furthermore, given the size conditions under which cooperative defense occurs in both fiddler crabs and chipping sparrows, reciprocity is unlikely to be involved. However, the size conditions under which *A. burtoni* engage in cooperative defense allow for the unexplored possibility of reciprocity to also contribute to cooperation in this species (Taborsky, 2013).

Can we rule out the possibility that the neighbor is simply defending his own territory? If this were the case, one would expect the conditions under which the neighbor and resident engage in aggressive defense to be similar. Several lines of evidence, however, suggest otherwise. First, the neighbor is significantly less aggressive to the intruder than is the resident, suggesting that the threat is not perceived as strongly. Second, the resident displays consistently high aggression to the intruder, while the aggressive displays of the neighbor are highly condition-dependent. Third, the conditions under which neighbor and resident modulate their

aggressive displays to the intruder differ. The resident adjusts his behavior depending on the size of the neighbor, while the neighbor responds to the size of the intruder. In addition, and maybe most importantly, we identify neural correlates of behavior in the neighbor which are not represented in the resident (see below). Despite these lines of evidence suggesting that the observed neighbor behavior is indeed akin to cooperation, future research can strengthen our interpretation. For example, the behavior of the neighbor toward the intruder should be examined when the resident is absent. Furthermore, an additional approach would be to vary the familiarity of the resident. One would predict that a neighbor should engage less in cooperative defense when the resident is less familiar. Lastly, cooperation often achieves an end situation that could not be brought about individually. As our experimental design did not allow fights to be resolved, we were unable to address how neighbor behavior affects the likelihood that the resident retains his territory. We did, however, find that the resident is less aggressive to the intruder when the neighbor engages, suggesting that the neighbor's behavior would indeed affect the outcome of the fight. Even if the neighbor attacks the intruder with the aim of evicting him from his own territory, the resident benefits, as more total aggression likely equals increased likelihood of eviction. Detailed field studies, which are beyond the scope of our study, would be needed to demonstrate to what extent neighbor engagement increases the likelihood that the resident retains his territory.

4.3. Residents exploit neighbor behavior

We found that residents are more aggressive to the intruder than are neighbor males. This is not surprising given that an intruder poses a direct threat to the resident—loss of territory—whereas the threat to the neighbor is not as immediate. The costs of losing one's own territory very likely exceed the costs of a new, unfamiliar neighbor. Interestingly, there appears to be behavioral modulation during territory defense as we find that resident males pay close attention to both the size and behavior of the neighboring male during a simulated territorial intrusion. Most strikingly, resident males exhibit fewer aggressive displays to the intruder when the neighbor is large. The resident male does not appear to be suppressed by the larger neighbor, but rather may exploit the behavior of his partner, as we find that forcing neighbor defection (when the neighbor is larger than the resident) increases the frequency at which the resident exhibits aggressive displays to the intruder. Importantly, total male directed aggressive displays and total intruder received aggressive displays did not differ with forced neighbor defection. This observed coordination with neighbor behavior and size may serve as a mechanism to reduce the costs of aggression, as less aggressive defense may be needed to evict an intruder when help is received from the neighboring male.

4.4. Neural correlates of cooperative defense

Cooperative behavior is common across animals, with many of the most intriguing examples within teleost species. Despite the many instances of cooperation, the neural mechanisms have not yet been examined in any detail in any system (Soares et al., 2010; Weitekamp and Hofmann, 2014). Here, we calculate an “Engagement Index” as a measure to dissociate cooperative behavior from aggressive behavior. For the neighbor, this measure consists of the residuals from the regression between aggressive displays to the intruder and Intruder–Neighbor body length, the strongest predictor of aggressive displays to the intruder. In this way, positive residuals, for example, suggest the neighbor is being more cooperative than predicted based on size. We find this index to be remarkably informative. Specifically, we find that the Engagement Index in neighbors is associated with reduced neural activation within subregion Dm-1 of the putative basolateral amygdala homolog and increased neural activation within the magnocellular

neurons of the POA, including increased activation of the parvocellular and magnocellular dopaminergic cell populations of the POA.

The amygdala is important to processing the salience and current value of social stimuli (Adolphs, 2010; Amaral, 2006). In monkeys the amygdala complex plays a context-dependent role in evaluating environmental stimuli for potential threats and in detecting relevant dangers (Amaral, 2003). It has been proposed to modulate social behavior by inhibiting interaction with threatening novel conspecifics (Amaral, 2003). Here, reduced neural activation in part of the amygdala homolog is associated with an increase in the Engagement Index. This region may be evaluating both the degree of threat posed by the intruder, as well as the relevance of the threat in a neighboring territory. The strong association we measured between reduced neural activity and increased cooperative behavior is consistent with this region having an inhibitory effect on behavior.

We also find that increased activity in the magnocellular subdivision of the POA (the putative homolog of the supraoptic nucleus in mammals (O'Connell and Hofmann, 2011; Moore and Lowry, 1998)) is associated with increased cooperative behavior according to the Engagement Index, which measures how likely an individual is to engage in cooperative defense independent of body size. Across vertebrates, the POA serves a fundamental role in regulating a wide variety of social behaviors, including inter-male aggression, male sexual behavior, and maternal care (O'Connell and Hofmann, 2011). In our experimental paradigm, cooperation is expressed as an increase in aggressive displays, but importantly, total aggression explains only a small part of the variation in the Engagement Index ($R^2 = 0.124$). Thus, while the role of the POA in mediating aggression is well known, it also appears to mediate a more complex type of social cognition in the form of cooperative behavior. Interestingly, in mammals the supraoptic nucleus is a production site for oxytocin (OT), a neuropeptide involved in affiliative behavior and social cognition (Ross and Young, 2009), from which it is released into the circulation. In *A. burtoni*, the mPOA contains a high number of isotocin (OT homolog) neurons (Huffman et al., 2012), thus the increased activity we measured in this region may be reflective of isotocin neuron activity mediating cooperative behavior via increased circulating isotocin levels.

We find that increased dopamine neuron activity in the POA is associated with the Engagement Index in neighbors. Dopamine in the POA mediates male sexual motivation (Dominguez and Hull, 2005) as well as maternal behavior (Numan, 2007). Interestingly, our Engagement Index may be measuring differences in motivation between individuals. Future experiments will dissociate these processes in detail. Furthermore, it is possible that dopamine is interacting with other neurochemicals in this region, such as oxytocin, contributing to the observed behavioral modulation. OT is known to interact with dopamine to influence various reward-related behaviors, including pair bonding (Young and Wang, 2004) and maternal care (Shahrokh et al., 2010).

In human subjects, some insights into the neural basis of cooperation have been obtained through functional imaging studies of reciprocity (Rilling et al., 2002, 2012; Watanabe et al., 2014). For example, individuals who cooperated in an iterated Prisoner's Dilemma (direct reciprocity) exhibited neural activity in brain regions involved in reward processing, including the nucleus accumbens, the caudate nucleus, and cortical regions, which the authors posit function to inhibit the self-ish choice to not reciprocate (Rilling et al., 2002). Furthermore, a separate study found that intranasal OT increases the response of the caudate nucleus to reciprocal cooperation (Rilling et al., 2012). A study of indirect reciprocity found differences between reputation-based reciprocity and pay-it-forward reciprocity, with only the latter being associated with neural activation in the anterior insula, a region that processes empathy and emotional reward (Watanabe et al., 2014). While reciprocity is common in human societies, the existence of reciprocity in non-human animals is more contentious, often because it is difficult to demonstrate experimentally (Taborsky, 2013). However, studies such as ours offer novel insights into the decision-making

process during cooperation and may be able to shed new light on the debate. In our system, reciprocity between neighboring males has not yet been examined but the involvement of the reward system we have demonstrated here makes this an intriguing possibility for future study.

4.5. Neighbor behavior encoded in hippocampus homolog in resident

The hippocampus is known for its role in declarative memory, spatial processing, and social recognition. However, it is also proposed to have an important role in social cognition and context-dependent decision-making more generally (Rubin et al., 2014). We find that neural activity in area DI of the resident, assessed by detection of protein of the immediate early gene *c-Fos*, is negatively correlated with intruder-directed aggression from the neighbor, suggesting that the teleost homolog of the hippocampus might, in part, serve a similar function. Importantly, this relationship appears robust, as a related study showed a positive relationship between intruder-directed aggression in the neighbor and mRNA expression of the immediate early gene *egr-1* in the resident (Weitekamp et al., unpublished data). This is interesting given that we show that residents modulate their aggressive behavior based on whether the neighbor is engaging in defense behavior. The hippocampus-like structure appears to play a critical role in this behavioral modulation and contributes to integrating relevant social information to generate an appropriate behavioral response, which in this case relates to exploiting the behavior of the social partner.

4.6. Patterns of neural processing differ between neighbor and resident

We performed hierarchical clustering on correlation matrices containing behavior and physiology data. Bootstrap resampling identified several significant clusters. Notably, in residents, aggressive displays to the intruder and the Engagement Index formed a cluster with T and with subdivisions of the basolateral amygdala homolog. In neighbors, these same behavior measures, along with T, formed a cluster with activity in dopaminergic cell groups in the pPOA, mPOA, and striatum homolog. Thus, it appears that the reward system may be not critical to aggressive defense behavior per se, but rather mediates cooperation in this context. This result is consistent with the functional imaging studies of cooperation in humans described above, which demonstrated a critical role for reward processing, though interestingly in a very different behavioral context. It would be interesting to examine whether pharmacological perturbation of the dopamine pathway affects aggressive defense differently between residents and neighbors. Lastly, in residents we find that the hippocampus homolog is closely associated with pPOA and mPOA, while in neighbors this region is most closely associated with subregions of the amygdala homolog. It is possible that the hippocampus homolog is serving as a sort of cognitive mediator, encoding the perceived relative threat of the intruder based on spatial location. In sum, our results reveal for the first time how the distinct behavioral roles of two individuals responding to the same stimulus are reflected in disparate activity patterns in central nodes of the social decision-making network.

4.7. Conclusion

Cooperation is ubiquitous in nature, yet the neural mechanisms have not been well studied (de Waal and Ferrari, 2010; Brosnan et al., 2010). This is likely due, in part, to the difficulty of conclusively demonstrating that cooperative behavior is indeed taking place in a given social context. In the present study, we have encountered similar challenges and some important behavioral experiments remain to be performed. Crucially, however, our neural activity data, alongside the behavior data, strongly point to the intriguing conclusion that residents and neighbors perceive and process the presence of an intruder in very different ways. In addition, we find several strong correlations between

the Engagement Index in the neighbor and neural activity in key nodes of the social decision-making network, relationships that are absent in the resident. Interestingly, the importance of an individual's role in territory defense could be missed with behavioral observations alone. We believe that more novel insights likely await to be revealed by future studies that attempt to examine the neural and molecular mechanisms underlying cooperative behavior.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.yhbeh.2017.01.001>.

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References

- Adolphs, R., 2010. What does the amygdala contribute to social cognition? *Ann. N. Y. Acad. Sci.* 1191, 42–61 (Mar).
- Aires, R.F., Oliveira, G.A., Oliveira, T.F., Ros, A.F.H., Oliveira, R.F., 2015. Dear enemies elicit lower androgen responses to territorial challenges than unfamiliar intruders in a cichlid fish. *PLoS One* 10, e0137705 (Jan 17).
- Akçay, Ç., Beecher, M.D., 2015. Team of rivals in chipping sparrows? A comment on Goodwin & Podos. *Biol. Lett.* 11 (Jul 1; 20141043).
- Akçay, Ç., Wood, W.E., Searcy, W.A., Templeton, C.N., Campbell, S.E., Beecher, M.D., 2009. Good neighbour, bad neighbour: song sparrows retaliate against aggressive rivals. *Anim. Behav.* 78, 97–102 (Jul).
- Alcazar, R.M., Hilliard, A.T., Becker, L., Bernaba, M., Fernald, R.D., 2014. Brains over brawn: experience overcomes a size disadvantage in fish social hierarchies. *J. Exp. Biol.* (Feb 5; jeb.097527-).
- Amaral, D., 2003. The amygdala: is it an essential component of the neural network for social cognition? *Neuropsychologia* 41, 517–522.
- Amaral, D.G., 2006. The amygdala, social behavior, and danger detection. *Ann. N. Y. Acad. Sci.* 1000, 337–347 (Jan 24).
- Arnott, G., Elwood, R.W., 2009. Assessment of fighting ability in animal contests. *Anim. Behav.* 77, 991–1004 (May).
- Backwell, P., Jennions, M., 2004. Coalition among male fiddler crabs. *Nature* 430, 2822 (Jul 22).
- Báez-Mendoza, R., Harris, C.J., Schultz, W., 2013. Activity of striatal neurons reflects social action and own reward. *Proc. Natl. Acad. Sci. U. S. A.* 110, 16634–16639 (Oct 8).
- Balleine, B.W., Delgado, M.R., Hikosaka, O., 2007. The role of the dorsal striatum in reward and decision-making. *J. Neurosci.* 27, 8161–8165 (Aug 1).
- Blumstein, D.T., Daniel, J.C., 2007. Quantifying Behavior the JWatcher Way. Sinauer Associates, Sunderland, MA, USA.
- Brosnan, S.F., Bshary, R., 2010. Cooperation and deception: from evolution to mechanisms. *Philos. Trans. R. Soc. B Biol. Sci.* 365, 2593–2598 (Aug 2).
- Brosnan, S.F., de Waal, F.B.M., 2002. A proximate perspective on reciprocal altruism. *Hum. Nat.* 13, 129–152 (Mar).
- Brosnan, S.F., Salwiczek, L., Bshary, R., 2010. The interplay of cognition and cooperation. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 365, 2699–2710 (Sep 12).
- Chen, C.-C., Fernald, R.D., 2011. Visual information alone changes behavior and physiology during social interactions in a cichlid fish (*Astatotilapia burtoni*). *PLoS One* 6, e20313 (Jan).
- de Waal, F.B.M., Ferrari, P.F., 2010. Towards a bottom-up perspective on animal and human cognition. *Trends Cogn. Sci.* 14, 201–207 (May).
- Desjardins, J.K., Hofmann, H.A., Fernald, R.D., 2012. Social context influences aggressive and courtship behavior in a cichlid fish. *PLoS One* 7, e32781 (Jan).
- Detto, T., Jennions, M.D., Backwell, P.R.Y., 2010. When and why do territorial coalitions occur? Experimental evidence from a fiddler crab. *Am. Nat.* 175, E119–E125 (May).
- Díaz-Muñoz, S.L., DuVal, E.H., Krakauer, A.H., Lacey, E.A., 2014. Cooperating to compete: altruism, sexual selection and causes of male reproductive cooperation [Internet]. *Anim. Behav.* 88, 67–78 (cited 2014 Jan 22).
- Dominguez, J.M., Hull, E.M., 2005. Dopamine, the medial preoptic area, and male sexual behavior. *Physiol. Behav.* 86, 356–368 (Oct 15).
- Dugatkin, L.A., 1997. Cooperation Among Animals [Internet]. ([cited 2015 Jan 15]; Available from: <http://agris.fao.org/agris-search/search.do?recordID=US201300309410>).
- Eichenbaum, H., 2004. Hippocampus: cognitive processes and neural representations that underlie declarative memory. *Neuron* 44, 109–120 (Sep 30).
- Elfstrom, S., 1997. Fighting behaviour and strategy of rock pipit, *Anthus petrosus*, neighbours: cooperative defence [Internet]. *Anim. Behav.* 54, 535–542 (Sep).
- Elwood, R.W., Arnott, G., 2012. Understanding how animals fight with Lloyd Morgan's canon. *Anim. Behav.* 84, 1095–1102 (Nov).
- Fawcett, T.W., Mowles, S.L., 2013. Assessments of fighting ability need not be cognitively complex [Internet]. *Anim. Behav.* 86, e1–e7 (cited 2013 Nov 22).
- Felix-Ortiz, A.C., Tye, K.M., 2014. Amygdala inputs to the ventral hippocampus bidirectionally modulate social behavior. *J. Neurosci.* 34, 586–595 (Jan 8).
- Fernald, R.D., Hirata, N.R., 1977. Field study of *Haplochromis burtoni*: quantitative behavioural observations. *Anim. Behav.* 25, 964–975 (Nov).
- Fischer, J.B., 1954. Evolution and bird sociality [Internet]. In: Huxley, J., Hardy, A.C., Ford, E.B. (Eds.), *Evolution as a Process*. Allen and Unwin, London, pp. 71–83 (cited 2014 Apr 13).
- Fox, H.E., White, S.A., Kao, M.H.F., Fernald, R.D., 1997. Stress and dominance in a social fish [Internet]. *J. Neurosci.* 17, 6463–6469 (Aug 15 [cited 2014 Apr 15]).
- Frostman, P., Sherman, P.T., 2004. Behavioral response to familiar and unfamiliar neighbors in a territorial cichlid, *Neolamprologus pulcher*. *Ichthyol. Res.* 51, 8–10 (Aug).
- Getty, T., 1987. Dear enemies and the Prisoner's dilemma: why should territorial neighbors form defensive coalitions? *Integr. Comp. Biol.* 27, 327–336 (Jan 1).
- Gleason, E.D., Fuxjager, M.J., Oyegbile TO, Marler, C.A., 2009. Testosterone release and social context: when it occurs and why. *Front. Neuroendocrinol.* 30, 460–469 (Oct).
- Goodwin, S.E., Podos, J., 2014. Team of rivals: alliance formation in territorial songbirds is predicted by vocal signal structure. *Biol. Lett.* 10, 20131083 (Jan 26).
- Grosenick, L., Clement, T.S., Fernald, R.D., 2007. Fish can infer social rank by observation alone. *Nature* 445, 429–432 (Jan 25).
- Harrell, F.E., 2008. Hmisc: Harrell Miscellaneous [Internet]. (Available from: <http://cran.r-project.org/web/packages/Hmisc/index.html>).
- Hofmann, H.A., 2003. Functional genomics of neural and behavioral plasticity. *J. Neurobiol.* 54, 272–282 (Jan).
- Huffman, L.S., O'Connell, L.A., Kenkel, C.D., Kline, R.J., Khan, I.A., Hofmann, H.A., 2012. Distribution of nonapeptide systems in the forebrain of an African cichlid fish, *Astatotilapia burtoni*. *J. Chem. Neuroanat.* 44, 86–97 (Jul).
- Hull, E.M., Dominguez, J.M., 2007. Sexual behavior in male rodents. *Horm. Behav.* 52, 45–55 (Jun).
- Kidd, C.E., Kidd, M.R., Hofmann, H.A., 2010. Measuring multiple hormones from a single water sample using enzyme immunoassays. *Gen. Comp. Endocrinol.* 165, 277–285 (Jan 15).
- Kidd, M.R., Dijkstra, P.D., Alcott, C., Lavee, D., Ma, J., O'Connell, L.A., et al., 2013. Prostaglandin F2 α facilitates female mating behavior based on male performance. *Behav. Ecol. Sociobiol.* 67, 1307–1315 (May 23).
- Kogan, J.H., Frankland, P.W., Silva, A.J., 2000. Long-term memory underlying hippocampus-dependent social recognition in mice. *Hippocampus* 10, 47–56 (Jan).
- Leiser, J.K., Itzkowitz, M., 1999. The benefits of dear enemy recognition in three-contender convict cichlid (*Cichlasoma nigrofasciatum*) contests. *Behaviour* 136, 983–1003.
- McGaugh, J.L., 2004. The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annu. Rev. Neurosci.* 27, 1–28 (Jan 24).
- Messias, J.P.M., Paula, J.R., Grutter, A.S., Bshary, R., Soares, M.C., 2016. Dopamine disruption increases negotiation for cooperative interactions in a fish. *Sci. Rep.* 6, 20817 (Feb 8).
- Milner, R.N.C., Detto, T., Jennions, M.D., Backwell, P.R.Y., 2009. Hunting and predation in a fiddler crab. *J. Ethol.* 28, 171–173 (Apr 11).
- Moore, F.L., Lowry, C.A., 1998. Comparative neuroanatomy of vasotocin and vasopressin in amphibians and other vertebrates. *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.* 119, 251–260 (Jun).
- Morgan, J.L., Curran, T., 1991. Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. *Annu. Rev. Neurosci.* 14, 421–451 (Jan 28).
- Munchrath, L.A., Hofmann, H.A., 2010. Distribution of sex steroid hormone receptors in the brain of an African cichlid fish, *Astatotilapia burtoni*. *J. Comp. Neurol.* 518, 3302–3326 (Aug 15).
- Nowak, M.A., 2006. Five rules for the evolution of cooperation. *Science* 314, 1560–1563 (Dec 8).
- Numan, M., 2007. Motivational systems and the neural circuitry of maternal behavior in the rat. *Dev. Psychobiol.* 49, 12–21 (Jan).
- O'Connell, L.A., Hofmann, H.A., 2011. The vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. *J. Comp. Neurol.* 519, 3599–3639 (Dec 15).
- O'Connell, L.A., Hofmann, H.A., 2012. Evolution of a vertebrate social decision-making network. *Science* 336, 1154–1157 (Jun 1).
- O'Connell, L.A., Fontenot, M.R., Hofmann, H.A., 2011. Characterization of the dopaminergic system in the brain of an African cichlid fish, *Astatotilapia burtoni*. *J. Comp. Neurol.* 519, 75–92 (Jan 1).
- O'Connell, L.A., Fontenot, M.R., Hofmann, H.A., 2013a. Neurochemical profiling of dopaminergic neurons in the forebrain of a cichlid fish, *Astatotilapia burtoni*. *J. Chem. Neuroanat.* 47, 106–115 (Jan).
- O'Connell, L.A., Rigney, M.M., Dykstra, D.W., Hofmann, H.A., 2013b. Neuroendocrine mechanisms underlying sensory integration of social signals. *J. Neuroendocrinol.* 25, 644–654 (Jul).
- Packard, M.G., Schroeder, J.P., Alexander, G.M., 1998. Expression of testosterone conditioned place preference is blocked by peripheral or intra-accumbens injection of alpha-flupenthixol. *Horm. Behav.* 34, 39–47 (Aug).
- Parikh, V.N., Clement, T.S., Fernald, R.D., 2006. Androgen level and male social status in the African cichlid, *Astatotilapia burtoni*. *Behav. Brain Res.* 166, 291–295 (Jan 30).
- R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rilling, J.K., Gutman, D.A., Zeh, T.R., Pagnoni, G., Berns, G.S., Kilts, C.D., 2002. A neural basis for social cooperation. *Neuron* 35, 395–405 (Jul).
- Rilling, J.K., DeMarco, A.C., Hackett, P.D., Thompson, R., Ditzen, B., Patel, R., et al., 2012. Effects of intranasal oxytocin and vasopressin on cooperative behavior and associated brain activity in men. *Psychoneuroendocrinology* 37, 447–461 (Apr).

- Riters, L.V., 2012. The role of motivation and reward neural systems in vocal communication in songbirds. *Front. Neuroendocrinol.* 33, 194–209 (Apr).
- Ross, H.E., Young, L.J., 2009. Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. *Front. Neuroendocrinol.* 30, 534–547 (Oct).
- Rubin, R.D., Watson, P.D., Duff, M.C., Cohen, N.J., 2014. The role of the hippocampus in flexible cognition and social behavior. *Front. Hum. Neurosci.* 8, 742 (Jan 30).
- Sachs, J.L., Mueller, U.G., Wilcox, T.P., Bull, J.J., 2004. The evolution of cooperation. *Q. Rev. Biol.* 79, 135–160 (Jun 24).
- Schroeder, J.P., Packard, M.G., 2000. Role of dopamine receptor subtypes in the acquisition of a testosterone conditioned place preference in rats. *Neurosci. Lett.* 282, 17–20 (Mar).
- Scott, A.P., Ellis, T., 2007. Measurement of fish steroids in water—a review. *Gen. Comp. Endocrinol.* 153, 392–400 (Jan).
- Scott, A.P., Hirschenhauser, K., Bender, N., Oliveira, R., Earley, R.L., Sebire, M., et al., 2008. Non-invasive measurement of steroids in fish-holding water: important considerations when applying the procedure to behaviour studies [Internet]. *Behaviour* 145, 1307–1328 (Oct 1 [cited 2014 Mar 24]).
- Shahrokh, D.K., Zhang, T.-Y., Diorio, J., Gratton, A., Meaney, M.J., 2010. Oxytocin-dopamine interactions mediate variations in maternal behavior in the rat. *Endocrinology* 151, 2276–2286 (May 1).
- Soares, M.C., Bshary, R., Fusani, L., Goymann, W., Hau, M., Hirschenhauser, K., et al., 2010. Hormonal mechanisms of cooperative behaviour. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 365, 2737–2750 (Sep 12).
- Summers, C.H., Watt, M.J., Ling, T.L., Forster, G.L., Carpenter, R.E., Korzan, W.J., et al., 2005. Glucocorticoid interaction with aggression in non-mammalian vertebrates: reciprocal action. *Eur. J. Pharmacol.* 526, 21–35 (Dec 5).
- Suzuki, R., Shimodaira, H., 2006. Pvcust: an R package for assessing the uncertainty in hierarchical clustering. *Bioinformatics* 22, 1540–1542 (Jun 15).
- Taborsky, M., 2007. Cooperation built the Tower of Babel [Internet]. *Behav. Process.* 76, 95–99 (cited 2015 Jun 24).
- Taborsky, M., 2013. Social evolution: reciprocity there is. *Curr. Biol.* 23, R486–R488.
- Taborsky, M., Taborsky, B., 2015. Evolution of genetic and physiological mechanisms of cooperative behaviour. *Curr. Opin. Behav. Sci.* (Nov). 10.1016/j.cobeha.2015.11.001.
- Taylor, P., Elwood, R., 2003. The mismeasure of animal contests [Internet]. *Anim. Behav.* 65, 1195–1202 (cited 2013 Nov 20).
- Temeles, E.J., 1994. The role of neighbours in territorial systems: when are they “dear enemies”? *Anim. Behav.* 47, 339–350 (Feb).
- Trainor, B.C., 2011. Stress responses and the mesolimbic dopamine system: social contexts and sex differences. *Horm. Behav.* 60, 457–469 (Nov).
- van der Meer, M.A.A., Ito, R., Lansink, C.S., Pennartz, C.M.A., 2014. Hippocampal projections to the ventral striatum: from spatial memory to motivated behavior [Internet]. In: Derdikman, D., Knierim, J.J. (Eds.), *Space, Time and Memory in the Hippocampal Formation*. Springer Vienna, Vienna, pp. 497–516.
- Watanabe, T., Takezawa, M., Nakawake, Y., Kunimatsu, A., Yamasue, H., Nakamura, M., et al., 2014. Two distinct neural mechanisms underlying indirect reciprocity. *Proc. Natl. Acad. Sci. U. S. A.* 111, 3990–3995 (Mar 18).
- Weitekamp, C.A., Hofmann, H.A., 2014. Evolutionary themes in the neurobiology of social cognition. *Curr. Opin. Neurobiol.* 28C, 22–27 (Jun 27).
- Wingfield, J.C., 1994. Regulation of territorial behavior in the sedentary song sparrow, *Melospiza melodia morphna*. *Horm. Behav.* 28, 1–15 (Mar).
- Wingfield, J.C., 2005. A continuing saga: the role of testosterone in aggression. *Horm. Behav.* 48, 253–255 (Sep; discussion 256–8).
- Wingfield, J., Hegner, R., Dufty, A., Ball, G., 1990. The “challenge hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies [Internet]. *Am. Nat.* 829–846 (cited 2014 Apr 13).
- Young, L.J., Wang, Z., 2004. The neurobiology of pair bonding. *Nat. Neurosci.* 7, 1048–1054 (Oct).