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Neuro-evolutionary patterning of sociality

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Evolutionary shifts in species-typical group size ('sociality') probably reflect natural selection on motivational processes such as social arousal, approach-avoidance, reward, stress/anxiety and dominance. Using four songbird species that differ selectively in sociality (one territorial, one modestly gregarious, and two highly gregarious species), we here examined immediate early gene (IEG) responses of relevant brain regions following exposure to a same-sex conspecific. The paradigm limited behavioural performance, thus species differences should reflect divergence in motivational and/or perceptual processes. Within the extended medial amygdala (which is involved in appetitive approach, social arousal and avoidance), we observed species differences in IEG response that are negatively graded in relation to sociality. In addition, brain areas that are involved in social stress and dominance-related behaviour (ventrolateral septum, anterior hypothalamus and lateral subdivision of the ventromedial hypothalamus) exhibited IEG responses that dichotomously distinguish the territorial species from the three gregarious species. The IEG responses of areas involved in reward (nucleus accumbens and ventral pallidum) and general stress processes (e.g. paraventricular hypothalamus, lateral bed nucleus of the stria terminalis and most areas of the lateral septum) do not correlate with sociality, indicating that social evolution has been accompanied by selection on a relatively discrete suite of motivational systems.

Keywords: sociality; coloniality; territoriality; evolution; bird; brain

1. INTRODUCTION

In mammals, a network of steroid-hormone-sensitive brain regions within the basal ('limbic') forebrain and midbrain has been implicated in a wide range of social behaviour processes (Newman 1999). As shown in figure 1*a* (Newman's model; Newman 1999), the nodes of this network are strongly interconnected. Each node is activated to some extent during multiple types of social interaction and the final behavioural output of the network probably depends upon the weighting of the various nodes, not a linear activation of one behavioural system or another. Increasing evidence suggests that this network is present in all vertebrate groups. For instance, we have recently traced the connections of a vocal–acoustic network in a teleost fish, and discovered that the circuitry for social vocalization is virtually identical to the mammalian social behaviour network shown in figure 1*a*. Vocal–acoustic sites are located in the preoptic area, anterior and ventromedial regions of the hypothalamus, and the midbrain (e.g. periqueductal grey and adjacent tegmentum), and each of these areas exhibits reciprocal connections with homologues of the septum and amygdala (Goodson & Bass 2002). Similarly, neural loci that are considered homologous to components of the mammalian network have been implicated in social behaviour processes in a variety of other vertebrates, including birds (Ball & Balthazart 2001; Sakata *et al.* 2002; Goodson & Evans 2004).

Although Newman proposed several variables that may produce species-specific responses within the network during social interactions, no comparative studies have previously been conducted that relate species differences in group size to differences in neural response patterns. Indeed, while the adaptive, ultimate significance of sociality has been extensively explored (Alexander 1974; Brown & Brown 1996), the proximate mechanisms that produce species differences in group size (e.g. behavioural, motivational and neural factors) are largely unknown. Proximate mechanisms related to stress, dominance, appetitive approach/avoidance and reward could hypothetically interact in multiple ways to yield species variation in social structure. The most relevant data on this topic come from microtine vole species that differ in mating systems. These studies demonstrate that reward circuitry components of the basal forebrain (e.g. neuropeptide systems of the nucleus accumbens and ventral pallidum) are particularly relevant for generating species differences in monogamous pair-bonding, and manipulations of these systems also influence general affiliative processes (reviews: Young *et al.* 2001; Lim *et al.* 2004). However, it remains unknown whether these circuits influence sociality differently between species that diverge in their species-typical group sizes, but not in their mating systems.

In the present investigation, we examined the patterns of immediate early gene (IEG) response within the social behaviour network and related structures during exposure to a same-sex conspecific, using males and females of four songbird species. Our goals were to determine what parameters of response reflect species differences in sociality (group size; results summarized in figure 1*b*) and what parameters may distinguish females and males. We employed antibodies against two IEG proteins, FOS (product of *c-fos*) and ZENK (product of a gene variably known as *Zenk*, *egr-1*, *Krox-24* and *zif-268*). FOS and ZENK proteins influence a variety of cellular processes, including neuroplasticity (Abraham *et al.* 1991; Bozon *et al.* 2002). These IEGs provide useful markers of neural

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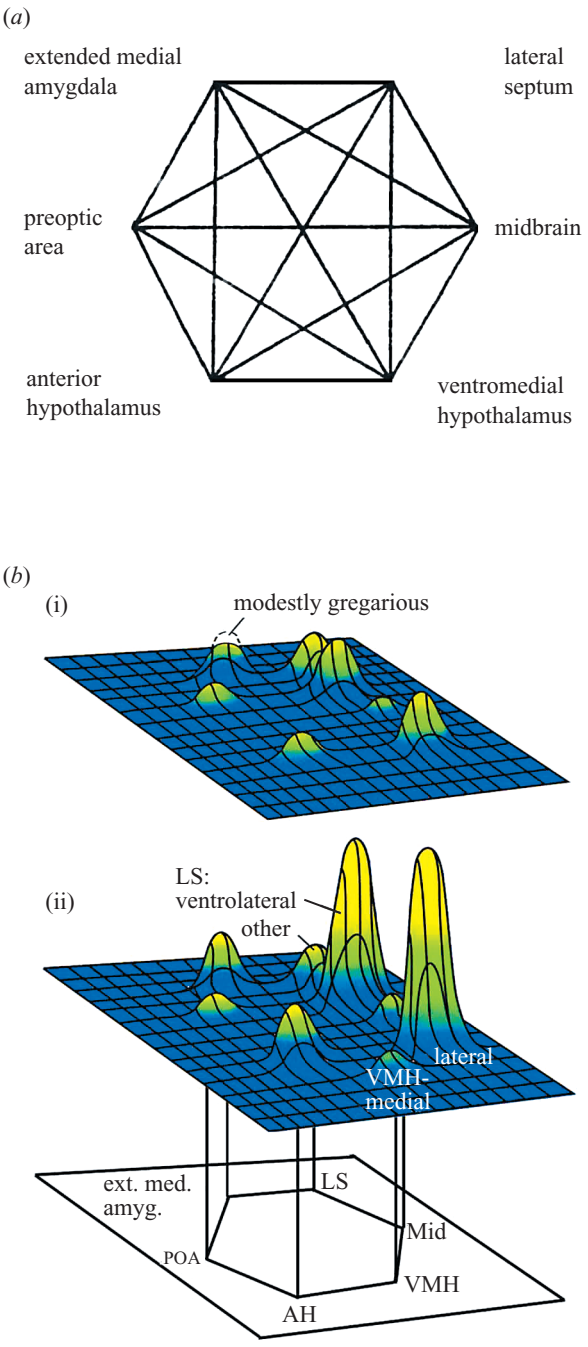

Figure 1. The social behaviour network and its responses to social stimuli in gregarious and territorial songbirds. (*a*) The network as originally described for mammals (modified from Newman 1999). As indicated, nodes of the network are extensively interconnected. All areas concentrate steroid hormones and are involved in multiple forms of mammalian social behaviour. (*b*) Schematic representation of the present data, showing responses of the network in gregarious (i) and territorial (ii) songbird species. As indicated by the dashed line, modestly gregarious species exhibit greater responses in the extended medial amygdala than do highly gregarious species, but less than the territorial species.

response that are complementary and only partly redundant (Ball & Balthazart 2001).

To isolate species-typical group size as a quasi-independent variable, we sought to control for multiple aspects of behaviour and ecology that may influence how an animal responds to social stimuli. Isolation of sociality is not possible in most vertebrate groups, as species differences in grouping behaviour are typically confounded with other relevant variables (e.g. mating system and ecological variables that influence endocrine systems). In addition, sociality in many species is condition-dependent, rendering controlled species comparisons difficult. The estrildid songbirds used here are all monogamous (typically pair-bonding for life), exhibit biparental care, breed semi-opportunistically dependent upon rainfall and inhabit arid and/or semi-arid habitat (Skead 1975; Goodwin 1982; Zann 1996). These species are the territorial violet-eared waxbill, *Uraeginthus granatina* (group size: two excluding dependent young; i.e. male–female pair), the moderately gregarious Angolan blue waxbill (group size: 8–40), and two colonial species, the zebra finch, *Taeniopygia guttata*, and the spice finch, *Lonchura punctulata* (group sizes: 90–300). These species exhibit only limited conditional variability in grouping behaviour such that species differences in sociality are stable across seasons and contexts. The zebra finch and spice finch are not closely related within the family Estrildidae and probably evolved their colonial social systems independently.

2. MATERIAL AND METHODS

(a) *Animals*

A total of 53 subjects were used (6–8 control and 6–8 experimental subjects for each of the 4 species, with approximately equal numbers of males and females; see below for descriptions of control and experimental conditions): violet-eared waxbill ($n = 4$ control males, 3 experimental males, 3 control females, 3 experimental females), Angolan blue waxbill ($n = 4$ control males, 3 experimental males, 4 control females, 4 experimental females), spice finch ($n = 3$ control males, 3 experimental males, 3 control females, 3 experimental females), zebra finch ($n = 3$ control males, 3 experimental males, 4 control females, 3 experimental females). Waxbills were wild-caught by mist net in South Africa and spice finches (probably of the Indian subspecies) were collected by a commercial supplier. These birds were maintained in captivity at least four months before they were killed. We have subsequently collected waxbills and successfully bred them in captivity within four months, thus this time-period is adequate for acclimation. Other behaviour also appears species-typical after captivity: violet-eared waxbills remain highly aggressive, regardless of whether they are housed with a pair-bond partner or not (Goodson 1998*b*), whereas wild-caught subjects of the gregarious species are quite docile (J. L. Goodson, personal observation). Zebra finches (native to Australia) were of domestic stock. Birds were housed indoors on a 14 L : 10 D photoperiod and were provided with finch seed mix, oyster shell and water *ad libitum*, with regular supplements of mealworms and greens. Collections and procedures were conducted legally and humanely under all applicable federal and state permits and in compliance with all applicable institutional and federal guidelines.

Experiments were conducted during the wild species' non-breeding season (February for spice finches; July for *Uraeginthus* species, with zebra finches tested concurrently). Time in captivity and photoperiod did not differ for any of the three wild species and subjects were maintained in same-sex housing for at least one month before testing. During this time-period, the territorial subjects were housed individually and gregarious subjects were group-housed; all subjects could see and hear conspecifics of both sexes in other cages (within 3 m in the same housing room).

Post-mortem inspections confirmed that all wild-caught subjects exhibited fully regressed gonads and no species differences in gonadosomatic index were observed (ANOVA $p > 0.10$). No evidence of physiological stress (e.g. enlarged adrenals) was found. By contrast, the domestic zebra finches exhibited fully developed oviducts (females) and gonads. Domestic zebra finches are reproductively robust and will breed readily under virtually all standard housing conditions (Zann 1996). However, as detailed below, the zebra finch and spice finch (both highly gregarious year-round) exhibited virtually identical IEG responses to same-sex social interactions, suggesting that gonadal state may not impact IEG responses of gregarious birds in non-reproductive social contexts.

(b) *Behavioural paradigm*

Two days before they were killed, each subject was placed individually into a small testing cage (31 cm × 20 cm × 36 cm: W × H × D). After 24 h acclimation, this cage was moved into a quiet room. The following day, a wire barrier was inserted into the cage, splitting it into left and right halves, with a single perch running parallel to the barrier on each side of the cage. For experimental subjects, a same-sex conspecific was then placed into the cage on the side opposite the subject. Control subjects were exposed to the barrier only. Behavioural observations were conducted for 10 min with the use of a curtain blind, focusing on song and agonistic behaviours (as described in Goodson 1998*b*; Goodson *et al.* 1999). Exposure to the stimulus lasted 90 min, at which time the subjects were perfused for immunocytochemistry.

(c) *Immunocytochemistry*

In addition to labelling tissue series for FOS or ZENK, we also multi-labelled the IEG material using a vasopressin antibody, and a third series included labelling for tyrosine hydroxylase and neuropeptide Y. The last two antibodies were used to provide a clear delineation of the chemoarchitectonic zones of the lateral septum (LS; Goodson *et al*. 2004), as the LS exhibits species-specific functional features (Goodson *et al.* 1999; Goodson & Bass 2001) and the LS zones are differentially implicated in agonistic behaviour, anxiety and/or stress (Goodson & Evans 2004). Vasopressin (or vasotocin in non-mammals) influences a wide variety of social behaviours, occasionally in a species-specific manner (Goodson & Bass 2001; Young *et al.* 2001). Results relating to vasotocin/IEG double labelling will be reported elsewhere.

Tissue preparation, fluorescent multi-labelling and the determination of antibody specificity was conducted as fully described elsewhere for other experiments (Goodson & Evans 2004; Goodson *et al.* 2004), which employed all of the above antibodies except FOS (which was used at a concentration of 1 : 1000, as was ZENK). The custom FOS antibody used here was generated against the avian FOS sequence of D'Hondt *et al.* (1999) and raised in mouse (ascites custom; Zymed Laboratories, South San Francisco, CA, USA). Pilot studies comparing this antibody to that of D'Hondt *et al.* (1999) demonstrate a similar distribution of labelling, and preadsorption of our custom antibody with 10 μM of antigen completely eliminated label.

(d) *IEG quantification*

IEG quantification and the delineation of areas were conducted using the methods previously employed for song sparrows (*Melospiza melodia*; Goodson & Evans 2004) and using the chemoarchitectural parcellation of the LS recently established for songbirds (Goodson *et al.* 2004). Slides were coded by an assistant who was unaware of treatment conditions. This assistant did not participate in data analysis. All further analysis was performed blind to the subjects' treatment groups. For each subject, 10× photomicrographs were generated for regions of interest using a Zeiss Axioscop microscope and an Optronics Magnafire digital camera linked to an Apple Macintosh G4 computer. For each structure, one to four sections were photographed bilaterally for analysis. Cell counts collected from photomicrographs were probably not biased by differences in cell number or density, as photomicrographs were obtained at a plane of focus in the middle of the tissue sections, which were quite thick (40 μm) relative to IEG-immunoreactive nuclear profile diameter (< 8 μm). In addition, photomicrographs for each IEG were obtained from a single tissue series, thus no quantification was conducted in adjacent sections. Only full, rounded nuclear profiles were counted. For each region of interest, a standardized box outline was created Adobe Photoshop 5.5 and the same box was then used for that area in all subjects. The boxes were superimposed on the photomicrographs and all IEG profiles within the boxes were quantified by placing dots over each profile and then counting the dots in NIH Image 1.63 (W. Rasband, National Institutes of Health). Raw cell counts were then converted into the number of profiles per 100 μm^2. Statistical analyses (ANOVA followed by Fisher PLSD) were performed using STATVIEW (SAS Institute, Inc., Cary, NC).

3. RESULTS

(a) *Behaviour*

Given that physical interaction was not possible in this paradigm, most subjects simply exhibited arousal (i.e. increased activity and inspection of the barrier, also seen in the control subjects with the insertion of the wire barrier) and did not sing or exhibit an agonistic response. Two experimental male zebra finches sang briefly after the introduction of the stimulus male and two experimental female violet-eared waxbills exhibited low-intensity agonistic responses (open gaping, a threat display; Goodwin 1982; Goodson 1998b). Hence, IEG response differences between experimental and control subjects should primarily reflect neural processing that is independent of behavioural performance. The generally low level of overt behaviour is probably not due to any stress induced by the testing paradigm, as we have regularly used the same cages and barriers to assess courtship behaviour in these species, and even with much shorter acclimation times, both wild-caught and domestic birds readily exhibit courtship and other sexual behaviours (J. L. Goodson, personal observation).

(b) *IEG responses*

After exposure to a same-sex conspecific, all nodes of the social behaviour network showed significant elevations in IEG proteins (table 1; locations of most structures are shown in figure 2*a*). We additionally quantified responses within six other structures that are connected to the social behaviour network. Of these, the medial septum complex, paraventricular nucleus of the hypothalamus and ventral pallidum showed a significant response to the social stimulus (table 1). A significant response was not observed for either IEG in the hippocampus, lateral bed nucleus of the stria terminalis, or nucleus accumbens.

Although these data are informative, our primary purpose here was to determine how activation in these areas *differs* in relation to subject sex and species-typical social structure. Therefore, to directly compare experimental

Table 1. Effect of treatment condition (control versus exposure to a same-sex conspecific) on FOS- and ZENK-ir nuclei per 100 μm^2, and differences between species and sexes on FOS and ZENK response indices (see § 3b). (Significant species differences are shown in figure 2. n.s., not significant.)

	condition (control vs. experiment)				species		sex	
area	control FOS-, ZENK-ir nuclei	experimental FOS-, ZENK-ir nuclei	$F_{1,1,3,37}$ (FOS,ZENK)	P (FOS,ZENK)	$F_{1,3,17}$ (FOS,ZENK)	P (FOS,ZENK)	$F_{1,3,17}$ (FOS,ZENK)	P (FOS,ZENK)
lateral septum								
caudal part, ventrolateral zone (LSc.vl)	2.21 ± 0.25, 6.33 ± 0.55	3.21 ± 0.42, 8.74 ± 0.86	8.069, 6.993	< 0.01, < 0.01	9.110, 1.159	< 0.001, n.s.	0.576, 0.291	n.s., n.s.
caudal part, lateral zone (LSc.l)	3.57 ± 0.23, 4.26 ± 0.54	6.23 ± 0.78, 8.68 ± 1.05	17.748, 23.339	< 0.001, < 0.0001	1.300, 3.845	n.s., < 0.05	0.374, 0.097	n.s., n.s.
caudal part, ventral zone (LSc.v)	2.88 ± 0.29, 4.96 ± 0.51	4.08 ± 0.36, 7.90 ± 0.72	9.831, 13.167	< 0.005, < 0.001	0.212, 0.110	n.s., n.s.	1.604, 1.145	n.s., n.s.
caudal part, dorsal zone (LSc.d)	3.74 ± 0.27, 4.54 ± 0.31	3.90 ± 0.29, 5.18 ± 0.42	1.699, 0.225	n.s., n.s.	1.539, 1.050	n.s., n.s.	2.268, 0.343	n.s., n.s.
rostral part (LSr)	1.78 ± 0.16, 3.12 ± 0.24	2.60 ± 0.30, 4.83 ± 0.45	6.909, 16.111	< 0.05, < 0.001	1.156, 2.590	n.s., n.s.	0.519, 0.047	n.s., n.s.
extended medial amygdala								
nucleus taeniae (Tn)	0.95 ± 0.21, 2.25 ± 0.35	1.54 ± 0.24, 3.66 ± 0.28	4.970, 10.657	< 0.05, < 0.005	4.078, 1.526	< 0.05, n.s.	0.079, 12.363	n.s., < 0.005[a]
medial bed nucleus of the stria terminalis (BSTm)	1.74 ± 0.13, 3.25 ± 0.18	2.73 ± 0.12, 5.07 ± 0.27	35.073, 33.607	< 0.0001, < 0.0001	10.772, 0.380	< 0.0005, n.s.	12.311, 0.900	<0.005[b], n.s.
Tn/BSTm combined	1.35 ± 0.10, 2.75 ± 0.21	2.14 ± 0.15, 4.36 ± 0.22	29.654, 28.547	< 0.0001, < 0.0001	7.927, 1.059	< 0.005, n.s.	1.573, 6.099	n.s., < 0.05[c]
preoptic area/hypothalamus								
preoptic area	0.58 ± 0.05, 3.53 ± 0.19	0.69 ± 0.07, 4.34 ± 0.27	1.662, 4.849	n.s., < 0.05	1.319, 1.286	n.s., n.s.	0.036, 1.606	n.s., n.s.
anterior hypothalamus (AH)	0.56 ± 0.07, 3.59 ± 0.25	1.53 ± 0.20, 6.62 ± 0.43	31.614, 46.974	< 0.0001, < 0.0001	4.121, 1.812	< 0.05, n.s.	0.037, 0.984	n.s., n.s.
ventromedial hypothalamus, medial part (VMH-med)	0.91 ± 0.11, 2.87 ± 0.34	0.78 ± 0.08, 3.13 ± 0.38	0.797, 0.103	n.s., n.s.	2.365, 3.233	n.s., < 0.05	0.716, 0.483	n.s., n.s.
ventromedial hypothalamus, lateral part (VMH-lat)	0.26 ± 0.05, 0.58 ± 0.07	2.25 ± 0.55, 4.98 ± 0.73	19.535, 50.702	< 0.0001, < 0.0001	4.099, 3.456	< 0.05, < 0.05	1.323, 0.807	n.s., n.s.
midbrain								
central grey (CG)	0.96 ± 0.19, 4.29 ± 0.43	1.39 ± 0.27, 5.40 ± 0.50	3.349, 3.314	n.s., n.s.	4.175, 3.001	< 0.05, n.s.	2.219, 0.516	n.s., n.s.
nucleus intercollicularis (ICo)	0.92 ± 0.16, 5.45 ± 0.58	1.20 ± 0.22, 7.72 ± 0.67	0.182, 7.073	n.s., < 0.01	4.262, 1.642	< 0.05, n.s.	0.591, 0.762	n.s., n.s.
CG/ICo combined	0.94 ± 0.17, 4.92 ± 0.49	1.29 ± 0.24, 6.48 ± 0.58	2.740, 4.505	n.s., < 0.05				

(*Continued.*)

Table 1. (*Continued.*)

area	condition (control vs. experiment)				species		sex	
	control FOS-, ZENK-ir nuclei	experimental FOS-, ZENK-ir nuclei	$F_{1,1,3,37}$ (FOS,ZENK)	P (FOS,ZENK)	$F_{1,3,17}$ (FOS,ZENK)	P (FOS,ZENK)	$F_{1,3,17}$ (FOS,ZENK)	P (FOS,ZENK)
associated structures								
paraventricular nucleus of the hypothalamus (PVN)	0.83 ± 0.08, 3.88 ± 0.31	1.35 ± 0.17, 5.11 ± 0.37	11.142, 5.330	<0.005, <0.05	2.389, 0.456	n.s., n.s.	0.347, 1.432	n.s., n.s.
lateral bed nucleus of the stria terminalis (BSTl)	3.85 ± 0.41, 21.78 ± 1.12	4.81 ± 0.48, 21.78 ± 1.26	2.442, 0.006	n.s., n.s.	0.692, 1.207	n.s., n.s.	0.001, 5.819	n.s., <0.05[d]
nucleus accumbens	0.55 ± 0.11, 3.57 ± 0.46	0.79 ± 0.11, 4.20 ± 0.43	2.679, 0.955	n.s., n.s.	1.219, 0.467	n.s., n.s.	0.232, 0.307	n.s., n.s.
ventral pallidum (rostral)	0.69 ± 0.31, 9.67 ± 5.77	0.98 ± 0.68, 10.37 ± 4.72	5.662, 0.113	<0.05, n.s.	1.879, 0.381	n.s., n.s.	0.094,3.619	n.s., n.s.
hippocampus (Hp)	0.54 ± 0.04, 0.57 ± 0.1	0.51 ± 0.05, 0.69 ± 0.12	0.201, 0.923	n.s., n.s.	1.852, 1.392	n.s., n.s.	0.483, 0.034	n.s., n.s.
medial septum + internal band (MS/MSib)	0.89 ± 0.07, 2.33 ± 0.20	1.30 ± 0.12, 3.28 ± 0.27	10.682, 7.829	<0.005, <0.01	2.853, 0.275	n.s., n.s.	0.006, 0.527	n.s., n.s.

[a] Males, 0.59 ± 0.45; females, 2.29 ± 0.35.
[b] Males, 0.63 ± 0.24; females, 1.29 ± 0.18.
[c] Males, 1.07 ± 0.36; females, 2.20 ± 0.31.
[d] Males, −2.74 ± 1.48; females, 2.25 ± 1.43.

subjects of different sexes and species, we created a response index by subtracting the mean number of ZENK- and FOS-immunoreactive (-ir) neurons observed for control subjects from that observed in individual experimental subjects. This was done separately for each sex and species, and data for experimental subjects were then compared using two-way ANOVAs (sex × species). The pattern of results for the two IEGs was complementary and only partly overlapping (ZENK response indices are shown in figure 2*d–g*; FOS response indices are shown in figure 2*h–m*). In general, FOS proved more responsive to the social stimulus than ZENK, and a broader pattern of species differences was observed with FOS (table 1; figure 2).

Males and females showed comparable IEG responses in all areas of the social behaviour network (ANOVA p-values for main effects of sex > 0.05), with the exception of ZENK responses in the extended medial amygdala, which were greater in females (table 1). In mammals, the extended medial amygdala is comprised of the medial amygdala and medial bed nucleus of the stria terminalis (BSTm; Newman 1999); homologous areas in birds are the nucleus taeniae (Thompson *et al.* 1998; Cheng *et al.* 1999; Absil *et al.* 2002) and BSTm (Aste *et al.* 1998), respectively. FOS responses for BSTm were also greater in females, but this was not evident for the extended medial amygdala as a whole (ANOVA $p > 0.10$; table 1). The sexes are therefore combined for presentation of species differences in figure 2, which presents no data for which sex differences were found. Outside of the social behaviour network, female-biased sex differences in ZENK response were observed only in the lateral BST (table 1).

As shown in figure 2*h*, FOS responses of the extended medial amygdala varied in relation to species-typical group size across the three wild species, such that the moderately gregarious Angolan blue waxbill exhibited a significantly smaller FOS response than the territorial violet-eared waxbill, but a significantly greater FOS response than the colonial spice finch. The two colonial species (one wild-caught, one domestic) did not differ significantly from each other on this measure or on any other measure in this dataset. Significant and similar species differences were observed for both of the extended amygdala components (table 1).

Within the LS, FOS and ZENK responses were exhibited in a zone-specific manner. Thus, although several chemoarchitectonic zones of the LS exhibited significant IEG induction following social exposure, species differences in this pattern were strongly localized to the ventrolateral and lateral zones of the caudal LS (table 1), with the territorial violet-eared waxbill exhibiting significantly greater IEG responses than the three more social species (figure 1*b* and figure 2*d*,*i*). Responses of other LS areas were virtually identical for all four species (e.g. ventral zone of the caudal LS; figure 2*e*,*j*).

In addition to the ventrolateral LS, species differences in IEG response were found for two other forebrain areas that are implicated in agonistic behaviour—the anterior hypothalamus (AH) and lateral subdivision of the ventromedial hypothalamus (VMH; Kollack-Walker & Newman 1995; Kollack-Walker *et al.* 1997; Delville *et al.* 2000; Goodson & Evans 2004). Responses for each of

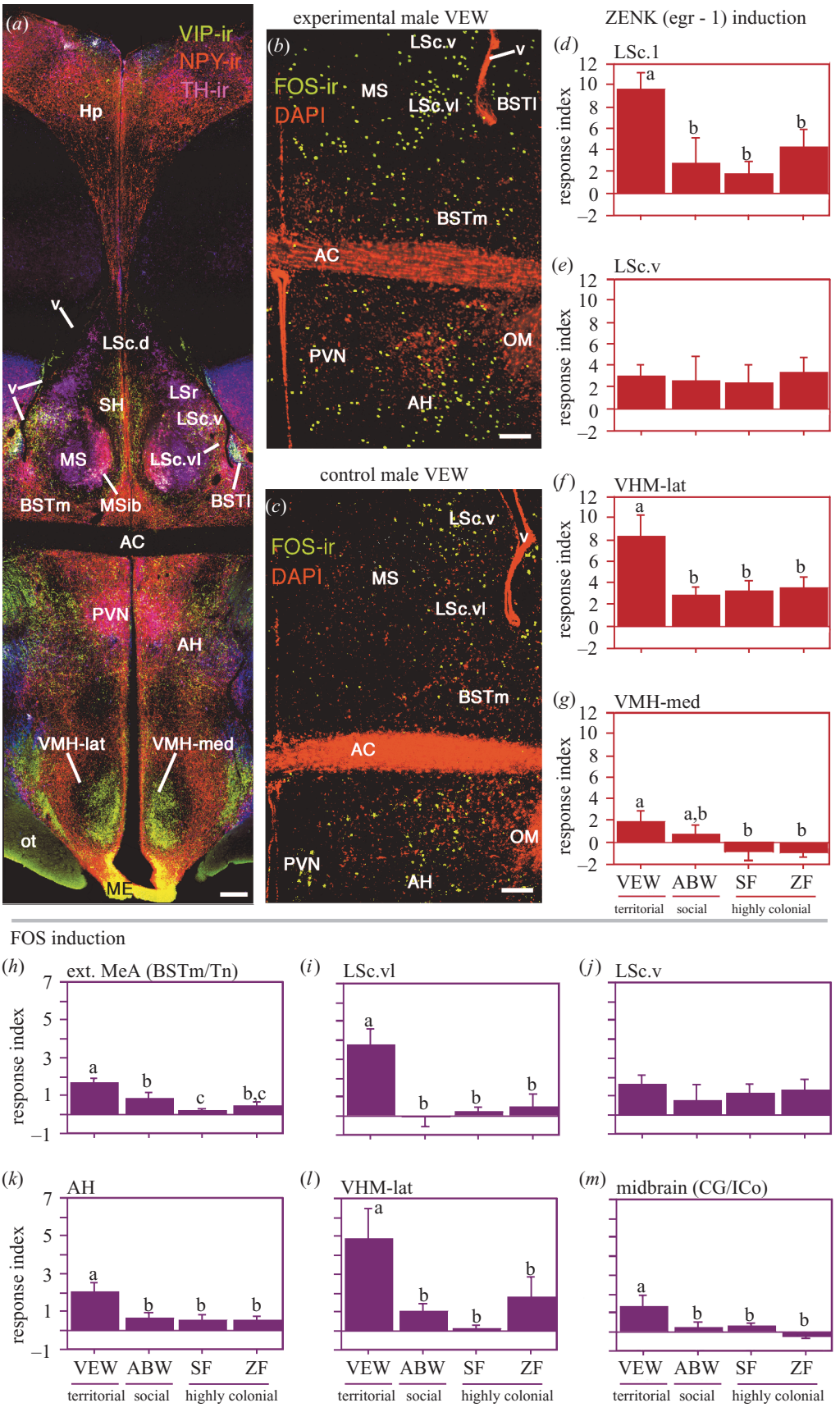

Figure 2. (*Caption opposite.*)

these areas were equivalent in the three social species but significantly greater in the territorial violet-eared waxbill (table 1; figure 1*b* and figure 2*f*,*k*,*l*). A weaker species difference was found for the medial aspect of the VMH (figure 2*g*), a region that may correspond in part to an area activated by female sexual behaviour (Meddle *et al.* 1999).

Figure 2. (*Opposite.*) Multiple components of the social behaviour network (visualized by chemoarchitecture) and their IEG responses to a same-sex social stimulus. (*a*) A coronal section of the midline forebrain of a zebra finch at the level of the anterior commissure (AC; modified from Goodson *et al.* 2004). Shown are multiple network components and associated structures as visualized by triple-label immunocytochemistry for neuropeptide Y (NPY; red), tyrosine hydroxylase (TH; purple) and vasoactive intestinal polypeptide (VIP; green). We have employed NPY and TH antibodies in conjunction with IEG antibodies to allow delineation of the septal nuclei and other areas. Scale bar, 200 μm. (*b*,*c*) FOS immunoreactivity (green) in experimental (*b*) and control (*c*) male violet-eared waxbills (territorial), showing responses of the experimental animal in the ventrolateral LS, BSTm and AH (structural detail provided by DAPI nuclear stain, pseudocoloured red). Scale bars, 100 μm. (*d–m*) ZENK (*d–g*) and FOS (*h–m*) response indices (experimental cell counts minus mean counts for controls; see § 3b) for components of the social behaviour network in the territorial violet-eared waxbill (VEW), the modestly gregarious Angolan blue waxbill (ABW) and two highly colonial species—the spice finch (SF) and zebra finch (ZF; unlike other subjects, ZF were domestic and in breeding condition). Response indices are shown as the number of immunoreactive nuclei per 100 μm^2 (means ± s.e.m.). Different letters above the error bars indicate significant species differences (Fisher's PLSD, $p < 0.05$) following significant ANOVA (sex × species; no main effects of sex were observed, thus male and female data are shown pooled). Abbreviations: AC, anterior commissure; AH, anterior hypothalamus; BSTm, medial bed nucleus of the stria terminalis; BSTl, lateral bed nucleus of the stria terminalis; CG, midbrain central grey; ext. MeA, extended medial amygdala; Hp, hippocampus; ICo, nucleus intercollicularis; LSc, caudal division of the lateral septum (dorsal, ventrolateral, lateral and ventral zones denoted as LSc.d, LSc.vl, LSc.l and LSc.v, respectively); LSr, rostral division of the lateral septum; ME, median eminence; MS, medial septum; MSib, internal band of the medial septum; ot, optic tract; OM, occipitomesencephalic tract; PVN, paraventricular nucleus of the hypothalamus; SH, septohippocampal septum; Tn, nucleus taeniae; v, lateral ventricle; VMH, ventromedial hypothalamus.

Finally, the territorial species exhibited a modestly larger FOS response in a cell band extending from the lateral midbrain central grey into the adjacent tegmentum (nucleus intercollicularis; table 1 and figure 2*m*).

As shown in table 1, numerous areas showed no evidence of species differences. These include the lateral BST, the paraventricular nucleus of the hypothalamus, and multiple zones of the LS (dorsal and ventral zones of the caudal LS, and the rostral LS)—areas that are implicated in a variety of general stress responses (Herman *et al.* 2003; Goodson & Evans 2004). Similarly, no species differences were found for the nucleus accumbens, ventral pallidum, preoptic area, hippocampus and medial septum complex.

4. DISCUSSION

The present data demonstrate that although numerous brain regions exhibit an IEG response to a same-sex social stimulus, sex and species differences in this response are relatively discrete, being found for only a few areas, or specific subdivisions of areas. Species differences that were negatively correlated with sociality were found for the extended medial amygdala (which is involved in social arousal and approach–avoidance behaviours; see below, second paragraph), with the modestly gregarious Angolan blue waxbill exhibiting a FOS response that was intermediate to that observed for the colonial and territorial species. In addition, the territorial violet-eared waxbill exhibited stronger FOS and ZENK responses within the lateral VMH and ventrolateral zones of the LS than did the three gregarious species, and a stronger FOS response in the AH. As discussed in the next paragraph, these last three areas are implicated in dominance-related behaviour and social stress. Sex differences in ZENK induction were found only within the extended medial amygdala (i.e. the nucleus taeniae and BSTm combined) and FOS induction showed sex differences only within the BSTm. Both of these sex differences were female-biased. Overall, the IEG responses observed here were modest, probably because the experimental subjects were allowed to interact with a same-sex conspecific only through a wire barrier. However, by using this paradigm, we were able to virtually eliminate the motor performance of social behaviours, thereby providing insight into neural function that is related to social perception and/or motivation, not activation of motor systems.

Although the role of the extended medial amygdala in social behaviour is complex, it is clearly related to social arousal, and probably plays an important role in the regulation of both social approach and avoidance. In Japanese quail (*Coturnix japonica*), lesions of nucleus taeniae have been shown to both increase and decrease appetitive sexual approach (Thompson *et al.* 1998; Absil *et al.* 2002). These divergent effects may relate to lesion placement (i.e. functional segregation within nucleus taeniae), although this has not yet been clearly established. Functional segregation has been more clearly established for the mammalian medial amygdala, with separate neuronal populations being implicated in general social arousal and sexual approach (review: Newman 1999). Lesions of the mammalian medial amygdala decrease sexual, agonistic and a variety of non-sexual affiliative behaviours (Lehman *et al.* 1980; Luiten *et al.* 1985; Kirkpatrick *et al.* 1994), and lesions of the BSTm decrease sexual behaviour in birds and mammals (Liu *et al.* 1997; Balthazart *et al.* 1998). A variety of social stimuli also elicit IEG responses in the extended medial amygdala of birds and mammals (reviews: Ball & Balthazart 2001; Newman 1999; also Goodson & Evans 2004). Combined, these results suggest that the extended medial amygdala promotes social contact in numerous behavioural contexts. However, IEG and lesion data also demonstrate that the medial amygdala can inhibit behaviour. The medial amygdala mediates pup avoidance behaviour in rats (Sheehan *et al.* 2000, 2001), and as noted above, some lesions in quail actually facilitate appetitive sexual approach (Absil *et al.* 2002). Thus, although the present data for songbirds show that the overall IEG response in the extended medial amygdala correlates negatively with species-typical group size, we suggest that even stronger species differences will be found once the specific neuronal populations that regulate approach and avoidance are identified and individually investigated.

Apart from the amygdala, all other species differences in IEG response dichotomously distinguish the territorial species from the three gregarious species. This was found for the ventrolateral LS, lateral VMH and AH. In both

mammals and birds, a variety of manipulations within these areas (e.g. lesions, electrical stimulation, neuropeptide and steroid hormone administration) influence aggression and/or agonistic communication (Barfield 1971; Phillips & Youngren 1971; Nyby *et al.* 1992; Ferris & Delville 1994; Goodson *et al.* 1999; Siegel *et al.* 1999) and IEG induction is also observed in these three sites after agonistic encounters and various social and non-social stressors (Kollack-Walker & Newman 1995; Campeau *et al.* 1997; Kollack-Walker *et al.* 1997; Delville *et al.* 2000; Goodson & Evans 2004). Subordinate male hamsters exhibit greater IEG induction within all three of these areas after aggressive encounters than do the dominant animals (Kollack-Walker *et al.* 1997), and IEG responses within the ventrolateral LS and AH of territorial song sparrows are negatively correlated with aggression (J. L. Goodson, A. K. Evans and K. K. Soma, unpublished observation). Importantly, while multiple zones of the LS in male song sparrows exhibit ZENK responses to social and non-social stressors (simulated territorial intrusion and capture/restraint stress, respectively), the ventrolateral zone of the caudal LS (i.e. the LSc.vl) is the only LS zone that shows a strong selectivity for the social stressor (Goodson & Evans 2004). Finally, lesion and IEG evidence from rats also demonstrate that the ventrolateral LS and VMH are part of a neural circuit that inhibits maternal response (Sheehan *et al.* 2000, 2001).

Combined, these data suggest that the AH, ventrolateral LS and lateral VMH function to regulate social behaviour in relation to the level of contextual stress, particularly in the case of dominance-related behaviour. This hypothesis of stress-related, context-dependent function is strongly supported by data on the role of the neuropeptide vasotocin within the caudal septum of territorial male sparrows, given that (i) endogenous vasotocin modulates ZENK responses of the LS to stress in song sparrows (Goodson & Evans 2004), and (ii) infusions of vasotocin into the septum of field sparrows (*Spizella pusilla*) increase the use agonistic song during the dawn chorus, but decrease overt aggression when the subject is faced with an actual territorial intruder (Goodson 1998a).

Importantly, the modestly gregarious Angolan blue waxbill exhibited responses in these dominance-related areas (ventrolateral LS, lateral VMH and AH) that were comparable to the highly gregarious, colonial species (spice finch and zebra finch), suggesting that motivational mechanisms related to aggression and/or social stress may not play a strong role in differentiating modestly and highly social species. Species differences were not observed in other areas that are less selectively responsive to a broad range of stressors (lateral BST, paraventricular nucleus of the hypothalamus, and multiple zones of the LS; Campeau *et al.* (1997); Herman *et al.* (2003); Goodson & Evans (2004)). Thus, differences in sociality are not correlated with widespread activation of generalized stress circuitry, nor are species differences observed for reward centres such as the nucleus accumbens and ventral pallidum, which are both implicated in pair-bonding and non-sexual affiliation in mammals (reviews: Young *et al.* 2001; Lim *et al.* 2004). Indeed, gradations in species-typical group size were found to relate only to the level of IEG response in the extended medial amygdala. This result may be broadly relevant for vertebrates, as recent findings demonstrate that a human phenotype characterized by social avoidance exhibits stronger whole-amygdala activation in response to novel facial stimuli than does a phenotype characterized by higher levels of social approach (Schwartz *et al.* 2003). In addition, our data suggest that a specific pattern of response within the social behaviour network may reliably evolve in conjunction with a given level of sociality, as we observed no differences between two songbird species that independently evolved coloniality (spice finch and zebra finch).

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