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Central Vasopressin and Oxytocin Receptor Distributions in Two Species of Singing Mice

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ABSTRACT

The neuropeptides arginine vasopressin (AVP) and oxytocin (OT) are key modulators of vertebrate sociality. Although some general behavioral functions of AVP and OT are broadly conserved, the detailed consequences of peptide release seem to be regulated by species-specific patterns of receptor distribution. We used autoradiography to characterize central vasopressin 1a receptor (V1aR) and OT receptor (OTR) distributions in two species of singing mice, ecologically specialized Central American rodents with a highly developed form of vocal communication. While both species exhibited high V1aR binding in the auditory thalamus (medial geniculate), binding in structures involved in vocal production (periaqueductal gray and anterior hypothalamus) was significantly higher in the more vocal species, *Scotinomys teguina*. In *S. xerampelinus*, receptor binding was significantly higher in a suite of interconnected structures implicated in social and spatial memory, including OTR in the hippocampus and medial amygdala, and V1aR in the anterior and laterodorsal thalamus. This pattern is concordant with species differences in population density and social spacing, which should favor enhanced sociospatial memory in *S. xerampelinus*. We propose that V1aR and OTR distributions in singing mice support an integral role for the AVP/OT system in several aspects of sociality, including vocal communication and sociospatial memory. J. Comp. Neurol. 516:321–333, 2009.

Indexing terms: mating system; Scotinomys; social behavior network; spatial memory; vasotocin; vocalization

Across vertebrates the arginine vasopressin (AVP) and oxytocin (OT) neuropeptide family is integral to a diverse range of social and reproductive behaviors. For example, the ancestral form of mammalian vasopressin, vasotocin (AVT) regulates aggression and singing in songbirds (Goodson, 1998; Goodson and Adkins-Regan, 1999), courtship behavior in newts and fishes (Thompson and Moore, 2000; Bastian et al., 2001; Salek et al., 2002; Grober et al., 2002), and mate calling in frogs (Marler et al., 1995). Likewise, isotocin (IT), an oxytocin homolog found in teleost fish, influences vocal production (Goodson and Bass, 2002) and stimulates social investigation (Thompson and Walton, 2004). In mammals, AVP and OT are also well known as modulators of a variety of cognitive and emotional processes, most notably, learning and memory, fear and aggression, and trust and selective affiliation (de Wied et al., 1976; Dantzer et al., 1987; Ferris et al., 1997; Cho et al., 1999; Winslow et al., 2000; Lim et al., 2004; Kirsch et al., 2005; Kosfeld et al., 2005).

The neural distributions of AVP/AVT and OT/IT immunoreactive fibers are relatively conserved across vertebrates (Moore and Lowry, 1998; Goodson et al., 2003, 2004; Rosen et al., 2008). Receptor distributions, however, can differ dramatically between closely related species (e.g., Insel et al., 1994; Beery et al., 2008). While comparisons across fishes, amphibians, birds, and mammals indicate that vasopressin and oxytocin and their homologs regulate many of the same types of social behaviors throughout the vertebrate lineage (reviewed in Goodson and Bass, 2001; Goodson, 2005), work in rodents has highlighted the species- and often sex-specific roles of AVP and OT in coordinating sociosexual and parental behaviors (reviewed in Young and Wang, 2004; Donaldson and Young, 2008). This combination of conservation and diversification in behavior and its underlying neural circuitry suggests that, while neuropeptide receptor distributions may respond rapidly to selection on behavioral phenotypes, general behavioral functions of the ancestral AVP/OT system are likely to be retained in mammals.

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The well-supported role of nonmammalian homologs of AVP and OT in vocal behavior in fishes, frogs, and birds indicates that regulation of vertebrate acoustic circuitry may be one such conserved function of vasopressin-related peptides (Goodson and Bass, 2001, and references therein; Goodson et al., 2003). Surprisingly, the potential importance of vasopressin and oxytocin in mammalian vocal communication has received little attention.

Here we describe oxytocin receptor (OTR) and vasopressin subtype 1a receptor (V1aR) expression patterns in two species of singing mice, *Scotinomys teguina* and *S. xerampelinus*, ecologically specialized Central American rodents that share a unique mode of social communication. Both species exhibit a complex vocal repertoire, which is used in both close-range and long-distance communication in a variety of social contexts. Most notable is a highly stereotyped advertisement call comprised of a rapidly articulated trill (up to 20 pulses/second, S.M. Phelps, unpubl.) that spans audible and ultrasonic frequencies (8–50 kHz; Hooper and Carlton, 1976; Miller and Engstrom, 2007). Because the roles of vasopressin and oxytocin in mammalian vocalization and auditory processing are largely unknown, establishing the distributions of V1a and OT receptors in the brains of highly vocal rodents is a critical first step to defining the functions of these neuropeptides in mammalian acoustic communication.

A second motivation for characterizing V1aR and OTR distributions in singing mice comes from interspecific differences in conspecific spacing, degree of maternal investment, and thermoregulatory demands, all factors that influence social structure (Komers and Brotherton, 1997; Ebensperger, 2001; Kokko and Jennions, 2008). Both species are montane but differ substantially in their elevational distributions: *S. teguina* occurs at mid-elevations (≈1,000–2,500 m), while *S. xerampelinus* is restricted to montane cloud forest and high altitude shrub and grasslands (≈2,000–3,500 m; Hooper, 1972). In *S. xerampelinus*, longer gestation, smaller litters, and slower pup development suggest greater maternal investment per offspring relative to *S. teguina*, a pattern consistent with altitudinal effects on life history traits in birds (Badyaev and Ghalambor, 2001). Likewise, while both species are social and neither are considered monogamous (Hooper and Carleton, 1976; Blondel et al., 2009), data from a sympatric site suggest substantial differences in density and spacing patterns (*S. teguina*, 163/ha; *S. xerampelinus*, 85/ha, B. Pasch, unpubl.). Avian patterns of space use and territoriality are regulated by AVT, and it has been suggested that the lack of a consistent correlation between V1aR distributions and mammalian monogamy may be explained by closer association of the AVP system with ecologically based species differences in space use, sociality, and aggression (Bester-Meredith et al., 1999; Goodson and Bass, 2001; Goodson et al., 2006). Thus, singing mice represent a potential model for studying how neural mechanisms of social behavior evolve in response to ecological pressures.

We used quantitative autoradiography to characterize V1aR and OTR expression patterns in *S. teguina* and *S. xerampelinus*. Specifically, our aims were to 1) describe the distribution and density of these neuropeptide receptors in rodents with a highly developed form of vocal communication, and 2) explore variation in receptor distributions in relation to species differences in social spacing and maternal investment.

MATERIALS AND METHODS

Animals

Scotinomys teguina and *S. xerampelinus* males and females used in this study were outbred lab-reared adults (age ≥60 days; Hooper and Carleton, 1976), derived from wild-caught individuals captured in Monteverde, Costa Rica (*S. teguina*) and Parque Internacional La Amistad, Panamá (*S. xerampelinus*). Both species were maintained at an ambient temperature of 19–22°C on a 12:12 light cycle, approximating the high elevation, tropical conditions associated with year-round breeding in the wild (Hooper and Carleton, 1976; S. Phelps, pers. obs.). Animals were housed in mixed-sex pairs in 9-gallon aquaria. Both species are insectivorous, but plant material comprises 16–28% of their diet in the wild (Hooper and Carelton, 1976). To approximate the animal portion of their diet, captive mice were given kitten chow ad libitum, together with live mealworms as enrichment. This diet was supplemented with a mixture of sunflower seeds, peanuts, and legumes.

Subjects were euthanized by CO_2 inhalation; brains were extracted immediately, frozen on dry ice, and stored at −80° C until sectioning. Brains from 16 *S. teguina* (7 males, 9 females) and 23 *S. xerampelinus* (9 males, 14 females) were used in the V1aR study; the brains of 18 *S. teguina* (8 males, 10 females) and 20 *S. xerampelinus* (10 males, 10 females) were used in the OTR study. All animal protocols were approved by the IACUC committee at University of Florida and were in accordance with the NIH *Guide for the Care and Use of Laboratory Animals*.

Tissue preparation and autoradiography

Four sets of coronal sections (20 μm thick, 100 μm apart) were cut in a cryostat, starting at the olfactory bulbs and extending caudally to the decussation of the corpus callosum at the level of the medial geniculate. Sections were thaw-mounted on Superfrost plus slides (Fisher Scientific, Pittsburgh, PA) and stored at −80° C until processing for autoradiography.

Autoradiography was performed using a 50 pM concentration of AVP receptor ^{125}I-linear-vasopressin (AVP; PerkinElmer, Oak Brook, IL, NEX3100) or 40 pM concentration of OT receptor ligand, ^{125}I-ornithine vasotocin (OVT; PerkinElmer, NEX254) following standard protocols (Insel and Shapiro, 1992). Briefly, thawed sections were fixed in 0.1% paraformaldehyde, washed in 50 mM Tris (pH 7.4), and incubated for 60 minutes with ^{125}I-labeled ligand in 50 mM Tris, 10 mM $MgCl_2$, 0.1% bovine serum albumin (BSA), and 0.05% bacitracin. Excess ligand was removed with 50 mM Tris / 10 mM $MgCl_2$ washes. Sections were rapidly air-dried and exposed to Kodak BioMax MR film along with ^{125}I-labled autoradiographic standards for 72 hours. Controls for nonspecific V1aR and OTR binding were incubated with an additional 50 μM of nonradioactive $(d(CH_2)_5{}^1,Tyr(Me)^2,Arg^8)$-vasopressin or (Thr^4,Gly^7)-oxytocin, respectively (Bachem, Torrance, CA, H-7710; H-5350).

Anatomical localization, analysis, and figure preparation

A subset of sections was stained for either cresyl violet (Fig. 1) or acetylcholinesterase to assist in defining neuroanatomical boundaries in regions with V1a and OT receptor binding. Structures were identified using the rat atlas (Paxinos and

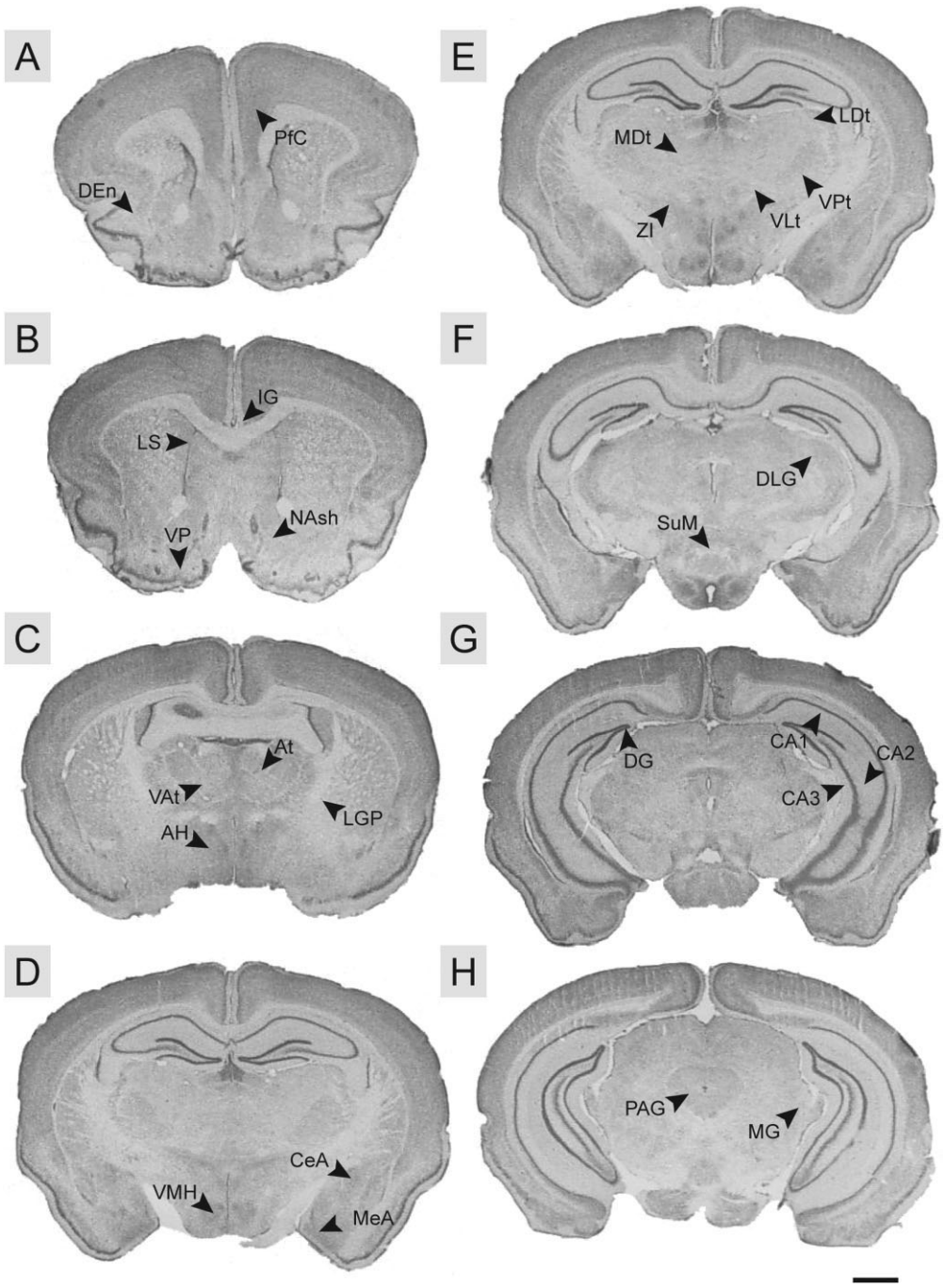


Figure 1.
Cresyl violet-stained sections from *S. teguina* showing the locations of structures in which V1aR and/or OTR binding were quantified. Arrowheads in **A–H** indicate dorsal endopiriform nucleus (DEn), prefrontal cortex (PfC), lateral septum (LS), indusium griseum (IG), nucleus accumbens shell (Nash), ventral pallidum (VP), ventral anterior thalamus (VAt), anterior hypothalamus (AH), lateral globus pallidus (LGP), anterior thalamus (At), ventromedial hypothalamus (VMH), medial (MeA) and central (CeA) amygdala, zona incerta (ZI), mediodorsal (MDt), laterodorsal (LDt), ventroposterior (VPt), and ventrolateral (VLt) thalamic nuclei, supramammillary nucleus (SuM), dorsal lateral geniculate (DLG), dentate gyrus (DG), CA1, CA2, and CA3 hippocampal fields, periaqueductal gray (PAG), and medial geniculate (MG). Gross neuroanatomy of *S. xerampelinus* appears identical. Scale bar = 1 mm.

Watson, 1998). Optical density measures for receptor binding were collected using the program NIH ImageJ (available at http://rsb.info.nih.gov/ij/). Each region of interest was measured bilaterally in three sections and averages of these readings were converted to decompositions per minute in rat brain tissue equivalent using an autoradiographic standard for each

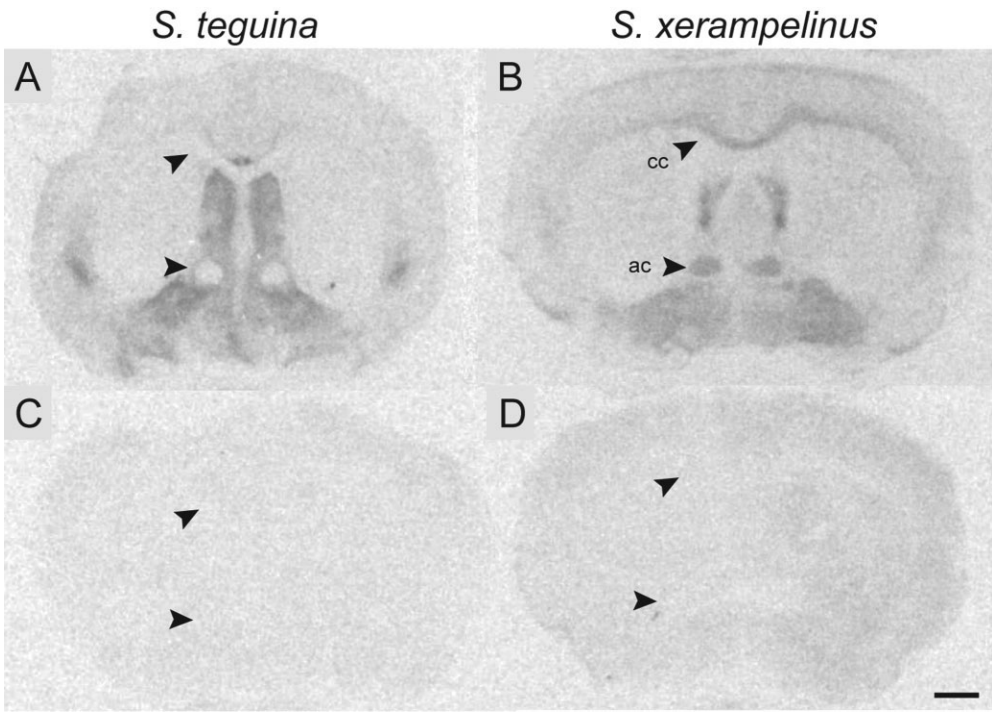


Figure 2.
Autoradiograms of ^{125}I-linear-AVP binding in singing mice in the absence **(A,B)** and presence **(C,D)** of selective receptor antagonist, (d(CH$_2$)$_5^1$,Tyr(Me)^2,Arg^8)-vasopressin. Arrowheads indicate corpus callosum (cc) and anterior commissure (ac), in which ^{125}I-linear-AVP is selectively bound in *S. xerampelinus* (B,D) but not in *S. teguina* (A,C). Scale bar = 1 mm.

film. Nonspecific binding was estimated from background levels of cortical binding on the same sections, averaged, and subtracted from mean specific binding for the corresponding region of interest. V1aR binding was quantified in 28 brain regions; OTR binding was quantified in 15 regions. Total brain V1aR and OTR binding were calculated for both species as the average of all measurements for each receptor type. V1aR fiber tract binding was not included in this comparison. Because this is the first description of V1aR and OTR distributions in *Scotinomys* we measured receptor density in all structures with appreciable binding and took note of structures in which lack of binding in *Scotinomys* was atypical of distributions reported for other species.

Data were analyzed in Statview (v. 4.57.0.0, Abacus Concepts, Berkeley, CA) using a two-way analysis of variance (ANOVA) with species and sex as between-subject variables. We used the method of Benjamini and Hochberg (1995) to adjust alpha-levels for multiple comparisons (V1aR, $\alpha = 0.022$; OTR, $\alpha = 0.03$). Comparisons with a significant main effect of sex, or species by sex interaction, were evaluated further with a Fisher's PLSD test.

Brain tissue sections were visualized by digitizing either films (autoradiograms) or slides (cresyl violet stains) on a Microtek Scan Maker 5900 at 1200 ppi with 8-bit gray-scale settings. Representative images of focal brain sections were imported into Adobe Photoshop CS3 (v. 10.0.1, San Jose, CA) and contrast and brightness were adjusted to minimize among-individual differences in nonspecific binding. Figures were assembled and labeled in Adobe Illustrator CS3 (v. 13.0.2).

RESULTS

^{125}I-linear-AVP (V1aR) and ^{125}I-OVT (OTR) binding in singing mice was abundant and specific (Figs. 2–4). Significant species differences were detected in the distributions of both neuropeptide receptors (Tables 1, 2). Sexual dimorphism was not detected in *S. teguina*. In *S. xerampelinus*, however, ^{125}I-OVT binding in the medial amygdala and CA1 field of the hippocampus was significantly higher in males. *S. xerampelinus* was also unique in having extensive ^{125}I-linear-AVP binding in the fiber tracts of many (Figs. 2B, 3K–O), but not all (Fig. 3F–J), brains examined. This surprising result does not seem to be a consequence of nonspecific binding: the presence of an excess of unlabeled AVP receptor ligand eliminated specific binding in all regions, including fiber tracts, and nonspecific binding was weak and homogeneous in both species (Fig. 2). ^{125}I-OVT-specific binding was similarly blocked by unlabeled OTR ligand (data not shown).

V1a receptor autoradiography

Total brain ^{125}I-linear-AVP binding did not differ between *S. teguina* and *S. xerampelinus* (ANOVA: $F_{(1,27)} = 0.02$, $P = 0.9$; Table 1). Specific binding was evident in structures throughout the brains of both species and interspecific differences were relatively complex, particularly in the thalamus (Fig. 3). Both species had comparably high binding in the lateral septum and in the centromedial and ventrolateral nuclei of the thalamus. Binding was moderate in the supraoptic nucleus, the paraventricular nuclei in the hypothalamus, and the medial and central amygdala. Moderate binding in the lateral globus

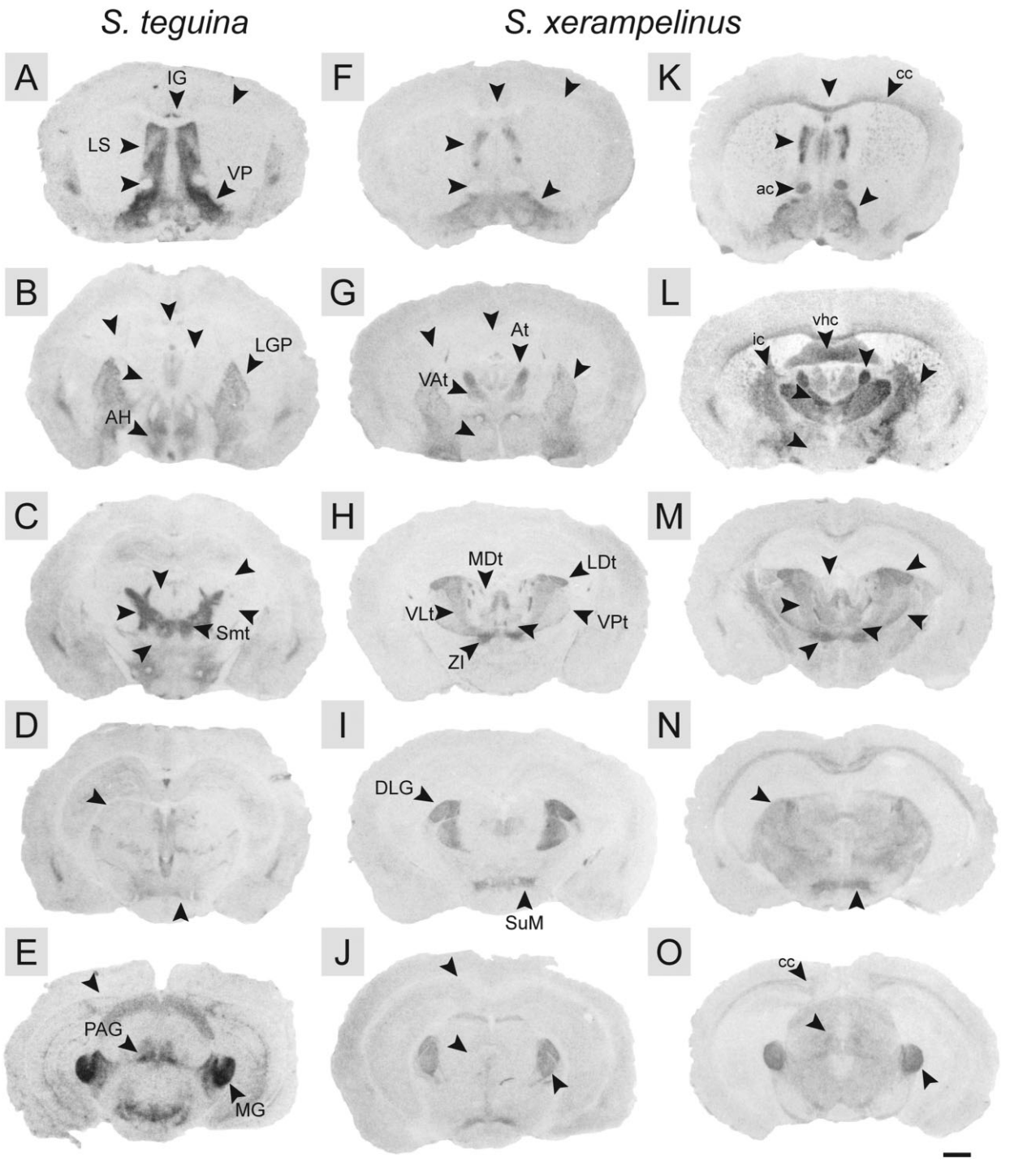


Figure 3.
Autoradiograms of V1aR binding in *S. teguina* **(A–E)** and in representative sections from *S. xerampelinus* without **(F–J)** and with **(K–O)** fiber tract binding. Arrowheads indicate lateral septum (LS), indusium griseum (IG), ventral pallidum (VP), corpus callosum (cc), and anterior commissure (ac) in A,F,K; anterior hypothalamus (AH), ventral anterior thalamus (VAt), anterior thalamus (At), lateral globus pallidus (LGP), internal capsule (ic), and ventral hippocampal commissure (vhc) in B,G,L; zona incerta (ZI), ventrolateral (VLt), mediodorsal (MDt), laterodorsal (LDt), ventroposterior (VPt), and submedius (Smt) thalamic nuclei in C,H,M; dorsal lateral geniculate (DLG) and supramammillary nucleus (SuM) in D,I,N; periaqueductal gray (PAG), medial geniculate (MG), and cc in E,J,O. Binding in MD and LD thalamus was polymorphic in both species: C is representative of *S. teguina* without binding in either structure, H and M are representative of *S. xerampelinus* with binding in both structures. Scale bar = 1 mm.

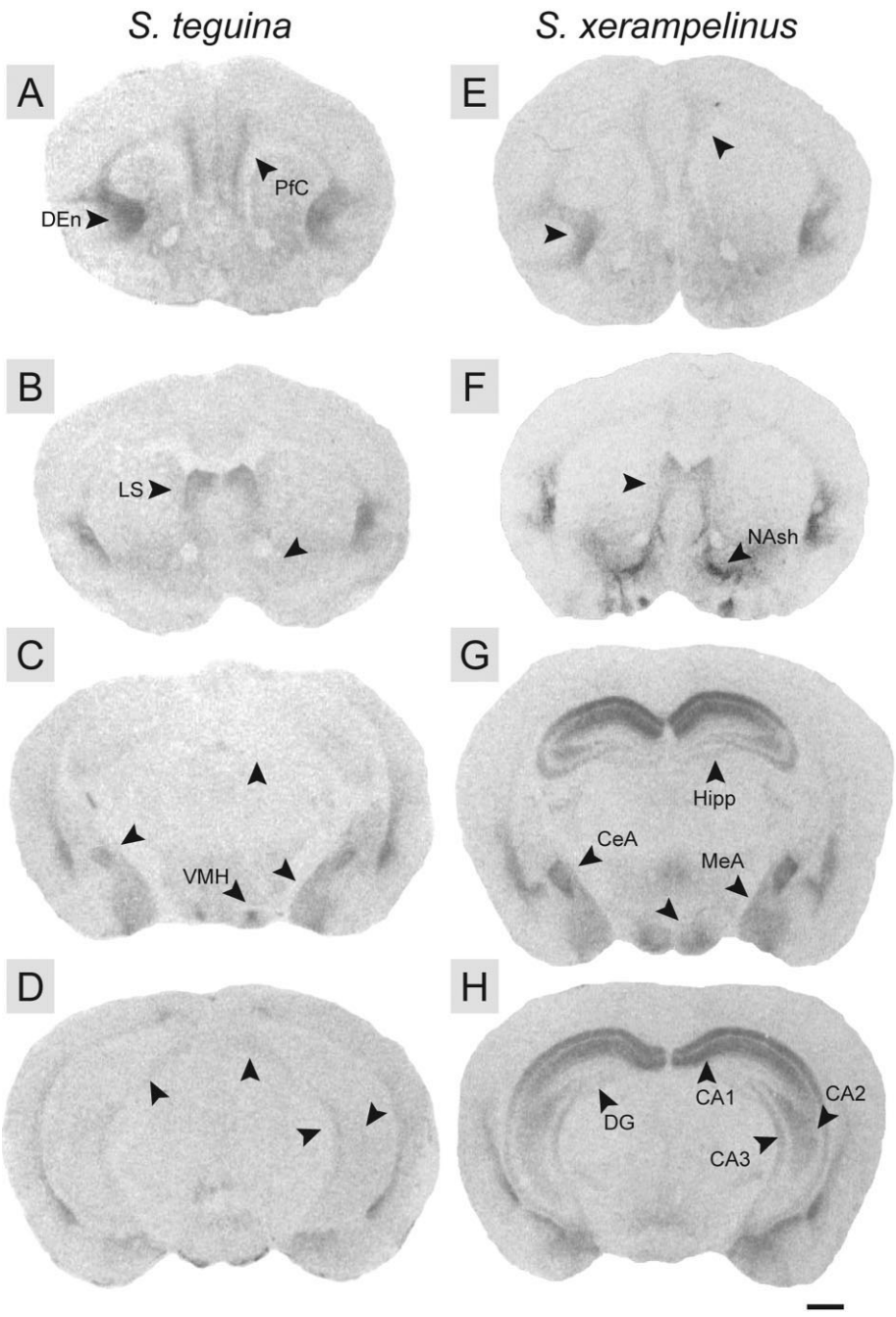


Figure 4.
Autoradiograms of OTR binding in *S. teguina* **(A–D)** and *S. xerampelinus* **(E–H).** Arrowheads indicate dorsal endopiriform nucleus (DEn) and prefrontal cortex (PfC) in A,E; lateral septum (LS) and nucleus accumbens shell (NAsh) in B,F; hippocampus (Hipp), ventromedial hypothalamus (VMH), central (CeA), and medial (MeA) amygdala in C,G; dentate gyrus (DG), and CA1, CA2, and CA3 hippocampal fields in D,H. Scale bar = 1 mm.

pallidus was also detected in both species (Fig. 3B,G,L); V1aR expression in this structure has not been reported previously in any rodent (reviewed in Beery et al., 2008, table 3). Binding was minimal or lacking in the hippocampus (data not shown), nucleus accumbens, medial preoptic area, and the accessory olfactory bulb. Binding in the forebrain tended to be higher in *S. teguina*, with significant differences in the anterior hypothalamus ($F_{(1,33)} = 17.8$, $P = 0.0002$; Figs. 3B,G,L, 5) and the ventral portion of the bed nucleus of stria terminalis (BNST; $F_{(1,35)} = 17.2$, $P = 0.0002$), and a trend toward higher binding in the medial and lateral BNST ($F_{(1,35)} = 3.5$, $P = 0.07$). Binding in the ventral pallidum was strong in both species, with a trend toward higher receptor density in *S. teguina* ($F_{(1,36)} = 5.2$, $P = 0.03$; Fig. 3A,F,K). A similar trend was observed in the main

TABLE 1. ^{125}I-linear-Vasopressin Specific Binding in Singing Mice (Mean ± SD dpm/mg Tissue Equivalent)

Brain region	*S. teguina*	*S. xerampelinus*	*P*
Main olfactory bulb	1638 ± 1174[(1)]	648 ± 1246	0.04
Accessory olfactory bulb	151 ± 249	68 ± 164	0.29
Ventral pallidum	7459 ± 3685[(1)]	5354 ± 1991	0.03
Lateral septum	6941 ± 2935	5607 ± 1879	0.09
Medial+lateral BNST	1419 ± 565	1027 ± 671	0.07
Ventral BNST	6476 ± 2420[1]	3865 ± 2420	0.0002
Lateral globus pallidus	3984 ± 2450	3011 ± 1430	0.15
Indusium griseum	5926 ± 2437[1]	57 ± 105	< 0.0001
Medial amygdala	4142 ± 2045	3299 ± 2145	0.23
Central amygdala	2325 ± 977	1935 ± 1373	0.36
Anterior hypothalamus	5331 ± 2517[1]	2470 ± 1425	0.0002
Lateral hypothalamus	3314 ± 1465	3583 ± 1812	0.64
Paraventricular hypothalamus	4119 ± 2345	3402 ± 1289	0.2
Supraoptic nucleus	4932 ± 2984	5292 ± 2455	0.8
Supramammillary nucleus	3898 ± 1562	7174 ± 2589[1]	0.0006
Anterior thalamus	38 ± 89	7858 ± 2977[1]	< 0.0001
Central medial thalamus	8562 ± 3889	9356 ± 2355	0.45
Reuniens (Thal)	3534 ± 2192	2327 ± 1670	0.07
Submedius (Thal)	8461 ± 3981[1]	4960 ± 1937	0.002
Zona incerta (Thal)	1854 ± 1061	4051 ± 1519[1]	< 0.0001
Mediodorsal thalamus	974 ± 1648	2182 ± 1696[(1)]	0.03
Laterodorsal thalamus	1188 ± 1928	3987 ± 4071[1]	0.02
Ventral anterior thalamus	2051 ± 3507	7075 ± 2879[1]	< 0.0001
Ventrolateral thalamus	8049 ± 3034	6202 ± 2837	0.06
Ventroposterior thalamus	174 ± 300	2392 ± 2014[1]	0.0001
Dorsal lateral geniculate	269 ± 322	3051 ± 2472[1]	0.0002
Medial geniculate	8231 ± 4868	5866 ± 2727	0.06
Periaqueductal gray	4639 ± 2701[1]	2145 ± 925	0.0003
Total brain[2]	3931	3866	0.9

[1]Significantly higher binding in species indicated.
[(1)]Not significant after correction for multiple comparisons, $\alpha = 0.022$.
[2]Calculated as the mean of specific binding across all structures.

TABLE 2. ^{125}I-Ornithine Vasotocin Specific Binding in Singing Mice (Mean ± SD dpm/mg Tissue Equivalent)

Brain region	*S. teguina*	*S. xerampelinus*	*P*
Prefrontal cortex	510 ± 245[1]	303 ± 119	0.002
Lateral septum	1137 ± 673	1504 ± 542	0.06
Caudate	65 ± 82	81 ± 45	0.37
Nucleus accumbens core	94 ± 106	157 ± 150	0.17
Nucleus accumbens shell	151 ± 384	497 ± 384[1]	0.002
Medial+lateral BNST	1128 ± 598	1372 ± 499	0.19
Medial amygdala	529 ± 296	1344 ± 731[1,2]	< 0.0001
Central amygdala	1200 ± 559	2382 ± 842[1]	< 0.0001
Medial preoptic area	817 ± 403	841 ± 439	0.98
Ventromedial hypothalamus	683 ± 593	830 ± 472	0.37
Dorsal endopiriform nucleus	1666 ± 735	1864 ± 920	0.48
Hippocampus CA1	24 ± 36	2776 ± 1264[1,2]	< 0.0001
Hippocampus CA2	16 ± 23	623 ± 353[1]	< 0.0001
Hippocampus CA3	114 ± 73	1652 ± 1202[1]	< 0.0001
Hippocampus dentate gyrus	681 ± 250	1649 ± 768[1]	< 0.0001
Total brain[3]	627	1189[1]	0.01

[1]Significantly higher binding in species indicated, $\alpha = 0.03$.
[2]Significantly higher binding in males.
[3]Calculated as the mean of specific binding across all structures.

olfactory bulb, albeit with lower binding in both species ($F_{(1,28)} = 4.9$, $P = 0.04$). Binding in the indusium grisium was highly concentrated in *S. teguina* and entirely lacking in *S. xerampelinus* ($F_{(1,36)} = 128.6$, $P < 0.0001$; Fig. 3A,F,K). In the thalamus, the submedius nucleus was the only structure with significantly higher binding in *S. teguina* ($F_{(1,33)} = 11.4$, $P = 0.002$; Fig. 3C,H,M). There were, however, trends toward higher binding in *S. teguina* in the reuniens ($F_{(1,35)} = 3.6$, $P = 0.07$) and ventrolateral ($F_{(1,36)} = 3.7$, $P = 0.06$; Fig. 3C,H,M) nuclei, and in the medial geniculate ($F_{(1,34)} = 3.5$, $P = 0.07$; Figs. 3E,J,O, 5). Binding in the lateral and dorsolateral periaqueductal gray (PAG) was present in both species, but significantly higher in *S. teguina* ($F_{(1,34)} = 16.1$, $P = 0.0003$; Figs. 3E,J,O, 5).

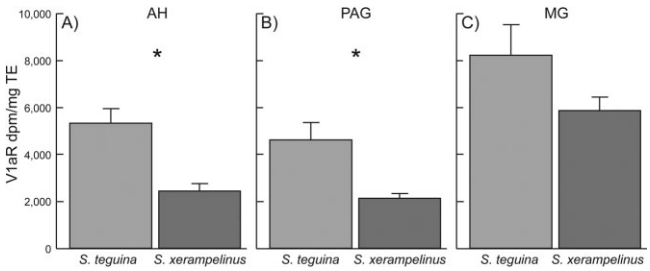
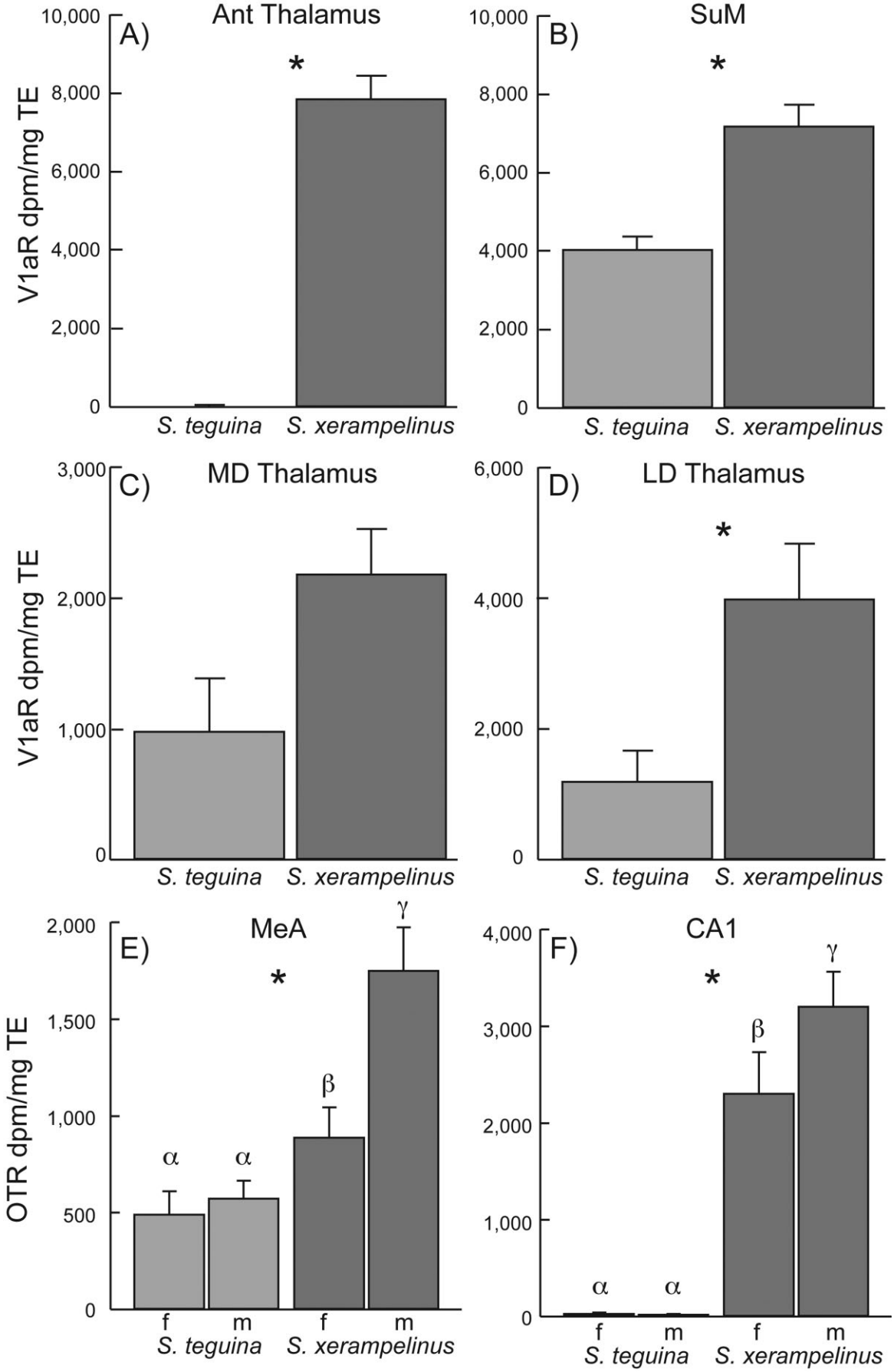


Figure 5.
V1aR binding in singing mice in structures implicated in vocal production and perception. **(A)** Binding in anterior hypothalamus (AH) and **(B)** periaqueductal gray (PAG) was significantly higher in *S. teguina* (light gray), with a trend in the same direction in **(C)** medial geniculate (MG). Asterisks denote significant comparisons; see Table 1 for *P*-values; bars represent standard error; dpm, decompositions per minute; TE, tissue equivalent.

Structures with significantly higher ^{125}I-linear-AVP binding in *S. xerampelinus* were strongly localized to the thalamus (Fig. 3B–E,G–J,L–O). Most strikingly, this species was characterized by highly concentrated AVP binding in the anterior thalamus, a distribution completely lacking in *S. teguina* ($F_{(1,35)} = 95.5$, $P < 0.0001$; Figs. 3B,G,L, 6). Thalamic binding was also stronger in *S. xerampelinus* in the zona incerta (ZI), the ventral anterior (VA) and ventroposterior (VP) nuclei, and in the dorsal lateral geniculate (DLG) (ZI: $F_{(1,34)} = 22.3$, $P < 0.0001$; VA: $F_{(1,35)} = 22.5$, $P < 0.0001$; VP: $F_{(1,37)} = 19.0$, $P = 0.0001$; DLG: $F_{(1,34)} = 17.4$, $P = 0.0002$). Likewise, binding in the laterodorsal (LD) thalamus was significantly higher in *S. xerampelinus* ($F_{(1,37)} = 6.5$, $P = 0.02$), with a trend in the same direction in mediodorsal (MD) thalamus ($F_{(1,37)} = 4.9$, $P = 0.03$; Figs. 3C,H,M, 6). However, binding in these structures was highly variable in both species: 10 *S. teguina* (63%; six females, four males) and one male *S. xerampelinus* (4%) completely lacked binding in the laterodorsal nucleus. Seven *S. teguina* (44%; three females, four males) and one female *S. xerampelinus* (4%) completely lacked binding in the mediodorsal nucleus. Four individuals, all *S. teguina*, lacked binding in both structures. Across species, there was a significant positive relationship between strength of binding in both structures (correlation = 0.62, $Z = 4.4$, $P < 0.0001$). When comparisons were restricted to individuals with binding there was no difference between species (LD: $F_{(1,25)} = 0.5$; $P = 0.5$; MD: $F_{(1,29)} = 1.6$; $P = 0.2$).

The supramammillary nucleus was the only extrathalamic structure in which binding was significantly higher in *S. xerampelinus* ($F_{(1,30)} = 14.7$, $P = 0.006$; Fig. 3D, I, N; Fig. 6). However, ^{125}I-linear-AVP binding was pervasive in the fiber tracts of 18 *S. xerampelinus* (78%; 12 females, 6 males). In these individuals, binding was detected in all regions containing compacted nerve bundles, including the corpus callosum, anterior commissure, fornix, optic tract, and fascicles in the caudate (Figs. 2B, 3K–O). Fiber tract binding was undetectable in all *S. teguina* and in two female and three male *S. xerampelinus*. Species differences, measured in the corpus callosum at the levels of the nucleus accumbens (cc1) and medial geniculate (cc2), were highly significant (cc1: $F_{(1,35)} = 24.1$, $P < 0.0001$; cc2: $F_{(1,30)} = 15.7$, $P = 0.0004$).


A)
Ant Thalamus
B)
SuM
C)
MD Thalamus
D)
LD Thalamus
E)
MeA
F)
CA1
V1aR dpm/mg TE
OTR dpm/mg TE
10,000
8,000
6,000
4,000
2,000
0
3,000
1,000
1,500
500
*
α
β
γ
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m
S. teguina
S. xerampelinus

Figure 6

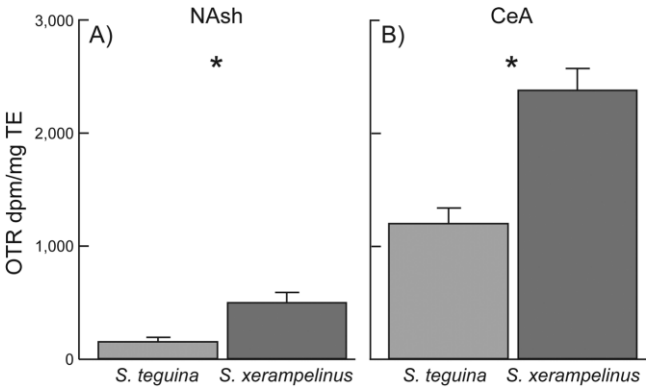

Figure 7.
OTR binding in singing mice in structures implicated in maternal behaviors. **(A)** Binding in nucleus accumbens shell (NAsh) and **(B)** central amygdala (CeA) was significantly higher in *S. xerampelinus* (dark gray). Asterisks denote significant comparisons; see Table 2 for *P*-values; bars represent standard error; dpm, decompositions per minute; TE, tissue equivalent.

OT receptor autoradiography

Total brain ^{125}I-OVT binding was significantly higher in *S. xerampelinus* ($F_{(1,15)} = 8.8$, $P = 0.01$), but both species were characterized by high binding in the medial and lateral compartments of the BNST, the dorsal endopiriform nucleus, and the lateral septum, with a trend toward higher binding in *S. xerampelinus* in the septum ($F_{(1,31)} = 3.8$, $P = 0.06$; Table 2; Fig. 4). Binding was moderate in both species in the medial preoptic area and ventromedial hypothalamus, and low in the caudate and nucleus accumbens core. The prefrontal cortex was the only region in which binding was significantly higher in *S. teguina* ($F_{(1,29)} = 12.2$, $P = 0.002$; Fig. 4A,E).

In *S. xerampelinus*, ^{125}I-OVT binding was significantly higher in the nucleus accumbens shell ($F_{(1,32)} = 10.9$, $P = 0.002$; Figs. 4F, 7), and in the amygdala in both central (CeA) and medial (MeA) nuclei (CeA: $F_{(1,33)} = 24.7$, $P < 0.0001$; MeA: $F_{(1,30)} = 21.3$; $P < 0.0001$; Figs. 4B,C,F,G, 6, 7). In the medial amygdala, an interaction between species and sex ($F_{(1,30)} = 5.2$; $P = 0.03$) was driven by strong sexual dimorphism within *S. xerampelinus*, with significantly higher binding in males (Fisher's PLSD: $P = 0.0007$; Fig. 6). Hippocampal binding in *S. teguina* was minimal or absent in CA1-3 and moderate in the dentate gyrus. Binding was contrastingly high in *S. xerampelinus* throughout the hippocampus, particularly in CA1 (CA1: $F_{(1,33)} = 90.4$; CA2: $F_{(1,34)} = 50.3$; CA3: $F_{(1,34)} = 27.5$; DG: $F_{(1,34)} = 27.2$; all, $P < 0.0001$; Fig. 4D,H). Within *S. xerampelinus*, binding in CA1 was significantly higher in males (Fisher's PLSD: $P = 0.03$; Fig. 6).

Figure 6.
V1aR **(A–D)** and OTR **(E–F)** binding in singing mice in structures implicated in social and spatial memory. Binding in anterior thalamus (Ant Thal), supramammillary nucleus (SuM), laterodorsal thalamus (LD Thal), medial amygdala (MeA), and CA1 was significantly higher in *S. xerampelinus* (dark gray), with a trend in the same direction in mediodorsal thalamus (MD Thal). Note that some individuals completely lacked V1aR binding in MD and/or LD thalamus; species differences in both structures are due to the higher frequency of binding in *S. xerampelinus*. Within *S. xerampelinus*, OTR binding in MeA and CA1 was significantly higher in males. Asterisks denote significant interspecific comparisons; see Tables 1 and 2 for *P*-values; Greek letters in E (MeA) and F (CA1) denote significant sex differences in *S. xerampelinus* (β, γ) but not in *S. teguina* (α, α); bars represent standard error; dpm, decompositions per minute; TE, tissue equivalent; f, females; m, males.

DISCUSSION

Comparison of the neural distributions of V1aR and OTR in two species of Central American rodents, *Scotinomys teguina* and *S. xerampelinus*, revealed a large number of interspecific differences in brain regions implicated in multiple aspects of social behavior, including communication and sensory processing, emotion and memory, and maternal care. Although divergence in V1aR and OTR distributions is common in rodents, several species differences in singing mice were particularly striking. These included higher V1aR binding in *S. teguina* in regions of the forebrain, contrasting with significantly greater concentrations of V1a receptors in *S. xerampelinus* in most thalamic structures, and higher OTR binding in *S. xerampelinus* throughout the brain. We discuss potential roles of AVP and OT in these interconnected circuits in relation to the unique ecologies of singing mice.

Another novel and unexpected finding was the V1aR fiber tract binding present in most *S. xerampelinus* brains. While we do not know whether these receptor populations are functional or effectively neutral, the absence of binding in controls (Fig. 2D), and the fact that some individuals lack fiber tract binding altogether (Fig. 3F–J), demonstrate that this result is not an artifact of nonspecific ligand binding. Although it is not unusual for receptors to be expressed in both neurons and glia (e.g., Nouel et al., 1997; Yu et al., 2008), this type of distribution has never been described for vasopressin receptors. Our current data could be interpreted either as glial expression of V1aR, or as localization of neuronal V1aR in axons. Localizing fiber tract receptor populations at the cellular level will be an important first step to exploring their function.

V1aR and OTR distributions in vocal-acoustic circuitry in singing mice

Regulation of acoustic circuitry is one of the most broadly distributed functions of vasopressin-related neuropeptides; despite multiple independent origins of vocalization in the vertebrate lineage, major nodes in the vocal-motor component of this circuit are homologous across birds, fishes, and mammals (Goodson and Bass, 2001). For example, OTR-like binding is present in several nuclei in the avian song system (Maney et al., 1997), and intraventricular AVT induces song in white-crowned sparrows (Leung et al., 2009). Injection of AVT or IT into the preoptic-anterior hypothalamic region (POA-AH) in the plainfin midshipman, a teleost fish, influences vocal production in a sex-specific manner (Goodson and Bass, 2000), while injection of oxytocin into the medial POA-AH induces mating-related vocalizations in female hamsters (Floody et al., 1998). Likewise, electrical stimulation of either the PAG or POA-AH evokes vocalizations in both fish and primates (Jürgens, 1994; Goodson and Bass, 2002). While the putative roles of vasopressin and oxytocin in mammalian auditory processing are undefined, work in teleost fish suggests that ancestral forms of these neuropeptides participate in the

integration of auditory input in midbrain and thalamic nuclei that are homologous to higher-order auditory nuclei in mammals (Goodson and Bass, 2002).

Given this evidence for the convergent recruitment of vasopressinergic neuropeptides to vocal-acoustic circuitry, we were interested in defining vasopressin 1a and oxytocin receptor expression patterns in singing mice. Although both species share a highly derived form of vocal communication, *S. teguina* has longer songs and sings more often (Miller and Engstrom, 2007; P. Campbell and S.M. Phelps, unpubl.). Surprisingly, neither species exhibited detectable V1aR binding in the medial preoptic area (MPOA), and OTR binding in this region was moderate in both. However, we found concentrated V1aR expression in two reciprocally connected structures implicated in vocal production, PAG and AH. In both structures, receptor density was significantly higher in *S. teguina*. Similarly, both species had strong expression in the auditory thalamus (medial geniculate nucleus), with a trend toward higher binding in *S. teguina* (Fig. 5).

In mammals, vocalization is one of an array of behavioral responses associated with PAG (e.g., lordosis in rodents and defensive rage in cats; reviewed in Behbehani, 1995; Jürgens, 2002). Likewise, AH is integral to male partner preference in ferrets (Paredes and Baum, 1995) and the action of AVP in AH is not exclusive to vocal production (e.g., Ferris et al., 1997; Albers et al., 2006). We also note that high V1aR binding in the medial geniculate is found in prairie voles (Insel et al., 1994; Wang et al., 1997), a species in which adult vocal communication is undocumented. Nevertheless, higher V1a receptor density in *S. teguina* in both vocal and auditory structures is noteworthy in light of species differences in vocal behavior. Although we think it unlikely that the action of AVP in AH and PAG is exclusive to vocal modulation in singing mice, strong receptor binding in both species and higher binding in the more vocal species suggest that this neuropeptide plays a functional role in the regulation of species-specific vocal behavior.

Recent documentation of adult vocal behavior in other genera in the family Sigmodontinae, including several species of *Peromyscus* (Wright and Brown, 2004; Kalcounis-Rueppell et al., 2006; Miller and Engstrom, 2007), invites more comprehensive comparative analysis of the relation between vocal communication and vasopressin and oxytocin receptor distributions in sigmodontine mice. Earlier studies of receptor binding in *Peromyscus maniculatus* and *P. californicus* focused on species differences in social mating system (Insel et al., 1991), paternal behavior and aggression (Bester-Meredith et al., 1999), and did not examine regions relevant to vocalization.

In the more distantly related laboratory mouse (*Mus*), V1aR is expressed in both AH and PAG, albeit at moderate levels (Dubois-Dauphin et al., 1996). It remains to be determined whether AVP in these regions is involved in the vocal behavior of adult males (e.g., Holy and Guo, 2005). Likewise, the finding that oxytocin knockout mouse pups vocalize less than wild-type controls when separated from their dam has been interpreted as a byproduct of reduced sensitivity to social isolation (Winslow et al., 2000; Winslow and Insel, 2002). It is possible, however, that neural oxytocin in vocalization-related regions such as MPOA plays a more specific role in the modulation of vocal production in *Mus*.

V1aR and OTR distributions in sociospatial circuitry in singing mice

The most striking species differences in receptor distributions were localized to spatial memory circuits; these patterns were paralleled by significant differences in receptor densities in circuitry critical to social recognition. Across these circuits, receptor binding was higher in *S. xerampelinus* (Fig. 6). Most notably, OTR binding in the hippocampus was minimal or lacking in *S. teguina* and contrastingly high in *S. xerampelinus* and V1aR binding in the anterior thalamus was present only in *S. xerampelinus*. Both brain regions are integral to the acquisition and consolidation of spatial memory and are reciprocally connected (Swanson and Cowan, 1977; Sikes and Vogt, 1987; Aggleton and Brown, 1999). V1aR binding was also significantly higher in *S. xerampelinus* in the supramammillary nucleus, which projects to the hippocampus and is directly involved in spatial working memory (Vertes and McKenna, 2000; Aranda et al., 2008). Although V1aR in laterodorsal and mediodorsal thalamus was variable in both species, binding in these regions was observed more frequently in *S. xerampelinus*. While LD and MD thalamus are implicated in a range of limbic functions, lesions to the LD nucleus cause spatial memory deficits in rats (van Groen et al., 2002) and damage to either nucleus contributes to amnesia in humans (Edelstyn et al., 2006; Cipolotti et al., 2008). Finally, although both species were characterized by strong OTR expression in the medial amygdala, binding was significantly higher in *S. xerampelinus.* While the MeA receives major input from the accessory olfactory bulb, it is also reciprocally connected with CA1 field in the hippocampus; it has been proposed that this bidirectional connection influences emotional learning (Petrovich et al., 2001; Kishi et al., 2006). Intriguingly, sex differences in OTR binding were detected in *S. xerampelinus* in both MeA and CA1 (Fig. 6), a pattern suggestive of a common oxytocin-modulated function.

Data from other rodent species support mnemonic functions for oxytocin and vasopressin in many of the above brain regions. In laboratory mice, OTR is highly expressed in both MeA and hippocampus (CA3 field; Ferguson et al., 2000): oxytocin knockout males exhibit normal spatial memory but fail to recognize familiar individuals and oxytocin injected into MeA restores social memory (Ferguson et al., 2001). Social recognition is similarly impaired in females with short-term silencing of MeA OT receptors (Choleris et al., 2007). Interestingly, targeted knockout of hippocampal OTR also impairs social recognition (Lee et al., 2008). Primiparous rats and mice exhibit enhanced hippocampus-dependent spatial memory (reviewed in Kinsley and Lambert, 2008), and experimental evidence demonstrates that oxytocin is critically involved in this phenomenon (Tomizawa et al., 2003). In male rats, injection of anti-oxytocin serum into ventral hippocampus inhibits social memory (van Wimersma Greidanus and Maigret, 1996) and AVP administration in dorsal hippocampus enhances spatial memory consolidation (Paban et al., 2003). Finally, variable V1aR expression in LD thalamus in male prairie voles has led to the suggestion that vasopressin in this structure modulates spatial representation of antagonistic social encounters (Ophir et al., 2008).

Social and spatial memory are typically studied in discrete behavioral paradigms. However, the neuroanatomical and experimental data summarized above, together with the results

of the present study, suggest that vasopressin and oxytocin facilitate the synthesis of these two types of memory. From a functional perspective, the ability to embed social memory in a spatial context is likely to be favored by selection under a range of social and ecological conditions. Lower population densities, greater conspecific spacing, and longer parental care in *S. xerampelinus* relative to *S. teguina* indicate that spatial orientation in relation to resources, nest sites, mates, and neighbors may be particularly important in this species. Higher V1aR and OTR expression in relevant mnemonic circuits in *S. xerampelinus* suggests a neural mechanism for enhanced sociospatial memory.

OTR in the maternal brain

Central oxytocin is a key modulator of mammalian maternal behaviors (Pedersen, 1997; Meaney, 2001). Because lower ambient temperatures and slower pup development at higher altitudes presumably require greater maternal investment from *S. xerampelinus* females, we looked for species differences in OTR expression in brain regions subserving maternal behavior. We found higher OTR binding in *S. xerampelinus* in two such structures: the central amygdala and the shell of the nucleus accumbens (Fig. 7). In rats, maternal responsiveness in virgin females and maternal aggression toward intruders are positively correlated with CeA OTR binding and OT levels, respectively (Champagne et al., 2001; Bosch et al., 2005). Likewise, individual variation in spontaneous maternal behavior in naive prairie voles is strongly associated with OTR density in the Nacc shell, and OT antagonist injected into this region disrupts maternal behavior (Olazábal and Young, 2006a,b). We speculate that ecological selection has shaped the neural substrates of maternal investment in *S. xerampelinus*, favoring greater responsiveness to pups and aggression toward potential predators. We note, however, that species differences were not observed in the lateral septum, BNST, and medial preoptic area, all of which are implicated in oxytocin-mediated maternal behaviors in other rodent species (Pedersen et al., 1994; Champagne et al., 2001; Olazábal and Young, 2006b).

Conclusions and perspectives

The comparative data presented here contribute to a recognized need to understand the neural correlates of social behavior from an evolutionary and ecological perspective (Goodson, 2005; Pollen et al., 2007). Until recently, comparative studies of V1aR and OTR in rodents focused mainly on the relation between receptor distributions and social mating system (Insel and Shapiro, 1992; Insel et al., 1994; Wang et al., 1997; but see Beery et al., 2008). However, as more species comparisons are added, consistent correlations between expression patterns and mating system break down, revealing that receptor distributions are not a blueprint for social structure, but rather a sample from a potentially large variety of neural phenotypes subserving species-typical social behaviors. For example, although elevated V1aR expression in the ventral pallidum is strongly associated with social monogamy in voles (Insel et al., 1994), significant pallidal V1aR is also found in nonmonogamous rodents, including both species of singing mice, a solitary species of tucu-tuco (Beery et al., 2008), and the polygynous laboratory mouse (Dubois-Dauphin et al., 1996). Although the V1aR-mediated action of AVP in the ventral pallidum is essential to pair bond formation in male prairie voles (Lim and Young, 2004), it may regulate social affect in ways that serve different functions in other taxa. Similarly, the role of the lateral septum in prairie vole pair-bonding can be contrasted with the broad conservation of aggression-related functions of septal AVP/AVT in both mammals and birds (Wang et al., 1994; Everts et al., 1997; Goodson, 1998; Goodson and Adkins-Regan, 1999; Bester-Meredith et al., 1999; Beiderbeck et al., 2007). These patterns are concordant with the concept of a vertebrate "social behavior network" in which general conservation of function is fine-tuned in the context of species-specific selective pressures (Newman, 1999; Goodson, 2005).

Based on the unique vocal behavior of *S. teguina* and *S. xerampelinus*, and species differences in ecology, we propose that V1aR and OTR distributions in singing mice support an integral role for the AVP/OT system in three aspects of sociality: vocal communication, sociospatial memory, and, to a lesser degree, maternal care and aggression. While testing these hypotheses awaits experimental manipulation, the emergent patterns of conservation and diversity for V1aR and OTR neural phenotypes in singing mice contribute to a broader view of the neural substrates of rodent sociality.

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