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Beating the boojum: Comparative approaches to the neurobiology of social behavior

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ABSTRACT

Neuropeptides coordinate complex social behaviors important to both basic and applied science. Understanding such phenomena requires supplementing the powerful tools of behavioral neuroscience with less conventional model species and more rigorous evolutionary analyses. We review studies that use comparative methods to examine the roles of vasopressin and oxytocin in mammalian social behavior. We find that oxytocin and vasopressin receptor distributions are remarkably variable within species. Studies of socially monogamous prairie voles reveal that pronounced individual differences in spatial memory structures (retrosplenial cortex and hippocampus) are better predictors of social and sexual fidelity than are areas known to regulate pairbonding directly, a pattern that seems to be mediated by the contributions of the neuropeptides to space use in natural settings. We next examine studies of individual and species differences in *cis*-regulatory regions of the *avpr1a* locus. While individual differences in social behaviors are linked to length of a microsatellite at the *avpr1a* locus, phylogenetic analyses reveal that the presence or absence of a microsatellite does not explain major differences between species. There seems to be no simple relationship between microsatellite length and behavior, but rather microsatellite length may be a marker for more subtle sequence differences between individuals. Lastly, we introduce the singing mouse, *Scotinomys teguina*, whose neuropeptide receptor distributions and unique natural history make it an exciting new model for mammalian vocalization and social cognition. The findings demonstrate how taxonomic and conceptual diversity provide a broader basis for understanding social behavior and its dysfunction.

1. Introduction

Neuropeptides and their targets coordinate phenotypes of tremendous conceptual and clinical importance (e.g. Insel, 1997; Kirsch et al., 2005; Meaney, 2001). Among such phenotypes, few are as interesting, complex and challenging as social behavior. Social behavior relies not only on interactions of elaborate neuronal circuits within a brain, but also on interactions between brains within a social group, and on the interaction of the group with its surroundings. Our understanding of social behavior reflects our broader ability to explain biological complexity using the core principles of gene function, heredity and evolution. In addition to its theoretical importance, social cognition is a major domain of dysfunction in autism, schizophrenia, and other disorders (American Psychiatric Association, 2000). In this review, we survey the role of two neuropeptide receptors, the vasopressin 1a (V1aR) and oxytocin (OTR) receptors in the regulation of social behavior among novel model species. Because the clinical relevance of these neuropeptides has been well reviewed (Bartz and Hollander, 2006; Carter, 2007; Insel, 1997; Swaab et al., 2005), we focus instead on a more neglected topic: the role of comparative biology in behavioral and molecular neuroscience. We argue that evolutionary and

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ecological approaches provide unique insights into the nature and consequences of natural variation in brain and behavior; we use studies of neuropeptides and social behavior to advance this thesis.

The father of behavioral endocrinology, Frank Beach, famously invoked Lewis Carroll's "Hunting of the Snark" as a cautionary tale (Beach, 1950; Carroll, 1898). In the poem, a ragtag hunting party set off in search of a snark, a mythical animal that assumed many forms, one of which was a boojum. While the snark was worthy quarry (good with greens, apparently), the hunter who captured a boojum was doomed to slowly disappear. Beach argued that by focusing our hunt for understanding on just one species, the laboratory rat, we were becoming a field too narrow to be relevant. In the words of Beach and Carroll, "the snark was a boojum." Contemporary studies rely ever more heavily on *Mus musculus* as a model species; given the tremendous success of *Mus* genetics and its impact on neuroscience, the need for taxonomic breadth may seem less compelling. On the contrary, we suggest there is a need not only for greater taxonomic diversity, but for a broader conceptual framework to make effective use of such diversity. That broader framework is supplied by evolutionary biology. We refer to this combination of taxonomic breadth and evolutionary perspective as the comparative approach.

There are at least three major benefits to supplementing biomedical neuroscience with comparative approaches. First, while major model species are well suited to laboratory housing and experimental manipulation, no single species can provide appropriate counterparts to all aspects of the human phenotype. Difficulties with social attachment, for example, characterize many autism spectrum disorders (American Psychiatric Association, 2000); because lab mice lack social attachment altogether, they are poor models for this aspect of the disorder. To find appropriate alternatives we must broaden the range of taxa examined. For example, prairie voles are small North American rodents that form long-term pairbonds (Getz et al., 1981) – because neurobiological and molecular techniques readily generalize from lab rodents to this less conventional species, the prairie vole has become a major model for social attachment (Young and Wang, 2004). In fact, the muroid rodents comprise roughly one third of all mammalian diversity, a group in which prairie voles and laboratory rodents are just three of >1300 species (Jansa and Weksler, 2004; Steppan et al., 2004). This group represents a tremendous range of potential models that remain under-utilized. A second reason to include comparative approaches in biomedical research is that when we focus on a small number of distantly related species, it becomes very difficult to extrapolate from one species to another. To generalize across species requires a more systematic sampling of taxa, and an explicit evolutionary framework for generalization (Felsenstein, 1985). The third motivation for using evolutionary approaches and non-traditional models is that they can illuminate the nature and origin of within-species variation. Model systems have often been bred to minimize variation in study subjects. While this may be useful in maximizing statistical power, it is worth noting that genetic variation is not a form of experimental error. If our understanding of a phenotype does not generalize across genetic backgrounds within a species, it seems even less likely to generalize to other species. Moreover, human disorders are forms of intra-specific variation. To understand dysfunction, we need to understand the nature, extent and origins of natural variation. Evolutionary and ecological perspectives are necessary for a complete understanding of natural diversity, and provide useful complements to the study of behavioral and molecular neuroscience in more traditional model species.

We now use a comparative perspective to explore variation in mammalian social behavior and the neuropeptides that regulate it. Specifically, we survey intra-specific variation in oxytocin and vasopressin systems and their consequences for behavior in natural settings; individual and species differences in the *cis*-regulatory sequences of the *avpr1a* locus. In closing, we introduce promising new models for studying the role of neuropeptides in social communication.

2. Surprises from the field: neuronal correlates of social attachment and sexual fidelity

Prairie voles are small rodents whose distribution range extends from the Midwestern United States, north through central Canada. Males and females form lifelong pairbonds, share a nest, defend a territory and raise young together (Getz et al., 1981, 1993). Although such "social monogamy" is common, most adults will live as single animals at some point in their lives (Getz et al., 1993). Indeed, up to 40% of males may opt for an un-paired non-territorial "wandering" phenotype (Getz et al., 1993). Single males and females are able to reproduce, but experiments in semi-natural enclosures suggest that single animals have significantly lower reproductive rates than paired animals (Ophir et al., 2008b). Although most animals pair, approximately 25% of young are sired by a mate outside of a pairbond (termed "extra-pair fertilizations"). These ecological data emphasize that sexual fidelity and social attachment are dissociable, and that within this generally monogamous species, there exists substantial variation in the extent to which animals exhibit either social or sexual fidelity. Such individual differences offer a rich opportunity to explore the basis for variation in social behavior.

To more fully characterize the ecological patterns of mating and social interactions among free-living prairie voles, we placed 6 sexually naïve males and 6 sexually naïve females into outdoor enclosures at natural densities. Because subjects were lab-reared but recently derived from wild-caught animals, we were able to minimize variation in age, weight and experience while preserving genetic diversity. We used radiotelemetry to localize all subjects 2 times per day. The experiment was repeated for a total of 8 replicate enclosures. We used location data to delineate the homerange of each subject, the extent of interactions between each subject and his or her neighbors (based on overlap of homeranges), and male–female pairbonding (based on shared exclusive space use between a male and female; for details see Ophir et al., 2008b,c). As reported elsewhere, single animals had larger homeranges and intruded on the homeranges of more neighbors (Fig. 1, also see Getz et al., 1981; Ophir et al., 2008c; Solomon and Jacquot, 2002). Paired animals, in contrast, had smaller, more exclusive homeranges (Fig. 1). Remarkably, patterns of space use within a paired or single "tactic" predicted whether animals would be successful or unsuccessful, and faithful or unfaithful (Ophir et al., 2008c). Paired males sired young when they maintained a small homerange and excluded intruding males. Single males, in contrast, were most successful when they roamed broadly. The vast majority of our females were both successful and paired, leaving few females who were single or unsuccessful and limiting the inferences we can draw. Nevertheless, the overall pattern of space use, pairing status and reproductive success was remarkably similar between males and females (Fig. 1). Males and females who mated outside a pair were also more likely to intrude on the homeranges of their neighbors. Thus sexual fidelity and space use co-vary, presumably because a cheating mate needs to encroach on neighboring territories to encounter extra-pair partners (Phelps and Ophir, in press).

The mechanisms of prairie vole pairbond formation have been well studied in the laboratory. Early insights came from examining species differences in the distribution of neuropeptide receptors (Insel et al., 1994). Prairie voles and their monogamous congeners, pine voles (*Microtus pinetorum*), both exhibit high

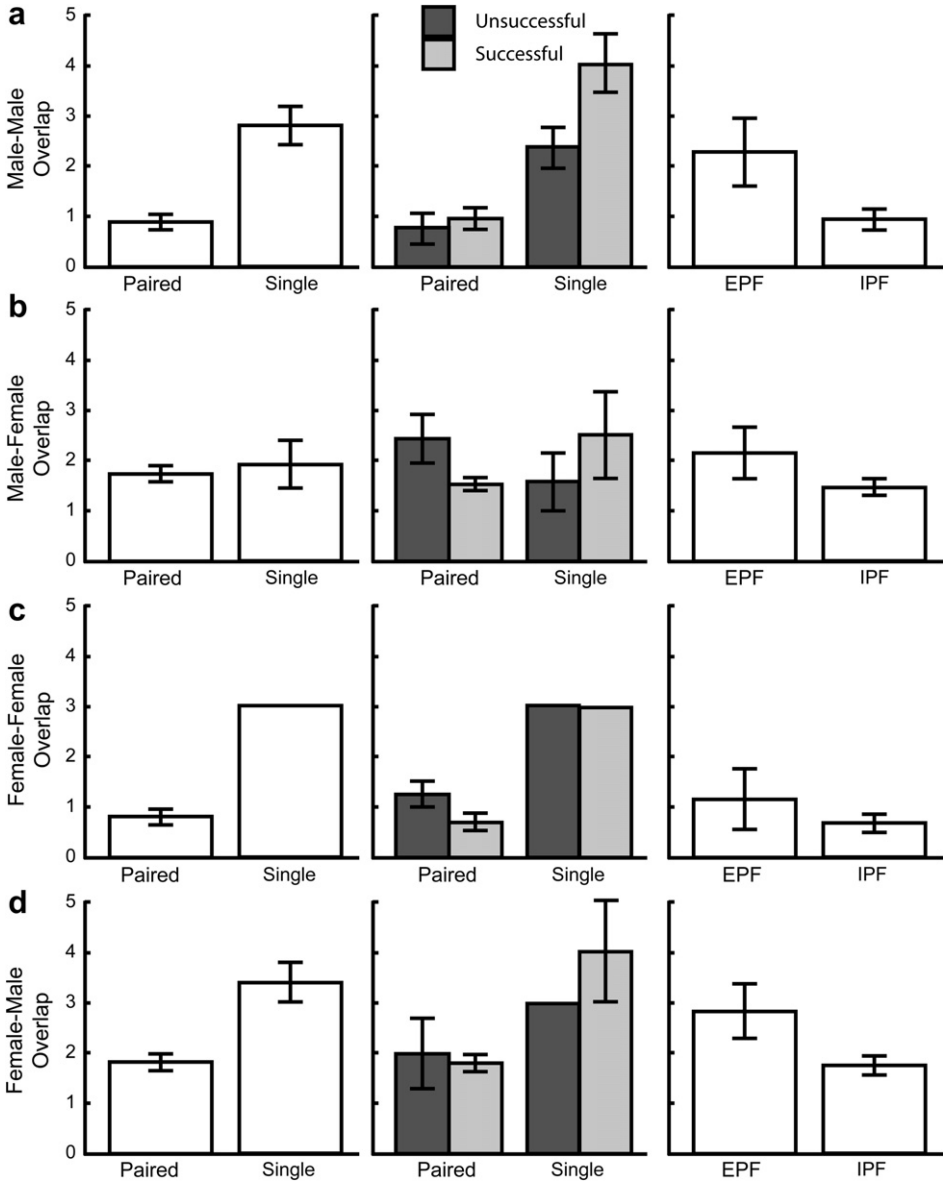

Fig. 1. Space use patterns in male (a,b) and female (c,d) prairie voles. a) Number of male homeranges overlapped by focal males: left, paired and single males ($P < 0.001$); middle, paired and single males split by mating success (defined as having sired offspring, success X pairing status interaction, $P = 0.04$), successful single males overlapped more males than either unsuccessful single males ($P = 0.002$) or successful paired males ($P < 0.001$); right, successful males split into those who mated faithfully (IPF) and those who did not (EPF; $P = 0.02$). Data from Ophir et al. (2008c). b) Number of female homeranges overlapped by focal males: left, paired and single males ($P > 0.05$); middle, pairing status X mating success interaction ($P = 0.04$), posthoc tests revealed successful single males overlapped more females than did successful paired males ($P = 0.02$), and successful paired males overlapped significantly fewer females than did unsuccessful paired males ($P = 0.02$); right, IPF and EPF males ($P = 0.05$). Data from Ophir et al. (2008c). c) Number of female homeranges overlapped by focal females: left, paired and single females ($P < 0.001$); middle, pairing status split by mating success; right, IPF and EPF females ($P > 0.05$). d) Number of male homeranges overlapped by focal females: left, paired and single females ($P = 0.001$); middle, paired and single females split by mating success; right, IPF and EPF females ($P = 0.025$). For female data, there were too few single females ($n = 5$, vs $n = 28$ paired females) to test for interactions between pairing status and mating success, but data are nevertheless broken down for comparison with male patterns.

levels of receptors for oxytocin and vasopressin in brain regions associated with reward, though their promiscuous congeners do not (Insel and Shapiro, 1992; Insel et al., 1994; Smeltzer et al., 2006). A combination of pharmacological and genetic manipulations reveal that oxytocin receptors in the nucleus accumbens and prefrontal cortex are both necessary for female prairie voles to form pairbonds (Liu et al., 2001; Ross et al., 2009; Young et al., 2001). Similarly, vasopressin receptors in the ventral pallidum and lateral septum are needed for male prairie voles to form pairbonds (Lim and Young, 2004; Liu et al., 2001; Wang et al., 1994). More dramatically, over-expression of the V1aR in the ventral pallidum of the normally promiscuous meadow vole promotes the formation of the specific social preferences that characterize pairbonding (Lim et al., 2004). These data established that oxytocin and vasopressin are important regulators of attachment. Given such findings, we asked how natural variation in receptor expression contributes to the substantial diversity of sexual and social fidelity exhibited by prairie voles in the field.

Over a series of studies, we examined the brains of un-manipulated, genetically diverse individuals to assess how much natural variation existed in the expression of neuropeptide receptors. For both V1aR (Phelps and Young, 2003) and OTR (Ophir et al., in press; Phelps, unpublished) we find remarkable variation in distribution and abundance – some structures are relatively stable in their

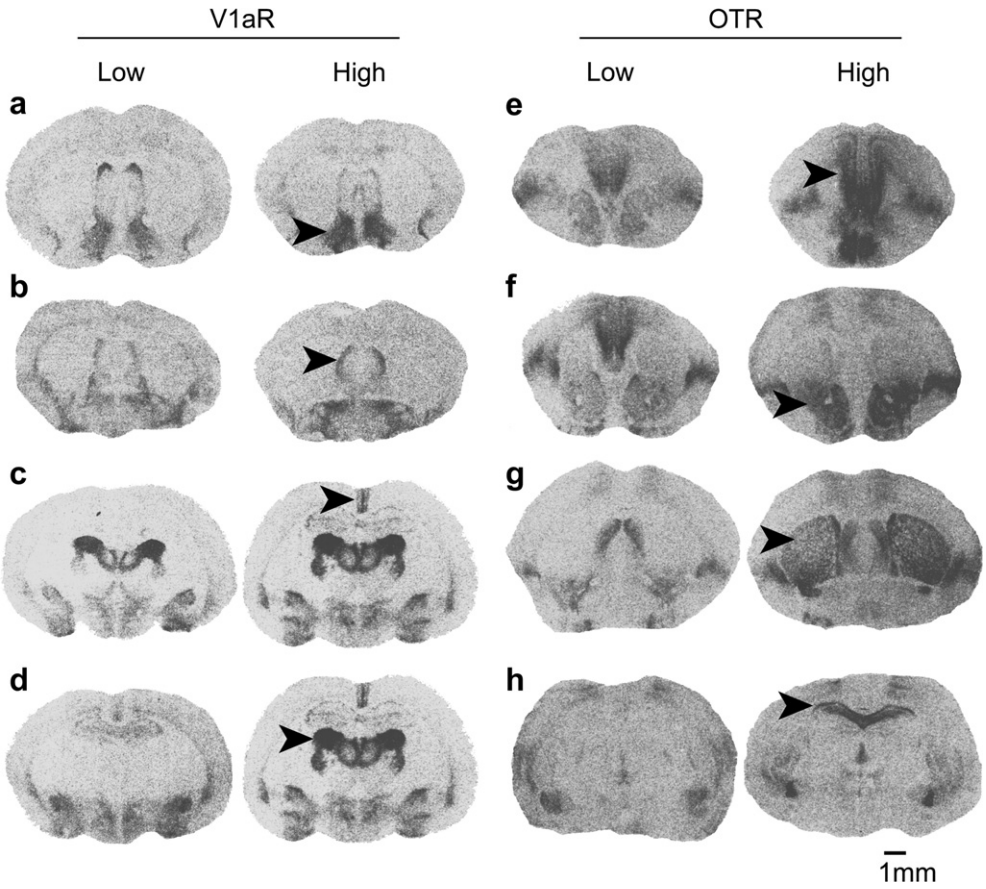

Fig. 2. Natural variation in neuropeptide receptor expression among prairie voles. Representative autoradiograms proceed rostral to caudal (V1aR, a–d; OTR, e–h). "High" and "Low" sections represent samples in which the indicated structure exhibited binding in the upper or lower quartile respectively. a) V1aR in ventral pallidum. b) V1aR in lateral septum. c) V1aR in retrosplenial cortex. d) V1aR in dorsal thalamus. e) OTR in prefrontal cortex. f) OTR in nucleus accumbens. g) OTR in caudate putamen. h) OTR in hippocampus.

expression, others vary widely across individuals. While V1aR in the ventral pallidum is consistently high, for example, individual differences in the retrosplenial cortex are pronounced (Fig. 2; Insel et al., 1994; Phelps and Young, 2003). This is particularly remarkable because the retrosplenial cortex is part of a spatial memory circuit that includes extensive connections with the hippocampus (Cooper et al., 2001; Harker and Whishaw, 2004; Maguire, 2001), and space use has proved to be an important attribute of social and sexual behavior in the field. In the case of OTR, brain regions involved in both pairbonding (nucleus accumbens, prefrontal cortex) and spatial memory (hippocampus) are highly variable (Ophir et al., in press). Do individual differences in these brain regions cause any of the variation we find in social attachment, space use or sexual fidelity? To test this hypothesis, we examined the brains of individuals from the field study described above.

We first asked whether paired and single males differed in terms of the density of V1aR expressed in either the ventral pallidum or lateral septum, two areas causally related to male pairbond formation. Contrary to our predictions, paired and single males did not differ in the abundance of V1aR in either structure (Fig. 3; Ophir et al., 2008c). We next focused on just those males who sired young, and compared the brains of males that sired young exclusively with their partner (intra-pair fertilizations, or IPF) to the brains of those that sired one or more young with a non-partner (extra-pair fertilizations, or EPF). Again to our surprise, we found that neither structure was associated with variation in sexual fidelity. To examine substrates of female pairing, we quantified OTR in the nucleus accumbens and prefrontal cortex (Fig. 3). As in males, we found that neither structure was associated with differences in either social attachment (pairing) or sexual fidelity (IPF/EPF). Overall these data demonstrate that receptor differences in classic pairbonding centers are not sufficient to explain natural variation in social or sexual fidelity. Although we suspect that individual differences in pairing might be mediated by experience-dependent peptide release, the contribution of other neuromodulatory systems is also plausible.

Although the mechanisms of pairbonding were clear candidates for mediating sexual and social fidelity in the field, correlations between space use, neighbor interactions and sexual fidelity suggest that spatial memory circuits may also be important for patterns of social and sexual behavior. We focused on V1aR abundance in the retrosplenial cortex of males and OTR abundance in the hippocampus of females, two highly variable expression domains. Perhaps not surprisingly, we find that neither retrosplenial V1aR (Ophir et al., 2008c) nor hippocampal OTR predict whether animals will pair or remain single (Fig. 4). They do, however, predict whether paired and single animals will be successful. Successful single males lack V1aR in retrosplenial cortex (Fig. 4) despite having normal levels of V1aR in the ventral pallidum and lateral septum (Fig. 3; Ophir et al., 2008c). Successfully paired males, in contrast, have normal levels of cortical V1aR. Similarly, successful paired females have high levels of OTR in the hippocampus, while successful single females do not (Fig. 4). Lastly, receptor expression in spatial memory circuits was highly predictive of sexual fidelity. Faithful males (IPF) have much higher levels of retrosplenial V1aR than unfaithful males (EPF), while faithful

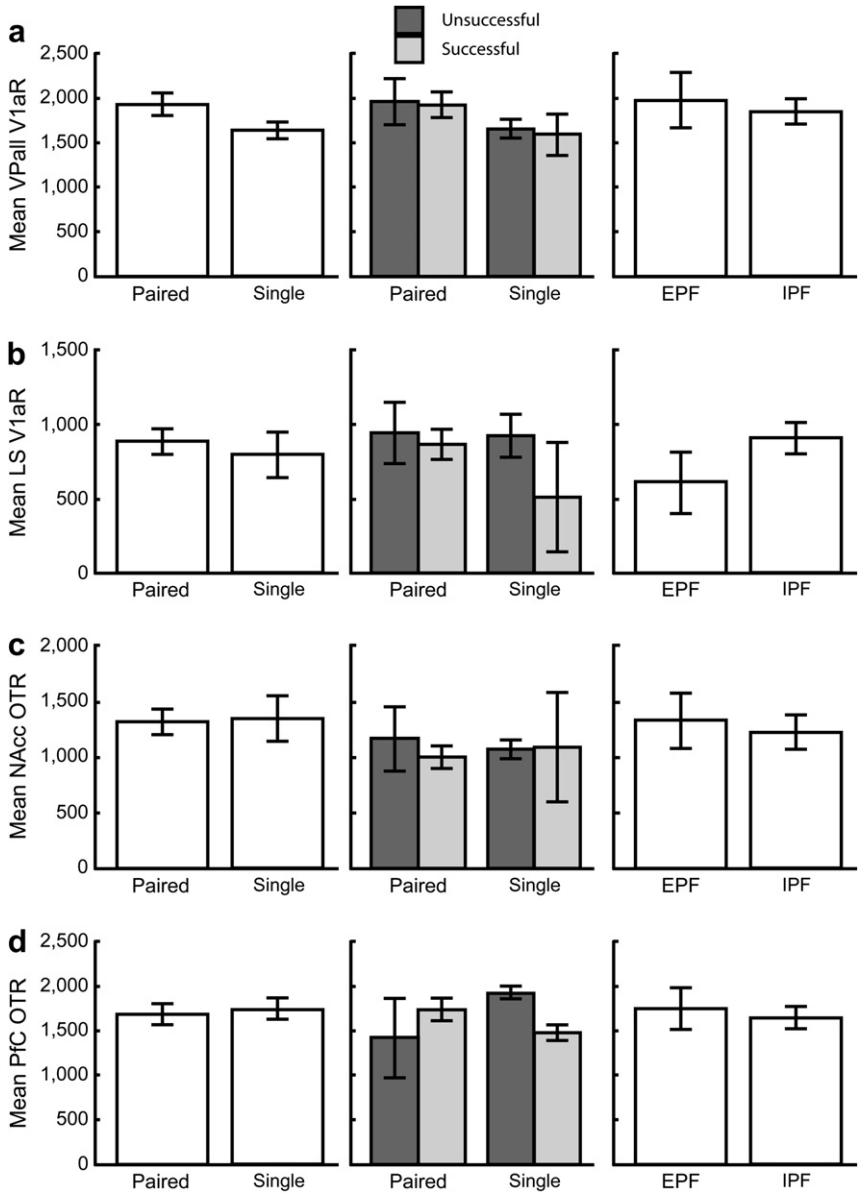

Fig. 3. V1aR and OTR binding in "pairbonding" regions of male (a,b) and female (c,d) prairie voles. a) V1aR in the ventral pallidum (VPall) of males: left, paired and single males; middle, paired and single males split by mating success; right, males who mated faithfully (IPF) and those who did not (EPF). b) V1aR in the lateral septum (LS) of males: left, paired and single; middle, pairing status X mating success; right, IPF and EPF males. c) OTR in the nucleus accumbens (NAcc) of females: left, paired and single females; middle, paired and single females split by mating success; right, IPF and EPF females. d) OTR in the prefrontal cortex (PfC) of females: left, paired and single females; middle, females split by pairing status and mating success; right, IPF and EPF females. All comparisons were non-significant ($P > 0.05$). Data in panels a and b from Ophir et al. (2008c).

females seem to have higher levels of hippocampal OTR than do unfaithful females. Overall we find that both sexes show a remarkable congruity in the relationship between receptor abundance in a spatial memory circuit and individual differences in space use and sexual fidelity.

Although neither OTR nor V1aR predicted whether animals would form pairs, low receptor abundance in spatial memory circuits was consistently associated with high neighbor overlap and low sexual fidelity. What explains the persistence of this neuronal and behavioral variation? In a more detailed analysis of V1aR, we found that paired males with high retrosplenial V1aR were not only more likely to sire young, they were also better at excluding other males from their territory and less likely to be cuckolded (Phelps and Ophir, in press). Conversely, males with low retrosplenial V1aR intruded on more territories and were more likely to obtain extra-pair fertilizations. While the increase in EPFs was a positive fitness gain for single males, in paired males it was accompanied by an increased probability of being cuckolded (Phelps and Ophir, in press). We suggest that high levels of V1aR in spatial memory circuits increases memory for cues associated with male–male interactions. Among paired males this translates into better mate guarding and higher sexual fidelity. Among single, non-territorial males, this may translate into actively avoiding sites of agonistic interactions. While such avoidance would seem sensible, it would also translate into fewer encounters with paired females and thus fewer chances for extra-pair fertilization. Indeed, recent theory on the evolution of mating systems demonstrates that female encounter rate is a major factor in predicting whether males are more likely to succeed as single, non-territorial males or as territorial mate-guarding residents (Kokko, 1999; Kokko and Morrell,

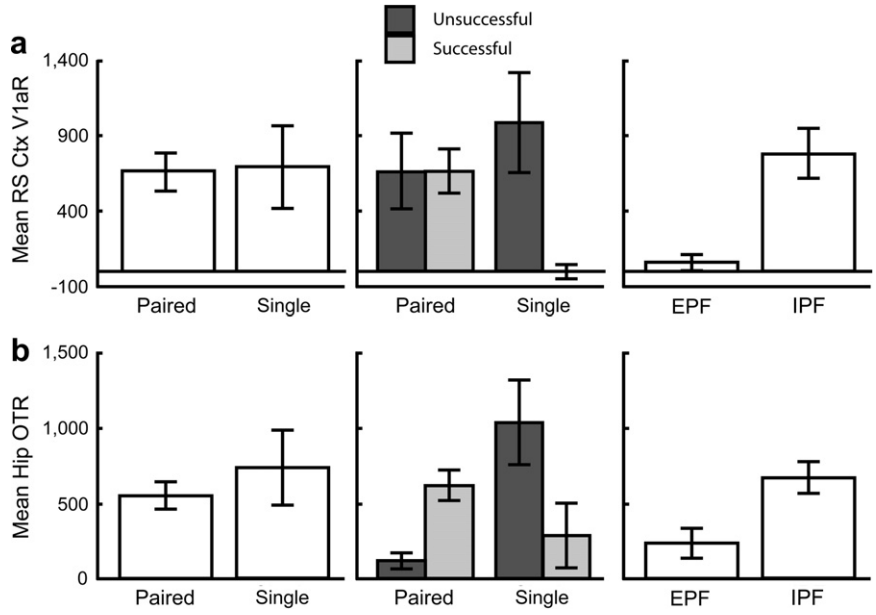

Fig. 4. V1aR and OTR binding in spatial memory regions of male and female prairie voles. a) V1aR in the retrosplenial cortex (RS Ctx) of males: left, paired and single males ($P > 0.05$); middle, paired and single males split by mating success (interaction, $P = 0.02$), successful single males had significantly lower retrosplenial V1aR binding than unsuccessful single males ($P = 0.01$); right, males who mated faithfully (IPF) and those who did not ($P = 0.01$). Data from Ophir et al. (2008c). b) OTR in the hippocampus (Hip) of females: left, paired and single females ($P > 0.05$); middle, paired and single females split by mating success; right, IPF and EPF females ($P = 0.06$). There were too few single females ($n = 5$, vs $n = 28$ for paired females) to perform statistics on animals split by mating success, but data are shown for comparison with male patterns.

2005; Sandell and Liberg, 1992). Overall the data strongly suggest that heterogenous selection on neuronal phenotypes permits the maintenance of variation in cortical V1aR.

Although our analysis of OTR in the hippocampus suffers from smaller numbers of single and unsuccessful females, the similarities in the data between sexes and neuropeptide systems are striking. It is well known that paired male prairie voles become aggressive toward novel anestrous females (Aragona et al., 2006; Getz et al., 1981; Winslow et al., 1993). Perhaps females with high hippocampal OTR exhibit higher site fidelity as a means to avoid aggressive neighbors, but this may be counterproductive when territory intrusions are needed to encounter a prospective mate. In both neuropeptide systems, variation in spatial memory circuits seems to persist because utility of receptor expression depends on the pairing status of males and females.

The analysis of OTR and V1aR among highly outbred populations revealed a surprising degree of neuronal variation, particularly in circuits related to spatial memory (Insel et al., 1994; Ophir et al., in press; Phelps and Young, 2003). By examining behavior in natural environments, we gained insights not only into the functions of such diversity, but into the evolutionary mechanisms of its persistence. Such studies illustrate how parallel investigations using traditional and non-traditional approaches can provide a more complete view of neurobiology and behavior. We now move down a level of analysis by focusing on individual and species differences in the regulation of the *avpr1a* locus, and its consequences for neuronal and behavioral phenotypes.

3. Comparative insights: individual and species differences in *avpr1a*

We have reviewed some of the profound differences in the distribution of neuropeptide receptors within and between species. Monogamous prairie and pine voles (*Microtus ochrogaster* and *M. pinetorum*) differ from promiscuous congeners in their ability to form pairbonds, and this is attributable to differences in the neuronal distribution of V1aR (Insel et al., 1994; Lim et al., 2004; Pitkow et al., 2001; Winslow et al., 1993; Young et al., 1999). The few amino-acid differences between receptors of these species do not seem to influence binding characteristics, and so it is clear that species differences in V1aR function reflect the regulation of the *avpr1a* locus (Insel et al., 1994). To investigate potential regulatory sequences, Young et al. (1999) examined DNA sequence 5′ of the *avpr1a* coding sequence. Although there are a number of small differences between species, the most obvious is the length of a microsatellite sequence with complex repeat motifs approximately 600 bp 5′ of the transcription start site. Monogamous prairie and pines voles both have long microsatellites, while promiscuous meadow and montane voles (*Microtus pennsylvanicus* and *Microtus montanus*) lack significant repetitive sequences at this site. The concordant patterns of microsatellite length, neuronal gene expression and mating system strongly suggested that the microsatellite length might drive species differences in neuronal phenotype, which in turn could cause differences in monogamy. To test this, Young et al. (1999) generated a transgenic mouse that expressed V1aR under the control of the prairie vole *avpr1a* promoter. They found that the transgenic mice expressed V1aR in a pattern that resembled the neuronal phenotype of prairie voles (Fig. 5). Moreover, vasopressin injections into the brains of transgenic mice elicited specific social preferences for individuals present at the time of injection. This behavior serves as a common measure of pairbonding, and is not exhibited by wild-type mice (Young et al., 1999). Together these data demonstrate that *cis*-regulatory sequences at the prairie vole *avpr1a* locus are capable of driving major changes in both neuronal phenotypes and related social behaviors.

The influence of *cis*-regulatory sequences on both brain and behavior suggested a primary role for the microsatellite in regulating the expression of *avpr1a*. To assess this, Hammock, Young and colleagues performed a series of elegant studies comparing *in vitro* gene expression under the control of the *avpr1a cis*-regulatory

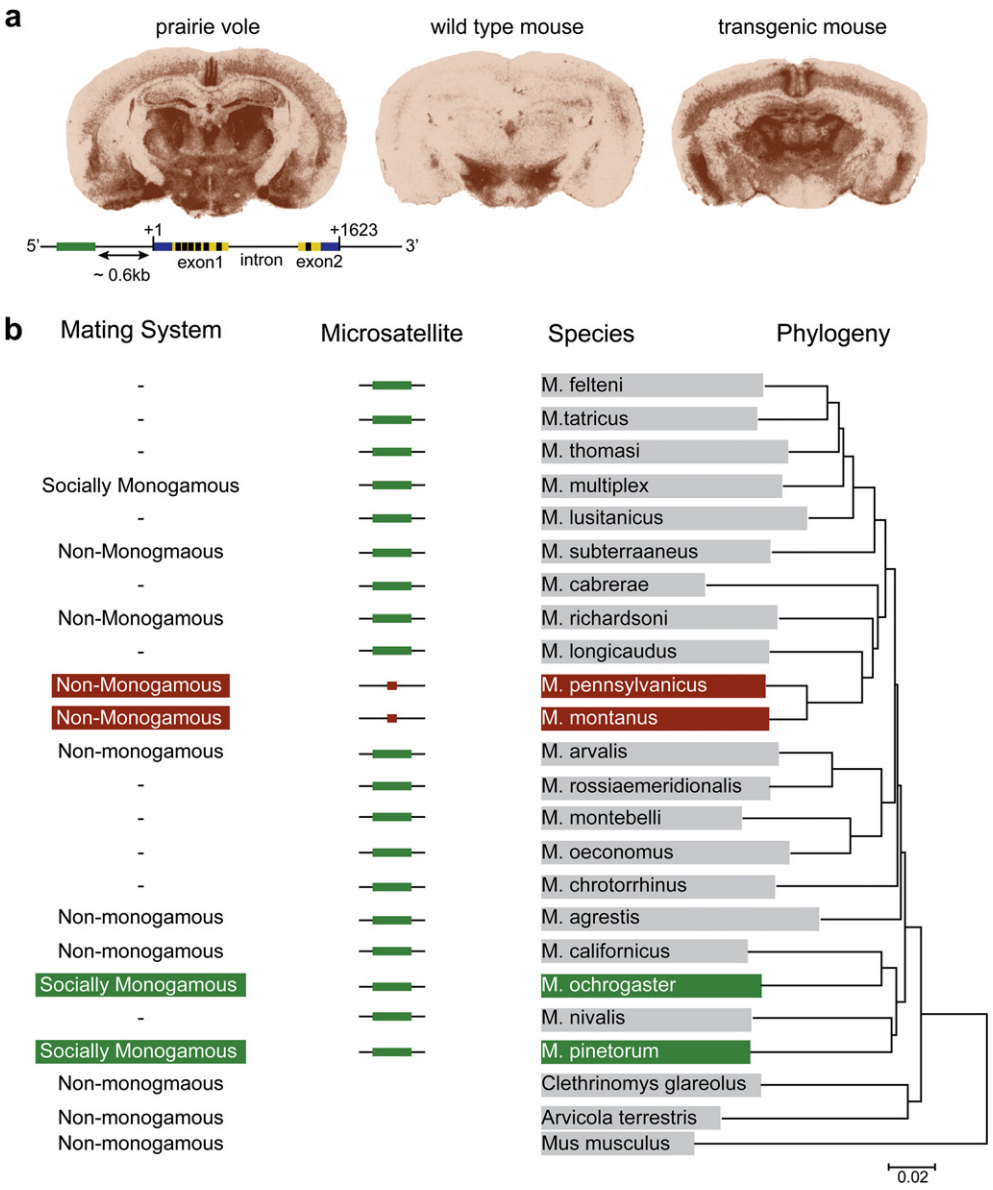

Fig. 5. Influences of *cis*-regulatory sequences on brain and behavior. a) Autoradiograms of V1aR binding in the brain of a prairie vole (right), a wild-type lab mouse (center) and a transgenic mouse engineered to express V1aR under the control of prairie vole *cis*-regulatory *avpr1a* sequences. The transgenic mice exhibited prairie-like V1aR abundance, and formed social preferences after vasopressin injection (see text; modified from Young et al., 1999). The structure of the prairie vole *avpr1a* locus is given on the lower left of the panel. b) The evolution of the long microsatellite thought to drive species-specific patterns of V1aR expression. Left panel indicates mating system, in which "social monogamy" refers to co-habitation of males and females, not sexual fidelity. Similarly, "non-monogamy" refers to a lack of male-female co-habitation. Center panel refers to the presence or absence of a long microsatellite motif. The right panel depicts the phylogenetic relationships of the species. Images adapted from Fink et al. (2006).

sequences with long microsatellites from the prairie vole locus, as well as homologous short sequences from promiscuous montane vole *avpr1a* locus (Hammock and Young, 2004). Although it was not feasible to examine the function of these sequences *in vivo*, they found that variation in microsatellite length alone caused variation in gene expression in a diverse array of non-neuronal cell types (Hammock and Young, 2004, 2005). Because microsatellite sequences are often highly variable, this provided an attractive hypothesis for explaining both between species and within species differences in V1aR distribution and social behavior. Indeed, male prairie voles with longer microsatellite alleles tend to spend more time in contact with a recent mate than do males with short alleles (Fig. 6) – a finding that suggested that short-allele males might be more likely to remain single and promiscuous in the field (Hammock and Young, 2005).

Based on the prairie vole work, researchers began to examine a series of four different microsatellite motifs in the human *avpr1a* locus – three are 5′ of the transcription start site, and a fourth is within the 2.2 kb intron (Thibonnier et al., 2000). Remarkably, three of the four have shown some association with autism-related social deficits (Kim et al., 2002; Yirmiya et al., 2006). One microsatellite in particular, located ~3.5 kb 5′ of the transcription start site and termed "RS3," has been linked to autism as well as to normal variations in social behavior and communication, including creative dance (Bachner-Melman et al., 2005), altruism (Knafo et al., 2008), and marital quality (Walum et al., 2008). Perhaps the most striking parallel comes from recent study by Walum et al. (2008), which reports that men with RS3 alleles of a specific length (334 bases) were scored as less "pairbonded" and reported higher rates of marital strife (Fig. 6).

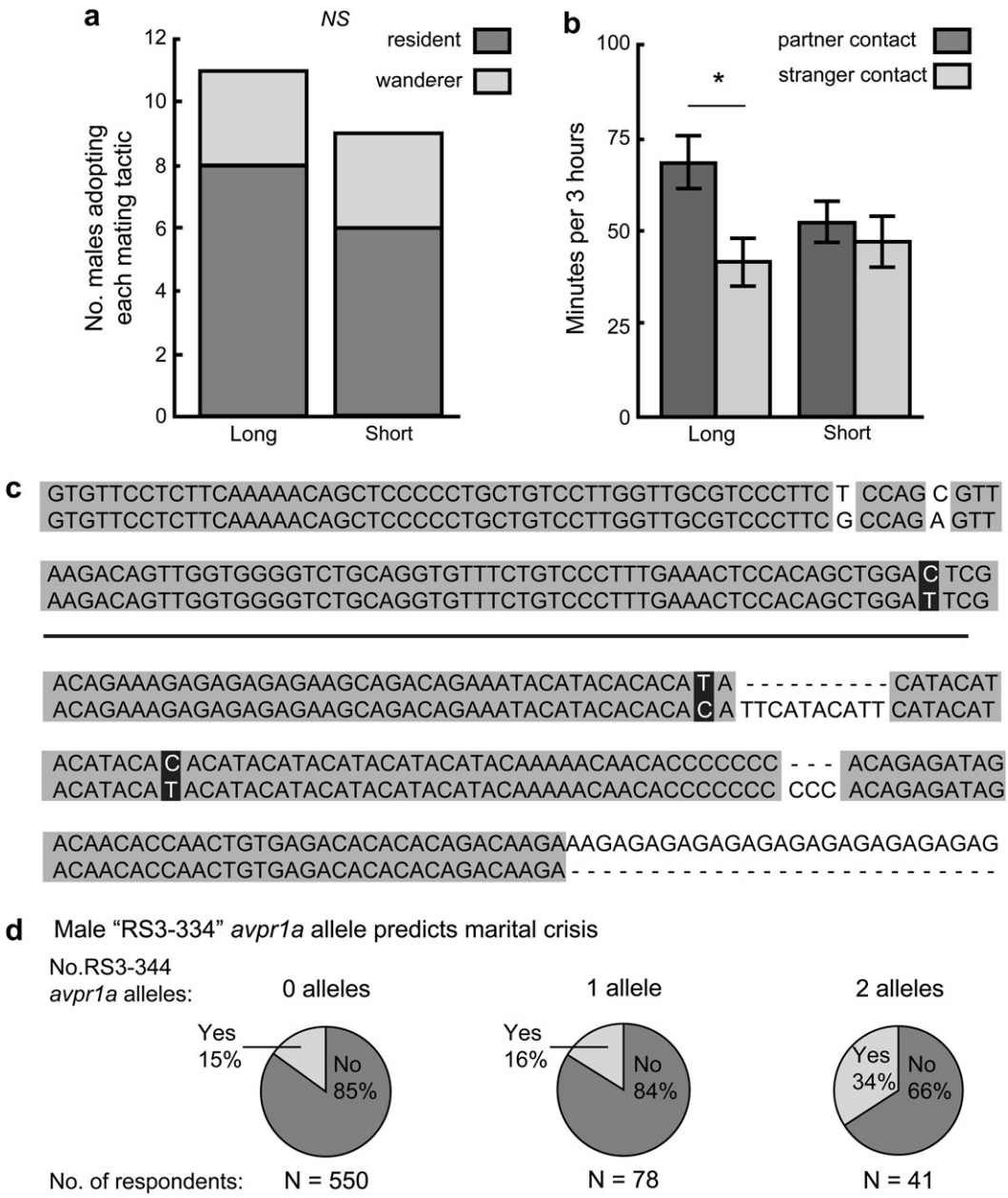

Fig. 6. Complex data relating *avpr1a* microsatellites to brain and behavior. a) Long microsatellites do not predict propensity to be single (wander) or paired (resident) among male prairie voles (Ophir et al., 2008a). b) Long microsatellites do increase the strength of partner preferences measures in the lab (from Hammock and Young, 2005). c) Alignment of long (top) and short (bottom) prairie vole *avpr1a* alleles. There is substantial sequence variation at multiple repeat motifs, as well as various single nucleotide polymorphisms (after Ophir et al., 2008a). Horizontal line indicates an intervening sequence has been omitted between upper and lower panels. d) Walum et al. (2008) report that a specific repeat length (334 bases) at the RS3 microsatellite of the human *avpr1a* locus predicts the probability a man has had a marital crisis that threatened divorce in the last year. The effects were sex specific (not shown).

To examine how well microsatellite length predicted social behavior across vole species, Fink et al. (2006) examined the lengths of *avpr1a* microsatellites in many *Microtus* species. When they mapped microsatellite length and mating system onto a phylogeny, their results came as a surprise. While it is true that the monogamous prairie and pine voles have long microsatellites, and the promiscuous meadow and montane voles do not, this distinction did not hold up across related species. Most vole species are promiscuous, but nevertheless have long microsatellites; indeed, meadow and montane voles appear to share the trait of short repeats not because they are promiscuous, but rather because of a single loss of the repetitive sequence in a recent common ancestor (Fig. 5). Similar studies have since looked at the distribution of the *avpr1a* RS3 and other microsatellites across a variety of primate species (Donaldson et al., 2008; Rosso et al., 2008). The presence of the RS3 microsatellite is widespread among great apes, but does not predict the mating system of a species. By examining the distribution of *avpr1a* microsatellites in an explicit evolutionary context, it becomes clear that the presence or absence of a repetitive motif does not explain species differences in *avpr1a*-mediated social behaviors. Does this inform our understanding of individual differences, either among voles or humans?

To examine the role of the prairie vole *avpr1a* microsatellite in natural pairbond formation, we genotyped the males from the field study described above. We found that, although we could detect associations between microsatellite length and V1aR abundance,

long-allele males were no more likely to pair or to be faithful (Ophir et al., 2008a). Indeed, published studies on individual differences in vole microsatellite length reveal inconsistent, sometimes contradictory associations between microsatellite length, behavior and V1aR distributions (Hammock et al., 2005; Hammock and Young, 2005; Ophir et al., 2008a). Alignment of two prairie vole alleles offers insight into this phenomenon: the microsatellite is a complex combination of repetitive sequences, and a long allele in one study is not necessarily equivalent to a long allele in another. In Fig. 6c, for example, the top allele is much longer than the bottom allele because of an expanded GA-repeat. The bottom allele, however, has longer poly-C and CATA repeats. Moreover, the two alleles differ at a number of single nucleotides in or near the repetitive sequence. It seems likely that the diverse results in studies of vole microsatellites reflect differences in the sampling of genetic diversity. Examining length alone confounds different repeat motifs, as well as other sequence polymorphisms that may co-vary with allele lengths. A closer examination of the data suggests this may be the case in humans as well. Few studies find an effect of allele length *per se* (e.g. Knafo et al., 2008; Levin et al., 2009), but instead find that a very specific allele length at one of the *avpr1a* microsatellites predicts human social behavior (Bachner-Melman et al., 2005; Kim et al., 2002; Wallum et al., 2008; Yirmiya et al., 2006). Which of the four *avpr1a* microsatellites predicts behavior varies from study to study, as does the effective allele length of a microsatellite. Importantly, linkage studies have repeatedly found that variation at the *avpr1a* locus is most predictive of human social phenotypes when the data are grouped by haplotype – that is, by the combination of microsatellite lengths across the *avpr1a* locus (Bachner-Melman et al., 2005; Kim et al., 2002; Yirmiya et al., 2006). Such haplotypes are the best approximation of the actual sequence of the *avpr1a* locus. This suggests that the sequence polymorphisms associated with an allele of a particular length, not the allele length itself, cause differences in brain and behavior. In humans, as in voles, researchers must examine specific DNA sequences to understand the behavioral and neuronal diversity attributable to regulation of the *avpr1a* locus. Not only have studies of voles elevated our understanding of human social behavior, but evolutionary analyses have refined our knowledge of both species, and have suggested useful foci for further study.

4. New models for new questions: neuropeptide receptors in singing mice

The increasing number of rodents with fully sequenced genomes (Mammalian Genome Project, http://www.broad.mit.edu/node/296), together with the extensive genomic resources available for *Mus*, suggest that this socially diverse and speciose order should be a fruitful source for alternative mammalian models. As described in the previous sections, prairie voles have already proven a remarkably informative model for the neural and molecular substrates of social attachment and associated disorders. Other recent examples include the deer mouse, *Peromyscus maniculatus*, which displays spontaneous stereotypic behaviors characteristic of obsessive compulsive and autism spectrum disorders (ASD; Korff et al., 2008; Lewis et al., 2007), and the diurnal fat sand rat, *Psammomys obesus*, a promising model for human seasonal affective disorder (Ashkenazy et al., 2009). Here we introduce an interesting new model of mammalian vocal communication known as the singing mouse.

The two species of singing mice, *Scotinomys teguina* and *Scotinomys xerampelinus*, are endemic to montane habitats in Central America (Hooper, 1972). Both species exhibit complex vocal repertoires used in close-range and long-distance communication in a variety of social contexts. Most conspicuous is a highly stereotyped advertisement song comprised of a rapidly articulated trill (up to 20 notes/second, S.M. Phelps, unpublished) that spans audible and ultrasonic frequencies (8–50 kHz; Hooper and Carleton, 1976; Miller and Engstrom, 2007). Advertisement songs, unlike the ultrasonic vocalizations made by lab mice, may be perceived at a distance by both conspecifics and predators; perhaps not surprisingly, we find that mice who score as more timid in open-field studies are also less likely to vocalize spontaneously (Crino, 2008). In addition, Alston's singing mouse, *S. teguina*, emits longer songs and sings more often than its congener *S. xerampelinus* (Miller and Engstrom, 2007; P. Campbell and S.M. Phelps, unpublished). Individual and species differences in the propensity to sing these elaborate songs may prove useful for understanding human communication and its disorders, such as verbal dyspraxia, social anxiety and selective mutism. In addition to the advertisement song, the mice produce reciprocal duet-like vocalizations when two individuals are in contact. One major characteristic of autism is a deficit in social reciprocity and communication (Lord et al., 2001; Tanguay et al., 1998); the mice seem like exceptionally good models for such phenotypes. Lastly, the two species differ substantially in their patterns of maternal investment, growth rates and spacing patterns, all of which are known to influence social structure in other species (Komers and Brotherton, 1997; Ebensperger, 2001; Kokko and Jennions, 2008). The mice thus offer a unique opportunity to study how vocal communication interacts with other domains of social cognition and behavior.

Despite the well-supported roles of non-mammalian homologues of vasopressin and oxytocin in vocal behavior in fishes, frogs and birds (Goodson and Bass, 2001; Goodson et al., 2003), the involvement of these neuropeptides in mammalian acoustic circuitry has received surprisingly little attention. Comparison of vasopressin 1a receptor (V1aR) distributions in singing mice revealed high binding in the auditory thalamus (medial geniculate) in both species, with a strong trend ($P = 0.06$) toward higher V1aR abundance in the thalamus of the more vocal *S. teguina* (Fig. 7; Campbell et al., 2009). Similarly, *S. teguina* exhibits significantly higher V1aR binding in structures involved in vocal production (periaqueductal grey and anterior hypothalamus; Campbell et al., 2009). These findings suggest that targeted manipulation of V1aR

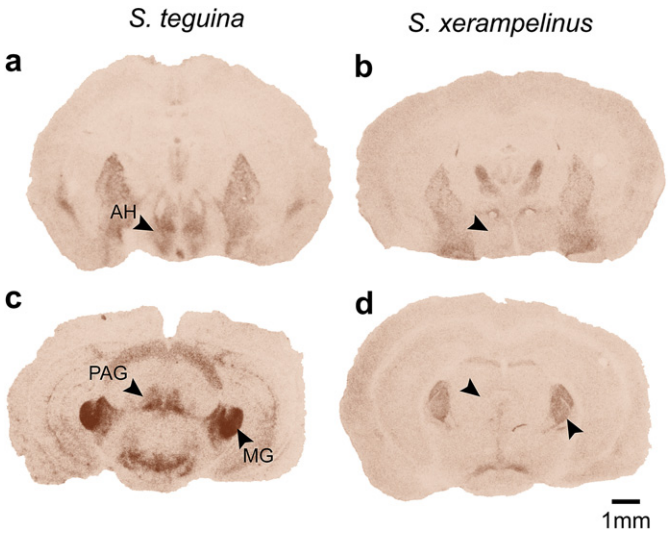

Fig. 7. V1aR binding in the brains of singing mice. *Scotinomys teguina* is the more vocal, mid-elevation species, and *S. xerampelinus* is less vocal and lives in cooler, higher elevations. *S. teguina* not only sings more often, it exhibits higher V1aR in the a) anterior hypothalamus (AH), and b) periaqueductal grey (PAG) and auditory thalamus (medial geniculate, MG). These structures have been implicated in motivational, motor and auditory aspects of vocalization. Figure modified from Campbell et al. (2009).

expression in singing mice could provide critical insight into the role of vasopressin in mammalian acoustic communication. While connections between autism and the vasopressin and oxytocin receptors are widely supported in association studies (Jacob et al., 2007; Kim et al., 2002; Lerer et al., 2008; Wu et al., 2005; Yirmiya et al., 2006), most experimental work in this area has focused on the relevance of this neuropeptide system to ASD-associated deficits in social affiliation (Lim et al., 2005; Winslow and Insel, 2002). The potential contribution of vasopressin to verbal deficits and atypical auditory processing, both common features of ASD diagnoses (Gervais et al., 2004; Muller et al., 1999), remains largely unexplored.

While species differences in acoustic circuitry are remarkable, differences in the ecology of the species suggest other brain regions may merit attention. The higher elevation singing mouse, *S. xerampelinus* exhibits higher maternal investment (e.g. slower pup development, Hooper and Carleton, 1976) and lower population densities (B. Pasch, unpublished), both of which correlate with important aspects of resource availability, social structure and associated space use. In parallel to these life history differences, *S. xerampelinus* has much higher levels of neuropeptide receptors in brain regions involved in spatial memory (OTR in the hippocampus, and V1aR in the anterior thalamus and supramammillary nucleus; Campbell et al., 2009). Data from other species demonstrate that oxytocin and vasopressin act in many of the above brain regions to regulate social and spatial memory. In mice and rats, for example, hippocampal oxytocin promotes social recognition (van Wimersma Greidanus and Maigret, 1996) and enhances spatial memory in primiparous females (Kinsley and Lambert, 2008; Tomizawa et al., 2003). In male rats, AVP administration in dorsal hippocampus enhances spatial memory consolidation (Paban et al., 2003). Congeneric species differences in receptor distributions in these structures have been reported in several other rodents (tucu-tucos, Beery et al., 2008; Insel et al., 1991), suggesting these expression domains are evolutionarily labile and ecologically important. Combined with our data from prairie voles, the role of neuropeptide receptors in socio-spatial memory seems remarkably underexplored. Singing mice provide a unique opportunity to combine studies of vocal communication, individual recognition and spatial memory to examine how neuropeptide receptors influence social cognition and its many substrates.

5. Summary and conclusions

The recent elucidation of neuropeptide contributions to mammalian social behaviors demonstrates how extending study from well established models to novel but related species can provide valuable new insights into complex social behaviors. By approaching the prairie vole from an evolutionary perspective, we identified a surprising degree of individual differences in neuronal phenotypes, and novel relationships between neuronal differences and complex social behavior. Structures like the hippocampus and retrospenial cortex, neglected contributors to rodent social behaviors, predicted social and sexual fidelity more accurately than did regions of the now classic "pairbonding circuit" (Young and Wang, 2004; Ophir et al., 2008c). Studies examining the natural variation in V1aR abundance and the prairie vole *avpr1a* locus not only provided a way to model variation in social attachment (Ophir et al., 2008a; Hammock and Young, 2005; Young et al., 1999), but led researchers to a novel candidate that predicts clinically relevant social behaviors (Kim et al., 2002; Yirmiya et al., 2006). Evolutionary analyses that examined the distribution of *avpr1a* microsatellites across species provided valuable context for interpreting the complex, seemingly contradictory data provided in different association studies (Donaldson et al., 2008; Fink et al., 2006; Rosso et al., 2008). And lastly, we introduced an exciting new model for examining how the neuropeptide regulation of social communication may interface with brain regions critical for other forms of social cognition.

At the level of the neural substrates of social behavior, one emerging theme is the neglected importance of spatial memory centers and their modulation by neuropeptides. The hippocampus, retrosplenial cortex and related structures have been implicated more broadly in the mapping of both space and time, in what is referred to in humans as episodic memory (Fink et al., 1996; Maguire, 2001). Episodic memory is in many ways a central part of subjective experience, and thought disorders like schizophrenia have been linked to dysfunction of the hippocampus and deficits in episodic memory (Harrison, 1999; Heckers et al., 1998). Interestingly, humans with long RS3 *avpr1a* alleles have higher levels V1aR mRNA in the hippocampus (Knafo et al., 2008), a finding associated with measures of altruism, but which has not been studied in the context of known hippocampal functions. Similarly, long RS3 alleles have been linked to acoustic pre-pulse inhibition, a common phenotype altered in schizophrenia (Levin et al., 2009). Understanding how episodic memory is influenced by neuropeptides, and how this interaction shapes social cognition, remains an exciting but largely unexplored area of basic and clinical research.

At a broad level, our common theme has been a reformulation of Frank Beach's classic argument (1950), modified to include both the taxonomic diversity Beach advocated and the evolutionary framework needed to effectively exploit such diversity. The availability of a densely annotated human genome, together with recent advances in sequencing and microarray technologies, have provided unprecedented insight into the molecular and neural substrates of human disorders with complex behavioral phenotypes, including autism, speech and language disorders, bipolar disorder and schizophrenia (Askland et al., 2009; Cichon et al., 2009; Cook and Scherer, 2008; Fisher et al., 2003; Stefansson et al., 2008; Szatmari et al., 2007). However, association studies require experimental validation. Can the lab mouse accurately represent the full spectrum of human disorders of affect, cognition and communication? Probably not, but we have shown how the classic biomedical approach, in which diverse questions are fit to a single model system, can be effectively supplemented by an intuitively logical but previously unfeasible approach: selection of a non-traditional system that optimizes the fit between question and organism. Indeed, modern sequencing methods make genomic approaches available for an unparalleled diversity of species (e.g. Clark et al., 2007; DeLong et al., 2006). Although technology has changed tremendously, one important aspect of biology has not: combining model systems and comparative methods continues to provide the best prospects for beating the boojum.

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