9 Monogamous Brains and Alternative Tactics: Neuronal V1aR, Space Use, and Sexual Infidelity among Male Prairie Voles

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9.1. Introduction

All of animal behavior can be regarded as a series of decisions—decisions about when to mate, forage, court, fight, or sleep, to name a few. The diverse decisions that underlie adaptive behavior often depend on the coordinated actions of many brain regions. Foraging and mate choice, for example, use sensory structures to recognize either food or prospective mates, reward structures to assign emotional valence to stimuli (Pfaus and Heeb 1997; Schultz 2006; Hoke et al. 2007), and motor regions to execute approach, avoidance, or the more specific actions required in each context. While each behavior relies on multiple mechanisms, each mechanism may also contribute to different decisions. Female water mites, for example, attend to vibrations on the surface of the water to detect their prey; males capitalize on female sensory design by using similar cues when courting (Proctor 1991). Although several authors have discussed the multiple uses of a sensory system (e.g., M. Kirkpatrick and Ryan 1991; Endler 1992; Christy 1995), the repeated use of other cognitive mechanisms has received less attention (see Sherry and Schacter 1987). For example, female guppies provided with orange foods develop incidental but significant preferences for orange males (Rodd et al. 2002), indicating that associations between color and value made when feeding are influencing seemingly unrelated mating decisions. To fully understand behavioral evolution, we must ask how distinct cognitive processes are coordinated to mediate a behavioral tactic; we must also ask how animals resolve conflicting demands made on common cognitive substrates. We explore the interactions among neural mechanisms and mating decisions in males of the socially monogamous prairie vole, *Microtus ochrogaster,* a species with alternative mating tactics. The results inform our understanding of both mating-system evolution and the complex interplay among the cognitive processes that govern animal behavior.

9.1.1. COGNITIVE ECOLOGY AND THE EVOLUTION OF MONOGAMY

A mating system emerges from the set of reproductive decisions made by individual males and females. The variables that shape individual tactics are subjects of a rich history of theoretical and empirical study that coincides with the very origins of behavioral ecology. Trivers (1972), for example, argued that sex differences in parental investment drive the elaboration of sexually dimorphic reproductive strategies; in this scenario, monogamy emerges when females and males are selected to exhibit similar levels of investment in young. Emlen and Oring (1977) emphasized the importance of ecological variables in shaping female distributions in space and time. In general, it is difficult to monopolize widely dispersed females, which favors monogamy; it is also difficult to defend females that breed synchronously, which favors a polygamous, nonterritorial, "scramble" competition for mates. Both treatments remain valid but have been refined by a series of theoretical studies that examine female polygamy (Kokko 1999; Ihara 2002; Wakano and Ihara 2005), mate guarding by males (Sandell and Liberg 1992; Kokko and Morrell 2005), and female mate choice (Kokko and Morrell 2005) in more detail. These studies demonstrate how mating patterns can emerge from complex interactions between decisions made by males and females—decisions influenced by a broad suite of social and environmental variables.

Given the multifactorial nature of mating decisions, they seem likely to rely on multiple cognitive processes. In vertebrates, for example, mate guarding emerges when males maintain a close proximity to mates and actively repel intruding males. One behavior requires selective social attachment; the other, selective aggression—two very different behavioral processes. The mechanisms of attachment are in many ways particularly interesting, because they utilize the neuronal circuits that underlie reward in many other contexts, including food, sex, and recreational drug use (Pfaus and Heeb 1997; Everitt and Robbins 2005; Schultz 2006; Hoke et al. 2007). Humans asked to view photographs of people with whom they are deeply in love show enhanced activation of a reward region called the ventral tegmental area (Aron et al. 2005; H. Fisher et al. 2005). This same region is activated by attractive faces in humans (Aron et al. 2005; H. Fisher et al. 2005) and by preferred mate signals in túngara frogs (Hoke et al. 2007). In prairie voles, pair-bonding and its attendant mate guarding are mediated by similar circuits (Young and Wang 2004). Not surprisingly, these reward mechanisms do not seem central to the neurobiology of aggression (reviewed in R. Nelson and Trainor 2007).

Attachment, attraction, and aggression, however, do not fully define mating tactics. Species differences in mating system, for example, are associated with differences in space use. Polygynous species are characterized by sex differences in home range size; in some taxa this is associated with sex and species differences in the size of the hippocampus, a brain region central to spatial navigation (Sherry et al. 1992; L. Jacobs 1996). Recent theoretical studies suggest that spatial navigation should prove central to mating tactics. The optimal level of mate guarding, for example, depends on how efficient males are when guarding mates (Kokko and Morrell 2005), on the number of females a nonterritorial male encounters when roaming (Sandell and Liberg 1992), and the number of females a territorial male can monopolize (Sandell and Liberg 1992). All three of these measures are influenced by a male's use of space, and all seem likely to draw on the mechanisms of spatial navigation. While a cohesive mating tactic requires coordinating multiple behaviors in response to diverse information, the contribution of multiple neural centers to individual and species differences in such tactics remains poorly understood.

9.1.2. A MODEL FOR MAMMALIAN MONOGAMY

Fewer than 3% of mammalian species are thought to be monogamous (Kleiman 1977; Komers and Brotherton 1997), and avian monogamy is much better studied in the field (e.g., H. Smith 1995; Temrin and Tullberg 1995; Owens and Bennett 1997; D. Westneat and Sherman 1997; Petrie and Kempenaers 1998; Griffith et al. 2002). Nevertheless, the extraordinary depth of work on the neurobiology of mammalian behavior is a useful resource for any exploration of neurobiology, cognition, and behavior. Nearly one-third of all mammals are Muroid rodents (Steppan et al. 2004), and this taxonomic diversity, coupled with a vast knowledge of their behavioral mechanisms, makes these species particularly promising models for the integrative study of a mating system. In this context, the prairie vole, *Microtus ochrogaster,* has emerged as a leading model for the study of monogamy.

Prairie voles are small Arvicoline rodents widely distributed in North America. Males and females form long-term associations characterized by shared nests, exclusive home ranges, and biparental care (Getz et al. 1981, 1993). Although most males pair-bond with females and exhibit a "resident" mating tactic, a significant minority forgo pair-bonding and adopt a "wandering" phenotype, characterized by large home ranges that overlap those of multiple males and females (Getz et al. 1981, 1993; Solomon and Jacquot 2002).

In the lab, male pair-bonding has been studied primarily in the context of the selective attachment formed after mating. Intracerebral injections of the neuropeptide vasopressin can promote such attachment, even in the absence

of mating (Winslow et al. 1993). Similarly, vasopressin antagonists block male pair-bonding but leave mating behavior unaltered (Winslow et al. 1993). In the central nervous system, the main target of vasopressin is the V1a receptor (V1aR). Among voles, species differences in the distribution of V1aR contribute to variation in mating system (Insel et al. 1994). Indeed, genetic manipulations that increase the abundance of V1aR in particular brain regions can cause males of a polygamous species to exhibit the selective affiliation characteristic of monogamous species (Young et al. 1999; Lim et al. 2004). Among the brain regions that have been studied, V1aR expression in the ventral pallidum seems to be particularly important for male attachment. Similarly, the medial amygdala regulates paternal care, and the lateral septum contributes to both pairing and paternal care (Young and Wang 2004). There are many other brain regions that express V1aR but whose contributions to behavior have not been investigated (Insel et al. 1994; Phelps and Young 2003). How does variation in neuronal V1aR abundance relate to the behavioral diversity evident in natural populations? Does the circuitry that underlies spatial navigation contribute to male tactics or their efficacy? We address these questions through field studies of V1aR function in prairie vole behavior.

9.2. Reproductive decisions, space use, and mating tactics

9.2.1. AFFILIATION, ATTACHMENT, AND MATING SUCCESS

In the wild, monogamous resident males defend small territories and exclude intruding conspecifics (e.g., Getz et al. 1993). The exact proportion of males that become residents varies somewhat from study to study, but residency is generally the more common tactic (Solomon and Jacquot 2002; Ophir et al. 2008b). In seminatural enclosures, for example, roughly 75% of males adopt a resident strategy, while the remaining 25% become wanderers (fig. 9.1; Ophir et al. 2008b). Although the enclosures exclude predators and limit movements of subjects, the overall pattern of space use resembles that of free-living animals (e.g., Getz et al. 1993). Why do animals adopt these distinct tactics? Are they evolutionarily equivalent, or is one tactic favored?

To assess the reproductive consequences of resident and wandering tactics, we placed sexually naive, adult male and female prairie voles into seminatural enclosures at natural densities. We then examined the paternity of embryos sired during the experiment. We found that residents had a significantly higher probability of fertilizing females than did wanderers (fig. 9.1; Ophir et al. 2008b). This probability of fertilization translated into a larger number of embryos sired by residents than by wanderers (fig. 9.1; Ophir et al. 2008b).
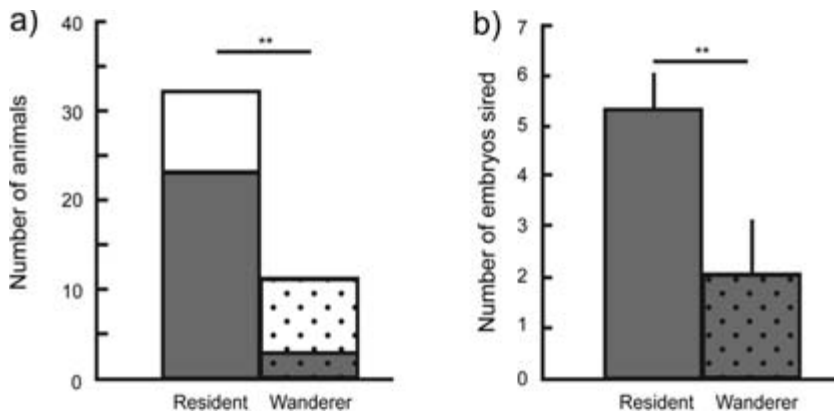
FIGURE 9.1. Mating success of residents and wanderers. a. Number of resident (solid) and wandering (stippled) males that mated successfully (gray) or did not mate successfully (white). Proportions were compared with a Fisher's exact text, **P = 0.01. b. Mean (±SE) number of embryos from males who were residents or wanderers. Means and standard errors are based on enclosure means ($N_{enclosure}$ = 8; T-test, **P < 0.01). Modified from Ophir et al. 2008b.

Fertilization rates are important but incomplete components of an animal's fitness: differences in longevity, condition, or parental care could substantially alter lifetime reproductive success. With respect to longevity, our data do not address whether there are differences in predation. We found that wanderers were in no better condition than residents (Ophir et al. 2008b), suggesting that the energetic costs of territory defense do not reduce the benefits of pairing. Getz and McGuire (1993) report that wanderers live longer than residents, but these differences are smaller than those we report for mating success. Considering the added advantages residents gain by caring for their young (Wang and Novak 1992), we suspect that our data underestimate the value of residency.

On balance, it appears that residency is a favored tactic. If so, wanderers must be making the best of a bad situation. What prevents wanderers from becoming successful residents? One explanation is that there is a paucity of receptive females. Puberty and ovulation in female prairie voles are induced by adult male olfactory cues (Carter et al. 1980; Dluzen et al. 1981); if females were insufficiently exposed to male pheromones, the number of receptive females would become limiting. Similarly, females may prefer the males who ultimately become residents to those who become wanderers. Although residents and wanderers are not known to differ in most gross morphological attributes (Solomon and Jacquot 2002; Ophir et al. 2007), resident males do have a longer anogenital distance (AGD) than wanderers (fig. 9.2a). Long
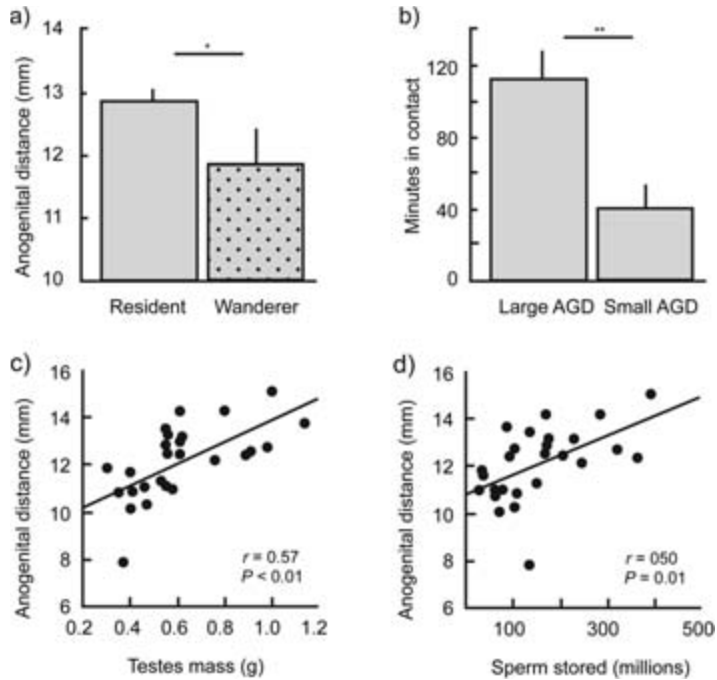
FIGURE 9.2. Anogenital distance (AGD), behavior, and fertility. a. Mean (±SE) AGD (mm) differed significantly between residents and wanderers (*P < 0.05). b. Mean (±SE) time (min) that females spent in side-by-side contact with males that had either longer or shorter AGD (**P < 0.01). AGD was significantly correlated with testis size (c) and sperm counts (d). Modified from Ophir and delBarco-Trillo 2007.

AGD is a marker for in utero masculinization and may serve as a proxy for other phenotypic differences detectable by females (Drickamer et al. 1995; Drickamer 1996; B. Ryan and Vandenbergh 2002). In the lab, prairie vole females prefer long-AGD males to short-AGD males (fig. 9.2b; Ophir and del-Barco-Trillo 2007). Potential reasons for such preferences include a preference for more aggressive males, which may translate into better territorial defense or infanticide deterrence. AGD is also positively correlated with testis size and sperm count in this same sample of prairie voles (figs. 9.2c and 9.2d; Ophir and delBarco-Trillo 2007). Because prairie voles form long-term pair-bonds, increased sperm production may translate into higher female reproductive success over a lifetime. Although the availability of responsive females may be limiting, one additional possibility suggested by the AGD data is that residents are simply more competitive and thus exclude future wanderers from available females.

9.2.2. SPACE USE AND HETEROGENEOUS SELECTION

One key attribute of reproductive tactics is how animals use space. Mate guarding by resident males, for example, includes both maintaining a close proximity to a mate and aggressively expelling intruders. Wandering males, in contrast, adopt a scramble tactic characterized by large home ranges and interactions with multiple prospective mates. Although males adopt one of two alternative reproductive tactics, they vary in how strictly they conform to the behavior that characterizes each tactic. We examined our radio-tracking and paternity data to determine whether there were differences in space use that predicted patterns of paternity and reproductive success within and between the two tactics.

As expected, wandering males had larger home ranges and overlapped more conspecifics (fig. 9.3; Ophir et al. 2008c). When we examined which space use patterns predict mating success, we found that successful wanderers used space differently from either unsuccessful wanderers or successful residents. Wanderers who were able to sire young roamed more broadly than unsuccessful wanderers: they exhibited larger home ranges, overlapped the home ranges of more males, and exhibited a trend toward overlapping the home ranges of more females (figs. 9.3a and 9.3c; Ophir et al. 2008c). Presumably this pattern maximized the rate that wanderers encountered potential mates and thus increased the probability of successful mating (Sandell and Liberg 1992). In contrast, resident males effectively exclude intruding males from their territories; on average, each resident home range overlaps the home range of less than one male. Based on theoretical treatments, resident males should maximize guarding when females exhibit moderate levels of multiple mating (Kokko 1999; Kokko and Morrell 2005). This small, actively defended home range is certainly consistent with such a tactic.

One very interesting treatment of monogamy emphasizes the trade-off that monogamous males must make between intrapair and extrapair paternity (Kokko and Morrell 2005; also see H. Smith 1995). Males who opt for intrapair paternity focus more on mate guarding at the cost of potential extrapair matings. Males who roam do so at the expense of mate guarding. Comparing space use of males who engage in intrapair (IPF) versus extrapair fertilizations (EPF) confirms these expectations. We find that EPF males—including both successful wanderers and philandering residents—exhibit patterns of conspecific overlap that resemble those of wandering males (fig. 9.3; Ophir et al. 2008c). This difference persisted after correcting for overall differences between residents and wanderers in the degree of overlap (Ophir et al. 2008c). Thus, even resident males increase extrapair paternity by venturing more often into surrounding environments.

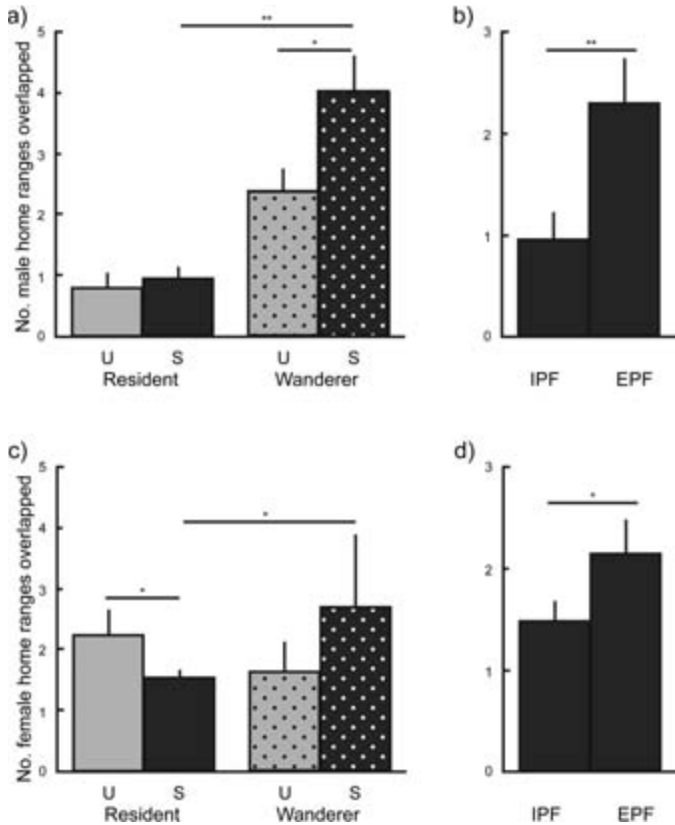In general, successful males adopt one of two strategies: they focus efforts

FIGURE 9.3. Space use among alternative male tactics. a. Mean (±SE) number of male home ranges overlapped by residents (solid) and wanderers (stippled) who were unsuccessful (U: light gray) or successful (S: dark gray) at siring young. An ANOVA revealed a significant effect of mating success ($P < 0.01$) and a mating success by reproductive tactic interaction (MS × RT, $P < 0.04$). b. The number of male home ranges overlapped by IPF and EPF males differed significantly (T-test, $**P < 0.01$). c. The number of female home ranges overlapped by successful and unsuccessful residents and wanderers exhibited a significant MS × RT interaction (ANOVA, $P < 0.05$). d. The number of female home ranges overlapped by IPF and EPF males. IPF = resident males that mated successfully only with their partner; EPF = wanderers that mated successfully plus residents that sired offspring outside the pair. Post hoc T-tests are reported in each panel ($**P < 0.01$, $*P \le 0.05$). Modified from Ophir et al. 2008c.

on mate guarding, or they maximize the number of females they encounter. This pattern suggests that selection operates against males with intermediate phenotypes. How do the brains of these animals reflect these evolutionary forces? In the next section, we examine natural variation in the neural expression of the vasopressin receptor, V1aR. We ask how natural diversity in brain

phenotype contributes to the probability that a male will adopt a resident or a wanderer tactic and to the efficacy of the tactic a male adopts.

9.3. Neural substrates of alternative tactics

Across a broad range of taxa, arginine vasopressin (AVP) and its nonmammalian homologue, vasotocin, influence a diversity of social behaviors, including mating, territorial aggression, and social memory (Dantzer et al. 1988; Ferris and Delville 1994; Goodson and Bass 2001; H. Caldwell et al. 2008). In prairie voles, vasopressin antagonists block the pair-bonding that normally follows repeated mating (Winslow et al. 1993; Lim et al. 2004). Similarly, vasopressin alone is able to produce the specific social attachments characteristic of pair-bonding even in the absence of mating (Winslow et al. 1993). The effects of vasopressin are not limited to attachment, however. Injections of vasopressin also trigger the onset of intense, selective aggression directed at intruding males (Winslow et al. 1993). Vasopressin also contributes to male paternal care (Bamshad et al. 1994; Wang et al. 1994, 1998). Thus, the neuropeptide coordinates many attributes of the resident tactic.

The behavioral effects of vasopressin are generally mediated by the V1a receptor (V1aR), the predominant receptor in the central nervous system. Variation in V1aR expression profiles among *Microtus* species mirrors behavioral differences related to mating system. In addition to substantial differences between species, there are also profound differences among prairie voles (fig. 9.4; Phelps and Young 2003; Ophir et al. 2008c). While vasopressin synthesis and release vary dramatically with recent experience, V1aR expression seems to be stable throughout adulthood (e.g., Poulin and Pittman 1993; Wang et al. 1997). Given that vasopressin coordinates the transition to a resident tactic through its actions on V1aR, we investigated whether individual differences in adult V1aR explain variation in mating tactic or associated behaviors.

9.3.1. MECHANISMS OF MALE PAIR-BONDINGS

The effects of vasopressin on attachment have been directly linked to two neural structures. Vasopressin actions in the lateral septum influence social memory and aggression in several rodents (Dantzer et al. 1988; Everts et al. 1997; Bester-Meredith et al. 1999; Bielsky et al. 2005) and influence pair-bonding and paternal care in prairie voles (Wang et al. 1998; Y. Liu et al. 2001; Young and Wang 2004). More extensively studied, however, is the role of the ventral pallidum, a key node in the reward pathway (Cardinal et al. 2002; Everitt and Robbins 2005). Extensive vasopressin release during repeated mating seems to drive the formation of social preferences for a mate through its influence on
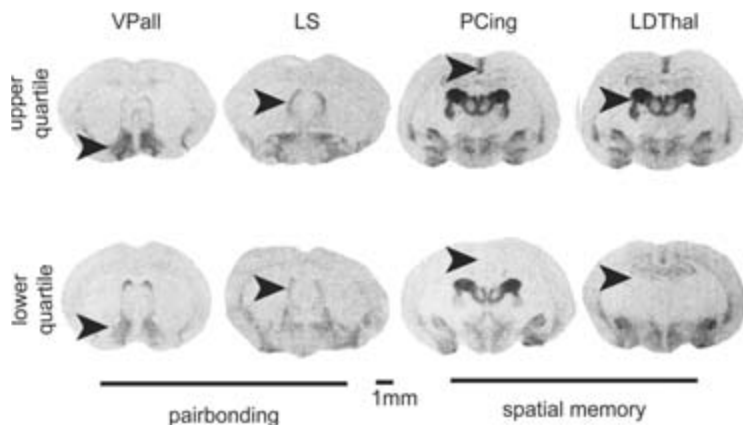
FIGURE 9.4. Natural variation in V1aR expression. Autoradiograms of brains in the upper and lower quartiles of ^{125}I-linear-AVP V1aR binding. Regions implicated in pair-bonding include the ventral pallidum (VPall) and lateral septum (LS). Regions implicated in spatial memory include the posterior cingulate/retrosplenial cortex (PCing) and laterodorsal thalamus (LDThal). Modified from Ophir et al. 2008c.

reward. This is supported by site-specific injections of hormone antagonists (Lim and Young 2004), by overexpression of V1aR receptors in the ventral pallidum (Pitkow et al. 2001; Lim et al. 2004), and by making transgenic mice that express V1aR under the control of the prairie vole V1aR regulatory sequence (Young et al. 1999). The ventral pallidum and lateral septum are part of a larger "pair-bonding" circuit, which links sensory information associated with a mate to the reward system (Young and Wang 2004). Given the extensive data on the importance of V1aR in monogamous behaviors, we first asked whether differences between wanderers and residents could be explained by natural variation in V1aR expression.

We predicted that the ventral pallidum and lateral septum would exhibit higher V1aR expression in resident males than in wandering males. This would suggest that wanderers were less responsive to vasopressin, and so less able to form pairs. To our surprise, we found no differences between residents and wanderers in the abundance of V1aR in either structure (fig. 9.5; Ophir et al. 2008c). These pair-bonding regions also failed to predict whether males exhibited sexual fidelity to their partners. Because we know that V1aR in these regions is necessary for pair-bond formation, the findings indicate that prairie vole males share a common propensity to form pair-bonds but that the use of these mechanisms is plastic. Presumably, this plasticity is attributable to variation in vasopressin release between tactics, though this has not been investigated. Because pairing substantially increases mating success, we suspect that
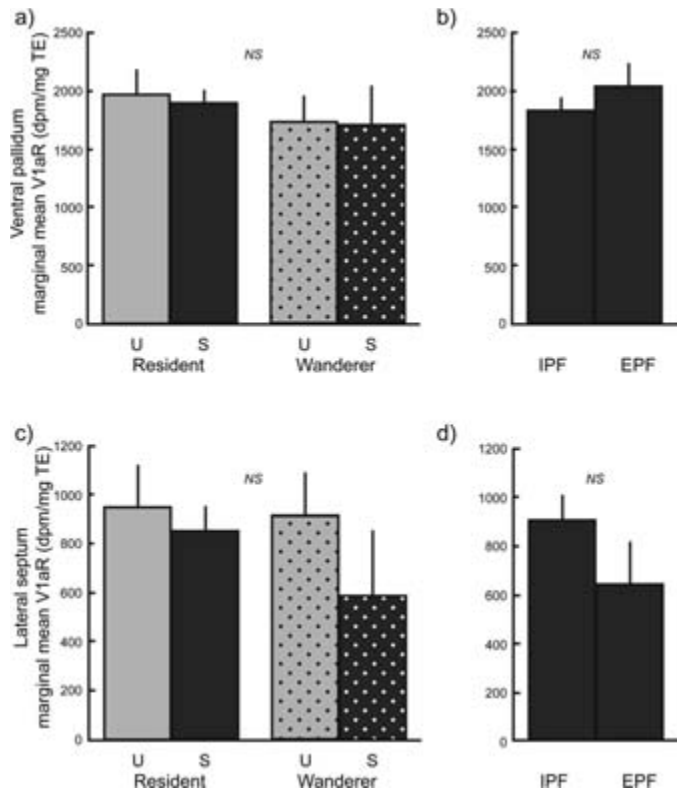
FIGURE 9.5. V1aR in pair-bonding regions does not predict tactic or fidelity. a. Mean (±SE) disintegrations per minute (dpm) in tissue equivalence (TE) of ^{125}I-labeled V1aR autoradiographic ligand binding in the ventral pallidum for successful (S) and unsuccessful (U) animals. Residents are depicted by solid bars and wanderers by stippled bars. S males fertilized at least one female. U males fertilized no females. b. Pallidal V1aR among IPF and EPF males. V1aR binding in the lateral septum among residents and wanderers (c) and among IPF and EPF males (d). There were no effects of residency, success, or sexual fidelity in either region ($P > 0.10$). Modified from Ophir et al. 2008c.

selection has cleared the standing variation in these structures. Thus, wanderers may be making the best of a bad situation, but their brains are ready to assume a favored strategy when the appropriate opportunity arises.

9.3.2. NEURAL SUBSTRATES OF SOCIOSPATIAL MEMORY

Vasopressin and V1aR are both integrally involved in pair-bond formation. However, pair-bonding is but one behavior modified by vasopressin. For example, a diverse literature documents vasopressin actions on memory consolidation and retention (DeWied 1971; Bohus et al. 1978; Egashira et al. 2004;

Hayes and Chambers 2005). Interestingly, two groups of structures vary dramatically in the abundance of V1aR between individual male prairie voles, both of which are implicated in memory (Phelps and Young 2003). These are the cingulate cortex and the dorsal thalamus.

Along the medial length of the cerebral cortex runs a fold called the cingulate cortex. Roughly midway between the rostral tip of the cortex and the caudal end is the posterior cingulate cortex, which then runs seamlessly into the more caudal retrosplenial cortex (Paxinos and Watson 2006). Both regions express V1aR in prairie voles, and both are highly variable in this expression (Insel et al. 1994; Phelps and Young 2003). The variation is present in both males and females, in mated and unmated animals, and in field caught and lab-reared animals (e.g., Phelps and Young 2003; Hammock and Young 2005; Ophir et al. 2008c). After examining well over 100 prairie vole brains, the two structures are always concordant—V1aR expression is either strong in both or weak in both (S. M. Phelps, personal observation). The posterior cingulate/retrosplenial cortex (PCing) has strong connections to the hippocampus, a brain region that maps the world in both space and time, thereby contributing to both spatial and "episodic" memory in humans and to spatial memory in many other taxa (Sherry et al. 1992; Cooper et al. 2001; Maguire 2001; Harker and Whishaw 2004). The PCing has been the subject of intensive recent interest for its involvement in both spatial and episodic memory in humans (Maguire 2001). In rats, lesions to PCing cause profound impairments in spatial memory and navigation (Harker and Whishaw 2004). In primates, the PCing sends projections to the laterodorsal thalamus (LDThal), which in turn projects to the hippocampus (van Groen and Wyss 2003; Shinkai et al. 2005). The LDThal has been the subject of less study, but given its neuroanatomical position and some limited behavioral data (van Groen et al. 2002), a role in spatial memory seems likely for this structure as well. Interestingly, the LDThal also exhibits profound variation in the expression of V1aR among prairie voles.

Given the natural diversity we observe in these structures, and the apparent disruptive selection we detected on space use patterns, we asked whether the abundance of V1aR in either structure was predictive of mating tactic, space use, or sexual fidelity among male prairie voles. We found that neither structure was responsible for becoming a resident or a wanderer (fig. 9.6; Ophir et al. 2008c). However, both were associated with the differences in space use that characterize successful wanderers and residents. Successful wanderers were characterized by larger home ranges and the overlap of more conspecifics; they also exhibited low V1aR expression in the PCing and LDThal. Residents were characterized by fewer conspecific overlaps and smaller home ranges; the PCing and LDThal of residents expressed higher levels of V1aR than did those of successful wanderers.
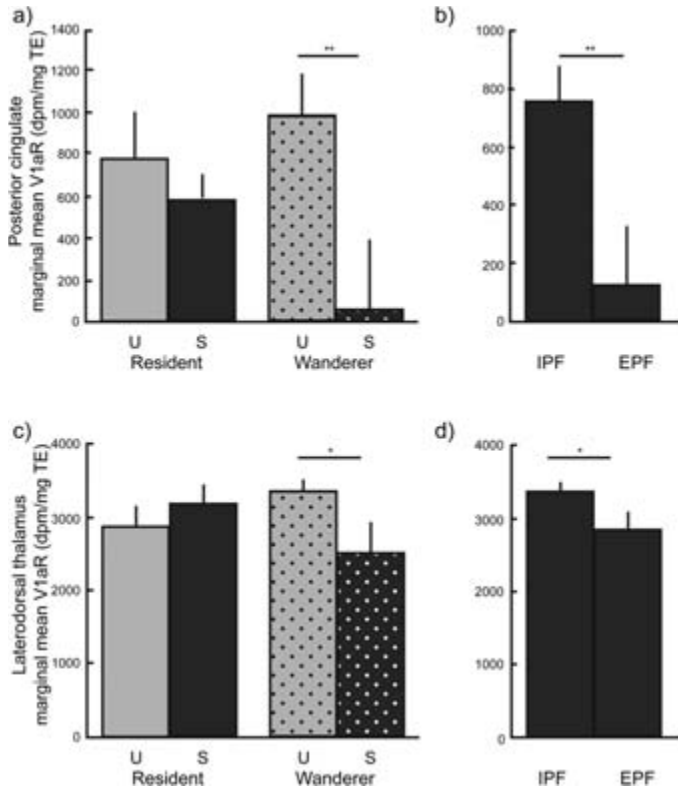
FIGURE 9.6. V1aR in spatial circuits predicts wanderer success and sexual fidelity. a. Mean (±SE) disintegrations per minute (dpm) in tissue equivalence (TE) of ^{125}I-labeled V1aR autoradiographic ligand binding in the PCing for successful (S) and unsuccessful (U) animals. Residents are depicted by solid bars and wanderers by stippled bars. MANOVA revealed a significant mating success by reproductive tactic interaction ($P < 0.05$) across both spatial memory structures. b. PCing V1aR in IPF and EPF males. V1aR binding in the LDThal among residents and wanderers (c) and among IPF and EPF males (d). Post hoc T-tests are reported in each panel (**$P < 0.01$, *$P < 0.05$). Modified from Ophir et al. 2008c.

Although V1aR in the pair-bonding circuit did not predict patterns of paternity, V1aR in the PCing was a particularly good predictor of male sexual fidelity. The prevalence of low V1aR expression among EPF males was attributable to both resident and wandering males, again mirroring patterns of conspecific home range overlap. This relationship between sexual fidelity and PCing V1aR expression also seems to hold across species (fig. 9.7). As discussed above, prairie voles are often socially monogamous but can exhibit alternative tactics. Their brains are characterized by high levels of PCing V1aR

in most individuals and the persistence of low PCing V1aR at lower frequencies (Phelps and Young 2003). Pine voles, thought to be genetically monogamous, exhibit consistently high levels of PCing V1aR (Insel et al. 1994). The polygamous montane and meadow voles, in contrast, exhibit no PCing V1aR whatsoever (Insel et al. 1994). This striking convergence of space use, sexual fidelity, and V1aR expression in spatial memory circuits suggests a common link between the cognitive demands of spatial navigation and the opportunity for EPF.

Male prairie voles thus possess the substrates for exhibiting pair-bonding, a preferred tactic, reflected in their uniformly high levels of V1aR in the ventral pallidum. It seems, however, that the males exhibit plasticity in the deployment of this machinery. Pair-bonding requires repeated bouts of mating with a single individual, with each bout releasing AVP, which modulates reward responses and forms attachments. By linking AVP release to mating bouts, the males have a neural mechanism for pairing with responsive females when they can be assured of paternity. In contrast, V1aR expression in spatial circuits may mediate a trade-off between the spatial demands of effective mate guarding and those of maximizing encounters with multiple females. Given evidence that PCing expression is highly heritable (Hammock and Young 2005), this raises the possibility that the variability in this and other spatial circuits may persist in a sort of balanced polymorphism. We return to this prospect in our conclusion. We now move down a level of analysis to focus on genetic mechanisms that underlie V1aR expression, with the ultimate hope of identifying allelic variation that could contribute to neuronal polymorphism.
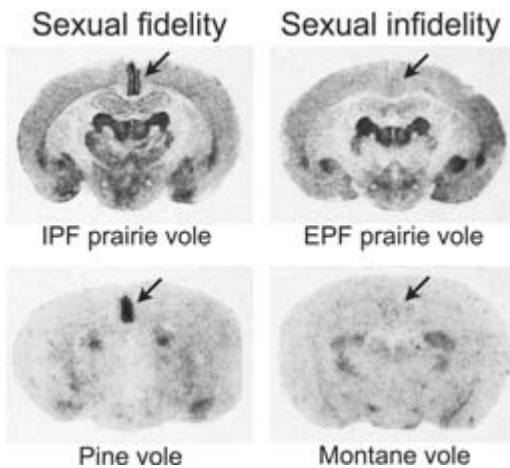

FIGURE 9.7. Individual and species differences in PCing. Sexually monogamous prairie voles and genetically monogamous pine voles both exhibit high levels of V1aR binding in the posterior cingulate/retrosplenial cortex. Sexually promiscuous prairie voles and polygamous montane voles both lack V1aR in the cingulate cortex. Pine vole and montane vole images from Insel et al. 1994.

9.4. Microsatellite polymorphisms and phenotypic diversity

The mapping of genotype to phenotype is central to an integrative under-standing of evolution in any context. For cognitive ecology, this will neces-sarily require investigating the relationship between variation at the level of individual genes and their function in neural circuits. In this regard, prai-rie vole V1aR expression and male monogamy again provide a useful model. The emergence of the resident tactic in the ancestors of modern prairie voles clearly required evolutionary changes in the pattern of *avpr1a* gene expression rather than changes in coding sequence (Young et al. 1999). As we have already reviewed, prairie voles differ from polygamous congeners in their neuronal pattern of V1aR expression, and these differences are caus-ally related to their capacity to form pair-bonds. In a seminal study, Young et al. (1999) generated a transgenic mouse that expressed the prairie vole *avpr1a* locus under the control of an upstream prairie vole noncoding se-quence. The transgenic mouse more closely resembled the V1aR phenotype of prairie voles than it did a wild-type mouse. Critically, intracerebral injec-tions of vasopressin caused the transgenic mice to form the specific social preferences characteristic of pair-bonding, while vasopressin injections into the wild-type mice had no effect. Within the ~1100 bases of the prairie vole regulatory sequence included in the transgene, the most conspicuous differ-ence between prairie voles and promiscuous congeners lay in the expansion of a microsatellite repeat near the transcription start site. Further analyses re-vealed that this repeat was shared by the monogamous pine vole (Young et al. 1999).

A series of elegant studies demonstrated that in cultured cells, a common model for gene expression studies, the microsatellite could alter gene expres-sion (Hammock and Young 2004, 2005). Hammock and Young (2005) found that male prairie voles with long and short microsatellite repeat lengths dif-fered in their neuronal V1aR abundance. This led the researchers to suggest that the prairie vole microsatellite length might cause both interspecies differ-ences in mating system and intraspecies variation in mating tactics. Whether the microsatellite caused interspecies variation was challenged by Fink et al. (2006), who demonstrated that a long microsatellite was a basal feature of the clade, and that the promiscuous meadow and montane voles shared a reduction in its length through descent. Although this made clear that having a long *avpr1a* microsatellite was not sufficient to predict monogamy, it did not address whether more subtle variation in length or sequence caused differ-ences between Microtine species or among prairie voles (Young and Hammock 2007). We set out to examine the latter in natural settings by genotyping the

animals in our preceding studies. Could long microsatellite alleles predict tactic? Or, more subtly, could they predict success within a tactic?

To simplify our analysis, we focused on males that had either two long alleles (both above median length) or two short alleles (both below median length). We found that long-allele males had generally higher V1aR abundance when averaged across all brain regions (Ophir et al. 2008a). We also found differences in two regions implicated in monogamous behavior, the ventral pallidum and medial amygdala (figs. 9.8a and 9.8c). The ventral pallidum is clearly causally related to pair-bonding (Lim et al. 2004). The medial amygdala conveys pheromonal information to the ventral pallidum and is important for paternal care (B. Kirkpatrick et al. 1994; Young and Wang 2004); thus, although the medial amygdala is not known to play a role in pair-bonding, a concordance between it and the ventral pallidum could coordinate residency.

Although the neuronal data were promising, our behavioral data revealed no significant differences between genotypes in any measure. We could not say that long-allele males behaved "more monogamously." Long-allele animals were no more likely to become residents, did not have smaller home ranges,
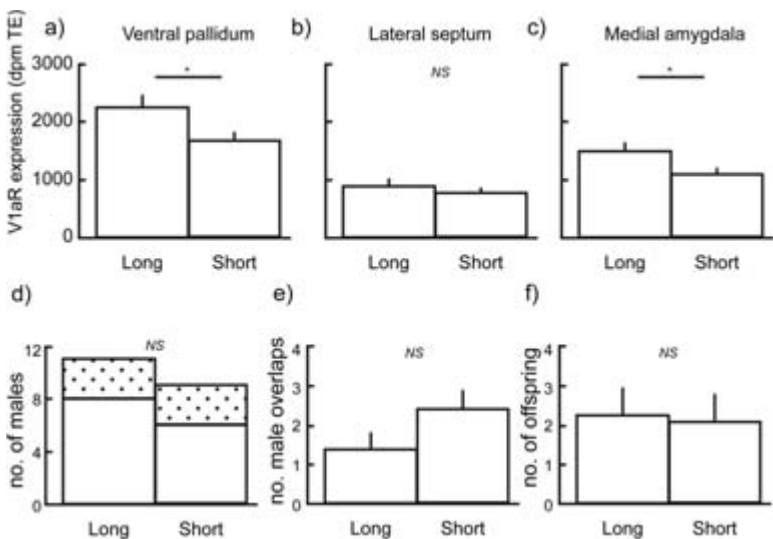

FIGURE 9.8. Microsatellite allele length influences V1aR abundance but not behavior or fitness. a–c. Long-allele males have higher V1aR in two nodes in the "pair-bonding circuit," the ventral pallidum, and the medial amygdala (T-test, *$P < 0.05$) but no differences in the lateral septum ($P > 0.10$). d–f. Despite differences in neural phenotype, we detect no effects on (d) the likelihood of becoming a resident (solid) or wanderer (stippled) (Fisher's exact, $P > 0.10$), (e) the number of male home ranges overlapped ($P > 0.10$), or (f) the number of offspring sired ($P > 0.10$). Data from Ophir et al. 2008a.
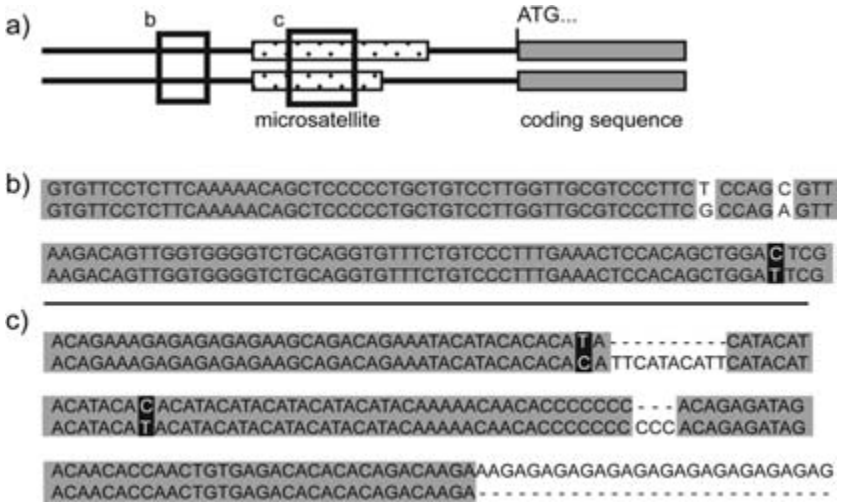
FIGURE 9.9. Nonhomologous origins of microsatellite length variation. a. Schematic of two *avpr1a* alleles with differing lengths of microsatellite repeats. Open boxes depict sites of sequences in panels b and c. b. Alignments of a long allele (top sequence) and short allele (bottom sequence), showing single nucleotide polymorphisms 5′ of the *avpr1a* microsatellite. c. Alignments of the same two alleles within the complex *avpr1a* microsatellite. Although the long allele has an expanded GA repeat, it also has shorter CATA and polyC repeat lengths. Thus, different repeat motifs within the microsatellite can make unique contributions to length. Focusing on allele length misses potentially important sequence variation within the microsatellite and in neighboring sequences. Modified from Ophir et al. 2008a.

nor did they overlap the home ranges of fewer conspecifics (figs. 9.8d and 9.8e; Ophir et al. 2008a). The conclusion that length had no influence on natural behavioral variation was also supported by the lack of allele-length effects on PCing and LDThal variation, the only brain regions that were associated with behavioral differences (see Ophir et al. 2008c).

These data demonstrate that microsatellite variation influences *avpr1a* expression but that this does not translate into behavioral differences in natural settings. Thus, the extraordinary variation that persists at the microsatellite can conceivably be attributed to its lack of influence on fitness. Indeed, long-allele males were no more or less likely to sire young than were short-allele males (fig. 9.8f).

Finding that allele length did not predict behaviors in our study led us to reexamine the data from the transgenic study by Young et al. (1999). If the length of the microsatellite did not drive a prairie vole pattern of V1aR expression and social behavior in transgenic mice, something else within the sequence of the transgene must have. We suggest that sequence variation within or near the microsatellite, rather than length alone, is responsible for the prai-

rie vole V1aR expression pattern and its influence on monogamy. The *avpr1a* microsatellite is a complex repetitive sequence, and there are many ways to produce equivalent lengths (fig. 9.9). Thus, within- and between-species differences in V1aR expression may yet map to sequence variation at this locus. If so, it will open up a rich array of experiments that can examine the persistence of genetic diversity and its contributions to the cognitive variation that underlies behaviors in natural environments.

9.5. Monogamy and cognitive ecology reconsidered

In a classic study, Bateman (1948) suggested that male fitness increases with number of mates, while female fitness does not. Emlen and Oring (1977) elaborated on this theme to suggest that females distribute themselves according to resources that influence their reproductive success, while males distribute themselves in a manner that maximizes the number of females they can monopolize. Thus, when females are widely dispersed in space, the effort required to monopolize females may preclude polygyny. Similarly, when female mating is synchronized with that of other females, males may be unable to monopolize multiple females simultaneously. Using game theory, Sandell and Liberg (1992) examined whether males should be territorial or nonterritorial as a function of female encounter rate, female defensibility, and the degree of resident advantage (the ability of a resident to dominate an intruder). The model demonstrated that the strategies reflected trade-offs in each of these domains, with increases in resident advantage favoring territoriality, and increases in female encounter rate favoring wandering.

Among socially monogamous animals, the common occurrence of female polygamy presents a conceptual challenge because it undermines male paternal investment and contradicts Bateman's view of female fitness as unaffected by the number of mates. One explanation emphasizes conflict over paternity, in which it benefits females to mate with extrapair males to gain either "good genes" or some other benefit, such as infanticide deterrence (Wolff and Macdonald 2004). Kokko and Morrell (2005) note that attractive males must make trade-offs between intrapair and extrapair paternity in order to maximize their fitness. The ideal male strategy emerges as a complex interaction between male attractiveness, female fidelity, and male mate-guarding efficacy. According to these models, males should mate-guard more when females occasionally attempt extrapair matings and when resident males are good at excluding intruders.

Many of the variables that should shape male tactics have correlates in the data we have reviewed. For example, the ultimate decision to mate-guard

requires a male to be confident of paternity (Trivers 1972; Kokko 1999; Kokko and Morrell 2005) and to be effective at monopolizing the female (Sandell and Liberg 1992; Kokko and Morrell 2005). From a proximate perspective, males form pair-bonds and become territorial only after 24 hours of mating (Insel et al. 1995), and this is mediated by the prolonged release of vasopressin during this period (Winslow et al. 1993). Vasopressin acts on reward structures to promote proximity, and this in turn facilitates mate guarding. Thus, high levels of V1aR in the ventral pallidum enable males to become mate-guarding residents once a prolonged mating has ensured both paternity and the more general ability to monopolize the female during estrus.

Because pheromonal cues activate vasopressin cells that project to many parts of the forebrain (Murphy et al. 1997; de Vries and Miller 1998; Young and Wang 2004), it seems likely that vasopressin modulates brain regions in response to social encounters of multiple sorts. Indeed, vasopressin has been implicated in a diverse suite of social behaviors (e.g., Dantzer et al. 1988; Ferris and Delville 1994; Goodson and Bass 2001). If V1aR in the ventral pallidum increases responses to mating rewards, what is the function of V1aR in the PCing? We hypothesize that high PCing V1aR facilitates memory for the locations of territory intrusions, which in turn increases a resident's ability to exclude intruders. If this is the case, why would low PCing V1aR persist? One clue comes from the fact that every male that obtained an EPF lacked PCing V1aR altogether (fig. 9.10; Ophir et al. 2008c). Wandering and paired males who intrude onto a neighbor's territory are likely to encounter a local resident. In most species, including many rodents, residents are dominant over intruders (Maynard Smith and Parker 1976; Gauthreaux 1978; Wolff et al. 1983; Yoder et al. 1996). By recalling the spatial context of such social defeats, intruding males could avoid repeated encounters with these males, but they will also encounter fewer females. Thus, low PCing might be regarded as a means of "adaptive forgetting" by wandering males and may incidentally promote extrapair paternity among resident males as well.

To examine this more closely, we calculated the proportion of matings obtained by males with high or low PCing V1aR expression. ("High" V1aR was defined as above 400 dpm/mg TE; "low" expression was defined as below 400 dpm/mg TE and is at or near background levels.) We found that half of wanderers with low PCing were able to mate, but none of the high-PCing wanderers were successful (fig. 9.10). Resident males with high PCing mated about as often as did residents with low PCing. This seems to suggest that high PCing is not advantageous for resident males. However, a closer look reveals that low-PCing males are nearly three times as likely to be cuckolded. Moreover, roughly one-third of the low-PCing resident matings were EPFs. Thus,
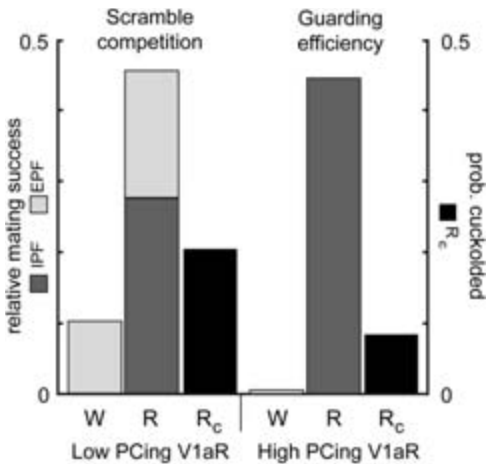
FIGURE 9.10. A balanced polymorphism in PCing V1aR abundance? Relative mating success was defined as the proportion of total fertilizations obtained by males of each class. W = wanderers; R = residents; dark gray bars correspond to success obtained through IPFs, and light gray bars to EPFs. On the same scale we have plotted the probability of being cuckolded by males of either brain phenotype (R$_c$, black bars). Half of the low-PCing wanderers were successful, while none of the high-PCing wanderers fertilized embryos. Similarly, all residents engaging in EPFs exhibited low PCing V1aR. While high-PCing males gained slightly fewer matings on average, they were much less likely to be cuckolded. Low PCing V1aR seems to be suited to scramble competition, and high V1aR to mate guarding.

it seems that high PCing V1aR maximizes mate-guarding efficiency, while low PCing facilitates a scramble tactic by increasing female encounter rates.

Interestingly, this interpretation is also supported by cross-species comparisons. Pine voles are widely dispersed (Fitzgerald and Madison 1983), presumably reducing potential female encounter rates and favoring mate guarding over wandering. This is reflected in both genetic monogamy (Marfori et al. 1997) and high levels of PCing V1aR (fig. 9.7; Insel et al. 1994). Montane voles have a very short breeding season triggered by new shoot growth, resulting in the synchronous breeding of many females (Negus and Berger 1977; Negus et al. 1977). Synchronously breeding females are difficult to defend, and this situation should favor a scramble tactic (Emlen and Oring 1977). Accordingly, the species is polygamous, is nonterritorial, and does not express V1aR in the PCing (Insel et al. 1994, 1995). The interspecific data, like the intraspecific data, suggest a trade-off between efficient mate guarding and female encounter rates mediated by cingulate V1aR.

Together these findings provide insight into both the evolution of mating systems and animal behavior more generally. The interaction between vasopressin and the ventral pallidum provides an interesting example of phenotypic plasticity in the neuroendocrine regulation of behavior. By making pair-bonding and residency contingent on repeated and prolonged mating, the mechanism ensures that a male is likely to be a successful resident before committing to the tactic. The association of cingulate V1aR variation with

intrapair and extrapair paternity highlights how behavioral specializations can make conflicting demands of cognitive substrates. Lastly, the combination of reward and spatial memory systems reveals how diverse mechanisms are needed to execute a cohesive and successful tactic. Although there are many causal details that remain to be explored, our results provide a glimpse of how integrative approaches may yield a more complete understanding of animal behavior. As new methods permit the manipulation of V1aR and other genes in natural environments, such studies promise to clarify both the mechanisms of natural behavior and the origins of behavioral diversity.

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