Hormones and Behavior 54 (2008) 694–702

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Field tests of *cis*-regulatory variation at the prairie vole *avpr1a* locus: Association with V1aR abundance but not sexual or social fidelity

Alexander G. Ophir *[,1], Polly Campbell [1], Kristen Hanna, Steven M. Phelps

Department of Zoology, University of Florida, PO Box 118525, Gainesville, FL 32611, USA

ARTICLE INFO

Article history:
Received 4 April 2008
Revised 17 July 2008
Accepted 17 July 2008
Available online 30 July 2008

Keywords:
Alternative mating tactics
Evolution
Gene regulation
Mating system
Microtus ochrogaster
Neuroendocrinology
Simple sequence repeats

ABSTRACT

The neuropeptide vasopressin and its receptor V1aR are broadly implicated in social behavior and play a central role in several key aspects of male mating tactics in voles. In the prairie vole, a microsatellite in the *cis*-regulatory region of the gene encoding V1aR (*avpr1a*) provides a potential genetic basis for individual variation in neural phenotype and behavior; recent studies found that allele length predicts V1aR expression and male social attachment in the laboratory. Here, we explore the relationship between *avpr1a* microsatellite length, V1aR neural phenotype, and field measures of monogamy and fitness in male prairie voles. We found significant effects of allele length on V1aR expression in structures integral to pairbond formation. These effects did not, however, translate to differences in mating tactics or reproductive success. Together, these data suggest that, while length polymorphism in the *avpr1a* microsatellite influences neuronal phenotype, this variation does not contribute significantly to male reproductive success and field behavior. We propose that previously reported behavioral effects may be mediated primarily by sequence variation at this locus, for which allele length is an imperfect proxy. By combining genetic, neuronal and ecological approaches, these data provide novel insights into the contribution of genotype to natural diversity in brain and behavior.

Published by Elsevier Inc.

Introduction

The neuropeptide arginine vasopressin (AVP) acts on the V1a receptor (V1aR) to coordinate a diverse range of mammalian social behaviors (Young et al., 2005). Experimental evidence demonstrates that V1aR distribution in the forebrain is integral to species-typical aspects of male sociosexual, parental and aggressive behavior in rodents (Bielsky et al., 2004; Ferris et al., 1997; Lim and Young, 2004). Interspecific differences in V1aR expression patterns correlate with mating system in voles (Insel et al., 1994; Young, 1999); comparative studies of monogamous and non-monogamous species demonstrate that manipulating AVP or V1aR can facilitate or eliminate pairbonding, a keystone behavior of monogamy (Lim and Young, 2004; Winslow et al., 1993). Based on these and other studies, Young and Wang (2004) proposed that male monogamy in the prairie vole (*Microtus ochrogaster*) is mediated by an integrated system of neural circuits, which depends critically on the action of AVP on V1aR in the ventral pallidum and lateral septum, and their interactions with regions like the medial amygdala, which influences paternal care and relays social information from the accessory olfactory bulb.

In natural settings, most prairie vole males adopt a monogamous "resident" tactic, which involves pairing with a single female, aggressively defending a territory, and providing parental care to young (Getz et al., 1993). In contrast, a significant minority of males adopt a "wanderer" tactic, which involves roaming broadly over multiple territories; although wanderers sire offspring, their space use indicates that they are not pairbonded (Ophir et al., 2008a, 2008b). The mechanistic origins for this behavioral diversity are not understood.

A microsatellite in the *cis*-regulatory region of the *Microtus* V1aR gene (*avpr1a*) provides a potential mechanism for the maintenance of adaptive diversity at this otherwise conserved locus (Young et al., 1999). The microsatellite is highly polymorphic in prairie voles and, in males, allele length is positively correlated with greater expression of "monogamous" behaviors (Hammock and Young, 2002, 2005). Based on these laboratory studies, Hammock and Young (2005) hypothesized that polymorphism at the *avpr1a* microsatellite locus explains the maintenance of resident and wanderer tactics in natural populations of prairie voles. Here, we test this hypothesis, and ask whether correlations between allele length, V1aR expression and male social behavior in the laboratory are present in freely behaving animals under semi-natural conditions.

If *avpr1a* microsatellite allele length modulates natural behavioral diversity in male prairie voles through V1aR expression, we expect that males with longer alleles should have higher V1aR expression in the ventral pallidum, lateral septum, medial amygdala, and other areas

* Corresponding author. 618 Carr Hall, Department of Zoology, University of Florida, PO Box 118525, Gainesville, FL 32611, USA.
E-mail address: aophir@ufl.edu (A.G. Ophir).
[1] Ophir and Campbell were equal contributors to this work and primary authorship is shared jointly.

0018-506X/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.yhbeh.2008.07.009

critical to monogamous behavior (e.g. Young et al., 2005). In the field, we expect that males with longer alleles should be more likely to become residents, exhibit higher mate fidelity, and employ more "monogamous" patterns of space use, including smaller home ranges and fewer conspecific home range overlaps. We have previously documented that resident males sire more young (Ophir et al. 2008a). Thus if microsatellite length is related to adaptive variation in male sociosexual behaviors, we also expect a correlation between allele length and male fitness, inferred from the number of offspring sired.

Materials and methods

Test animals and field enclosures

A total of 48 male and 48 female prairie voles were allowed to behave freely in semi-natural field enclosures. All individuals were laboratory-reared and group-housed, and were first, second or third generation descendents of wild-caught founders from Shelby County, Tennessee (TN) or Champaign County, Illinois (IL). Each enclosure trial consisted of animals taken from the same population. Earlier laboratory and field studies detected no behavioral or morphological differences between voles from the two sites (Ophir et al., 2007); population comparisons of V1aR expression and of *avpr1a* allele frequencies are provided in results.

All individuals were ear-tagged and weighed prior to initiating field enclosure trials. Animals were distributed into eight groups, each consisting of six nulliparous females and six adult, sexually mature males, standardized for age and body mass across enclosure trials. Vole densities in each enclosure trial were within the range reported for natural prairie vole populations (Getz et al., 1993; Taitt and Krebs, 1985).

The study was conducted using four field enclosures located on the University of Memphis (for details see Mahady and Wolff, 2002). Each enclosure measured 20×30 m and included a 4×5 grid with 4 m spacing. Vegetation within each enclosure consisted primarily of mixed pasture grasses and dicots. This experiment was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Florida (project number D289) and the University of Memphis (project number 0012), and was in accordance with the guidelines set by the UF and UM Animal Research and Care Committees.

Measures of space use, paternity and V1aR expression in 4 brain regions from these same subjects have been reported elsewhere (Ophir et al., 2008a, 2008b). In the current paper, we combine these data with *avpr1a* microsatellite lengths and V1aR measures in 11 new brain regions. All analyses are novel.

Radio telemetry and tissue collection

Each vole was fitted with a 1.9 g transmitter and collar (BD-2C, Holohil Systems Ltd., Carp Ontario) 2 days prior to introduction to the field. Animals were closely monitored before field introduction to ensure that the collars did not cause discomfort or alter behavior.

We ran a series of four trial blocks over the 2004 breeding season, each consisting of two simultaneous trials (Ophir et al., 2008a). In each trial, we allowed 3 days for animals to establish territories and find shelter. On the third day following introduction, we began taking telemetry readings twice daily (between 06:00 and 20:00 h) for at least 12 days, at varying time of day and enclosure order. Animals were tracked to within 1 m of their actual location. On day 20 we began trapping animals and removing them from the enclosure, allowing enough time for fertilization but not parturition (gestation is 21 days).

Immediately after trapping, we euthanized subjects with CO_2 followed by rapid decapitation to collect brains for V1aR autoradiography. Brains were dissected, frozen on powdered dry ice and stored at −70 °C until sectioning. We collected a tissue sample (liver or tail) from all animals for genetic analysis. We counted the number of embryos per pregnant female and measured each embryo from crown to rump. Embryo size corresponds roughly to the time since fertilization occurred, with each mm approximating one day (J. O. Wolff, personal communication).

Home range size, space use, and pair determination

We calculated 75% core minimum convex polygons (MCP) from the assembled *X* and *Y* coordinates to estimate the size of each principle-core home range (see Ophir et al., 2008a). From these MCPs, we determined the number of times a given male's home range overlapped those of other females or males, and which individuals demonstrated paired behavior.

Pairbonded prairie voles share and mutually defend a common home range (Getz et al., 1993). Thus, the home ranges of paired individuals should overlap their partner's home range more than any other conspecific. To determine pairs, we first estimated encounter rates between all possible pairs of males and females by taking the product of the proportion of the home range area a given male overlapped a given female, and *vice versa*. Next we calculated 'relative encounter rates' (RER) for each possible pair by dividing the encounter rate for a given pair by the sum of encounter rates for all opposite-sex individuals (Ophir et al., 2008a). We considered animals paired if they encountered one another more frequently than all other opposite-sex individuals combined (i.e. a mutual relative encounter rate ≥0.5).

In order to develop a continuous measure of male social fidelity, we also recorded the RER each male exhibited with his most-encountered female (RER_{MAX}). We used this measure to examine whether allele length sums correlated with the degree of selective attachment a male exhibited (high RER_{MAX} is interpreted as high attachment).

Genetic analyses

DNA was extracted using a Qiagen DNEasy kit (Qiagen Inc., Valencia, CA). Paternity assignment for embryos was based on four highly polymorphic microsatellite loci (MSMM6 and MSMM2 [Ishibashi et al., 1999]; MOE2 [Van de Zande et al., 2000]; AV13 [Stewart et al., 1998]). Amplification conditions and details of paternity analysis are reported in Ophir et al. (2008a).

Analysis of the *avpr1a* microsatellite was restricted to adult males. Because prairie voles carry an *avpr1a* pseudogene, which includes the 5′ regulatory region containing the microsatellite (Young et al., 1999), we used a nested PCR design to assure amplification from the functional copy of *avpr1a*. Primers used in the first reaction (Rxn 1) targeted a sequence unique to functional *avpr1a* (−1536.F 5′ CCACAAATAGACCAACGTTCTTAAG 3′ and +849.R 5′ AGTCTTCACGCTGCTGACAC 3′; names relative to transcriptional start site). Rxn 1 amplifications were carried out in a volume of 25 μl using 12.5 μl GoTaq Master Mix (Promega), ~180 ng template DNA and 0.1 mM of each primer. Rxn 2 comprised 0.01 μl Rxn 1 PCR product, 0.4 mM of each primer (florescent-labeled −1376.F 5′ AAACTCCACAGCTGGACTCG 3′ and −834.R 5′ GTTACTGTAGAAAGCCAGGTTCC 3′) and 6.25 μl GoTaq Master Mix in a 12.5 μl total volume. Annealing temperatures for both reactions were 58–60 °C. PCR products were run on a MegaBASE 1000 automated sequencer (Amersham Biosciences); allele size was quantified and scored in GeneMarker (v. 1.4). Because an excess of homozygotes detected in preliminary analyses implicated one or more null alleles, we re-ran Rxn 2 for all homozygotes, using combinations of original and alternate primers. Second alleles were recovered in 6/20 males with primers −1376.F and −788.R (5′ GGTTATTCCACATGTCCAGC 3′).

Population F_{ST} values for the *avpr1a* microsatellite locus were calculated in arlequin (v. 3.0; Schneider et al., 2000). Heterozygosity was calculated in fstat (v. 2.9.3.2; Goudet, 1995). Within-population deviations from Hardy–Weinberg equilibrium and heterozygote

Table 1
Correlations with summed allele lengths

	N	*r*	*P*
Brain			
OB	36	0.126	0.47
AOB	36	0.069	0.69
VPall	35	0.338	0.05
LS	36	0.074	0.67
BST	36	0.156	0.37
PCing	36	0.099	0.56
CeA	36	0.357	0.03
MeA	36	0.404	0.01
LD Thal	36	0.114	0.51
MD Thal	36	0.214	0.21
VP Thal	36	-0.023	0.89
AH	36	0.264	0.12
PaV	36	-0.226	0.18
SCN	36	-0.298	0.08
VMH	36	0.301	0.07
Total brain V1aR	34	0.365	0.03
Behavior			
No. of offspring	39	0.113	0.49
Embryo size	23	-0.018	0.94
Home range size	38	-0.001	0.99
Male–Male ER	38	0.036	0.82
Male–Male overlap	38	-0.217	0.19
Male–Female ER	38	-0.038	0.82
Male–Female overlap	38	-0.117	0.49
Home range size	38	-0.001	0.99
Male–Male ER	38	0.036	0.82
RER_{MAX}	40	0.127	0.43

Embryo size was measured from crown-to-rump and is an index of time since conception. Home range size represents 75% minimum convex polygons. ER=encounter rate defined in the Materials and methods section. RER_{MAX}=relative encounter rate between a male and the female with whom the male had the most extensive home range overlap. OB=olfactory bulb; AOB=accessory olfactory bulb; VPall=ventral pallidum; LS=lateral septum; BST=bed nucleus of the stria terminalis; PCing=posterior cingulate/retrosplenial cortex; CeA=central amygdala; MeA=medial amygdala; LD Thal=laterodorsal thalamus; MD Thal=mediodorsal thalamus; VP Thal=ventral posterior thalamus; AH=anterior hypothalamus; PaV=paraventricular nucleus of the hypothalamus; SCN=suprachiasmatic nucleus; VMH=ventromedial hypothalamus.

deficits were evaluated in genepop (v. 3.4, Raymond and Rousset, 1995).

We examined the relationship between allele length, brain and behavior using two metrics. In the first, we categorized males as having either 'long' or 'short' *avpr1a* microsatellite genotypes by ordering all alleles by length, splitting the distribution at the median (greater than median='long'; less than median='short'), and selecting the subset of males for which both alleles fell within the same range (N_{long}=11; N_{short}=11). To reduce potential confounds represented by males with alleles of widely divergent lengths, heterozygous males carrying both 'long' and 'short' alleles were excluded from this analysis. Because sample sizes using these criteria were sometimes low, we supplemented these data by summing the lengths of both alleles for all males (N=40) and correlating this variable with brain and behavior variables. (Other transformations of allele lengths [minimum length, maximum length or log(sum of lengths)] produced equivalent patterns.) These analyses complement one another, and both have precedents in previous studies (short–long, Hammock and Young, 2005; summed allele length, Hammock et al., 2005).

V1aR autoradiography

Four sets of coronal slices (20 μm thick at 100 μm intervals) were sectioned on a cryostat, mounted on Superfrost slides (Fisher Scientific) and stored at −80 °C until processing. To visualize V1aR binding, sections were lightly fixed, incubated with 50 pM ^{125}I-linear-AVP (Perkin-Elmer), washed in Tris buffer and air-dried (Insel et al., 1994). Sections were then exposed to film for 72 h alongside radiographic standards.

Films were digitized on a Microtek ScanMaker 5900 and optical density of V1aR binding was measured using NIH ImageJ software. To quantify V1aR binding in each brain region we took three measures and averaged them. Non-specific binding was estimated from background levels of cortical binding on the same sections, averaged, and subtracted from the mean specific binding for the corresponding region of interest (Young et al., 1997). We quantified V1aR expression in 15 brain areas (see Table 1). Total brain V1aR expression was quantified as the average of measurements for all 15 regions.

Results

Assessing avpr1a microsatellite genotypes

We successfully genotyped 19 Illinois-derived males (IL) and 21 Tennessee-derived males (TN) at the *avpr1a* microsatellite locus and identified 16 alleles. Allele length ranged from 508 to 585 bp (Fig. 1). A subset of males was selected based on genotype (see Materials and methods). To maintain samples large enough to have sufficient power, we included three males for which one allele fell at the median (2 'long' males; 1 'short' male). For all other males included in this analysis, both alleles fell within either the upper or lower third of the allele size distribution. In our second analysis (N=40), summed allele lengths for each individual ranged from 1026 to 1170 bp.

The two populations were significantly differentiated at this locus (F_{ST}=0.06, P=0.003), and both exhibited heterozygote deficits (IL, H_{Obs}=0.53, H_{Exp}=0.88; TN, H_{Obs}=0.76, H_{Exp}=0.88) and significant departures from the Hardy–Weinberg equilibrium (IL, P<0.0001; TN, P=0.02). However, 'long' and 'short' genotypes were distributed approximately equally between IL and TN males (long: TN=5, IL=6; short: TN=6, IL=5). Thus, despite significant differentiation between populations based on allelic identity and evidence for non-equilibrium dynamics at this locus, the length-dependent distribution of *avpr1a* microsatellite genotypes relevant to this study was consistent across populations.

V1aR expression and avpr1a microsatellite allele length

We quantified V1aR binding in 15 brain regions, from the olfactory bulb to the level of the anterior hypothalamus for 36 males behaving freely in mixed-sex, semi-natural outdoor enclosures. (The brains of 4 males were not recovered, and therefore were not included in these analyses; and the condition of the sections containing the VPall for

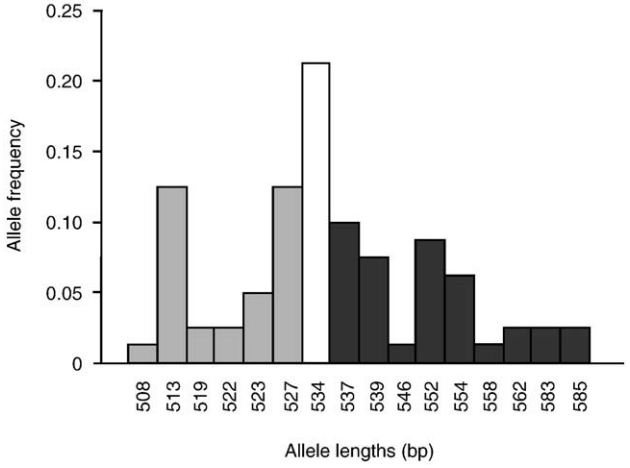

Fig. 1. Frequency distribution of *avpr1a* microsatellite alleles by the number of base pairs (bp). White bar represents the median, light gray bars encompass 'short' males, and dark gray bars encompass 'long' males.

one male was too poor to quantify.) Tennessee- and Illinois-derived males did not exhibit significant differences in V1aR binding in any of the 15 brain regions examined (1-factor ANOVA; all $Fs_{(1,\ 18)} \leq 3.76$; all $Ps \geq 0.07$). However, Illinois males tended to have more V1aR in the central amygdala ($P=0.07$), the lateral septum ($P=0.09$) and the bed nucleus of the stria terminalis ($P=0.11$; $P \geq 0.20$ for all other regions). The lack of significant differences in V1aR expression between the two populations of voles, further justified our pooling the animals (see Ophir et al., 2007).

As other studies have reported (Hammock et al., 2005; Phelps and Young, 2003), we found a high degree of individual variation in V1aR binding in some structures (e.g. posterior cingulate/retrosplenial cortex and thalamic nuclei) and relatively little in others (e.g. ventral pallidum and medial amygdala). Nonetheless, significant differences in V1aR expression between 'long' and 'short' *avpr1a* microsatellite genotypes were localized to the ventral pallidum (ANOVA; VPall: $F_{(1,\ 19)}=5.05$; $P=0.037$), medial amygdala (MeA: $F_{(1,\ 19)}=4.82$; $P=0.040$), and the ventromedial hypothalamus (VMH: $F_{(1,\ 19)}=4.83$; $P=0.041$; Figs. 2A, C). In all three regions, V1aR expression was higher in 'long' males. V1aR expression in the central amygdala (CeA) and the suprachiasmatic nucleus (SCN) exhibited non-significant trends toward differences between 'long' and 'short' genotypes (CeA: $F_{(1,\ 19)}=2.99$; $P=0.100$; SCN: $F_{(1,\ 19)}=2.45$; $P=0.134$; Figs. 2A, C). In all focal brain regions, except the SCN and the paraventricular hypothalamic nucleus (PaV), V1aR expression was consistently higher in 'long' males (Fig. 2). This trend was significant when V1aR expression was averaged across brain regions (total brain: $F_{(1,\ 18)}=5.17$; $P=0.035$; Fig. 2B).

Summed allele length was significantly correlated with V1aR expression in the VPall (Pearson's correlation; $N=35$, $r=0.34$, $P=0.05$; Fig. 3A), MeA ($N=36$, $r=0.40$, $P=0.01$; Fig. 3C), and CeA ($N=36$, $r=0.36$, $P=0.03$; Fig. 3E). We observed non-significant trends in the relationships between summed allele length and the VMH ($N=36$, $r=0.30$, $P=0.07$; Fig. 3B), SCN ($N=36$, $r=-0.30$, $P=0.08$; Fig. 3D), and AH ($N=36$, $r=0.26$, $P=0.12$). V1aR expression in all the other brain regions that we examined was not significant (Table 1). Most relationships between allele length and V1aR brain expression were positively correlated (Table 1), and total brain V1aR expression was significantly correlated with allele length ($N=34$, $r=0.36$, $P=0.03$; Fig. 3F). The overall pattern was consistent with our findings based on long vs. short comparisons. In both analyses, allele length was significantly and positively associated with the ventral pallidum and medial amygdala V1aR and total brain V1aR. The CeA, VMH and SCN exhibited consistent relationships across analyses, but the exact *P*-values fluctuated (Figs. 2, 3 and Table 1).

Behavior and avpr1a microsatellite allele length

Unlike V1aR expression in the brain, *avpr1a* allele length did not predict monogamous behavior in the field. Paired males ($N_{long}=8$; $N_{short}=6$) were just as likely to exhibit a 'long' as a 'short' genotype (Fisher's exact test; $P=1.0$; wandering males $N_{long}=3$; $N_{short}=3$; Fig. 4A). Similarly, summed allele length did not differ between residents and wanderers (ANOVA; $F_{(1,\ 34)}=0.129$; $P=0.72$; $N_{residents}=28$, $N_{wanderers}=8$; Fig. 4C). Allele length failed to predict whether animals exhibited sexual fidelity, infidelity or did not sire offspring (Figs. 4B, D). This was most evident when comparing summed allele lengths of males that produced EPF, IPF or no offspring ($N=5$, 17 and 18 respectively; $F_{(2,\ 29)}=1.103$; $P=0.34$; Fig. 4D). A similar pattern emerged when comparing 'long' and 'short' males, although the sample sizes in this analysis are anecdotal (IPF, $N_{long}=5$, $N_{short}=3$; EPF, $N_{long}=1$, $N_{short}=2$; Fisher's exact test; $P=0.55$; Fig. 4B).

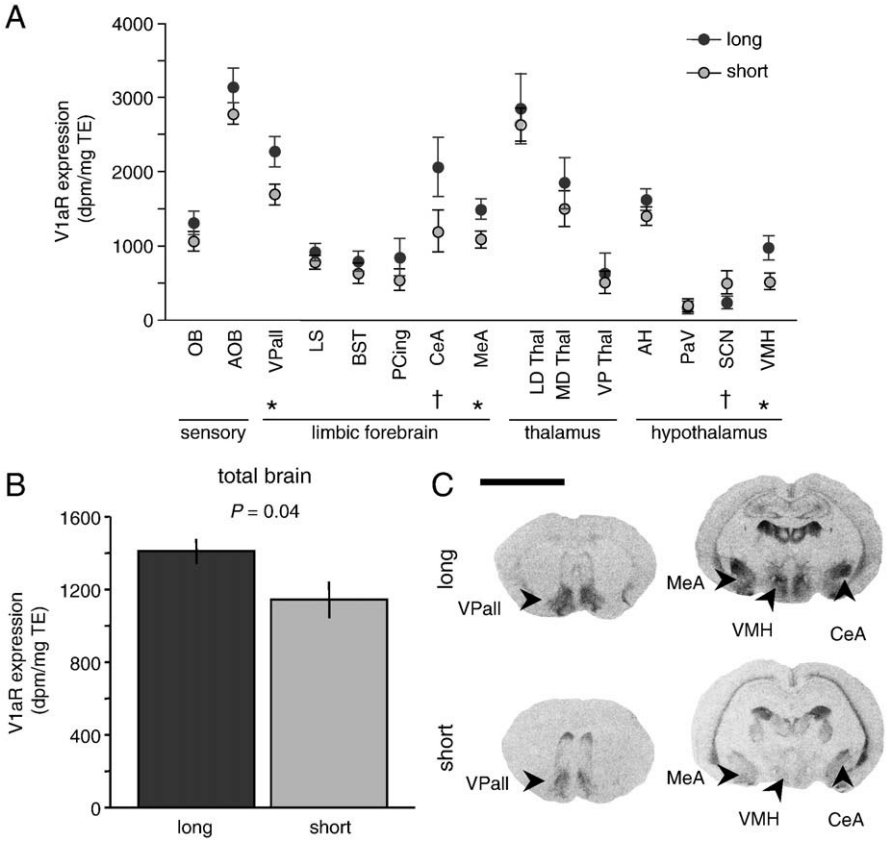

Fig. 2. (A) Mean (±SE) V1aR binding (in disintegrations per minute per milligram in tissue equivalence [dpm/mg TE]) for 'long' and 'short' males across the forebrain. '*' indicates $p<0.05$; '†' indicates a non-significant trend $p<0.15$. Abbreviations of neural structures are defined in Table 1. (B) Mean (±SE) total brain V1aR expression for 'long' (dark gray) and 'short' (light gray) males. (C) Audioradiograms of exemplar 'long' and 'short' V1aR binding patterns in the VPall, MeA, CeA, and VMH. Scale bar=5 mm.

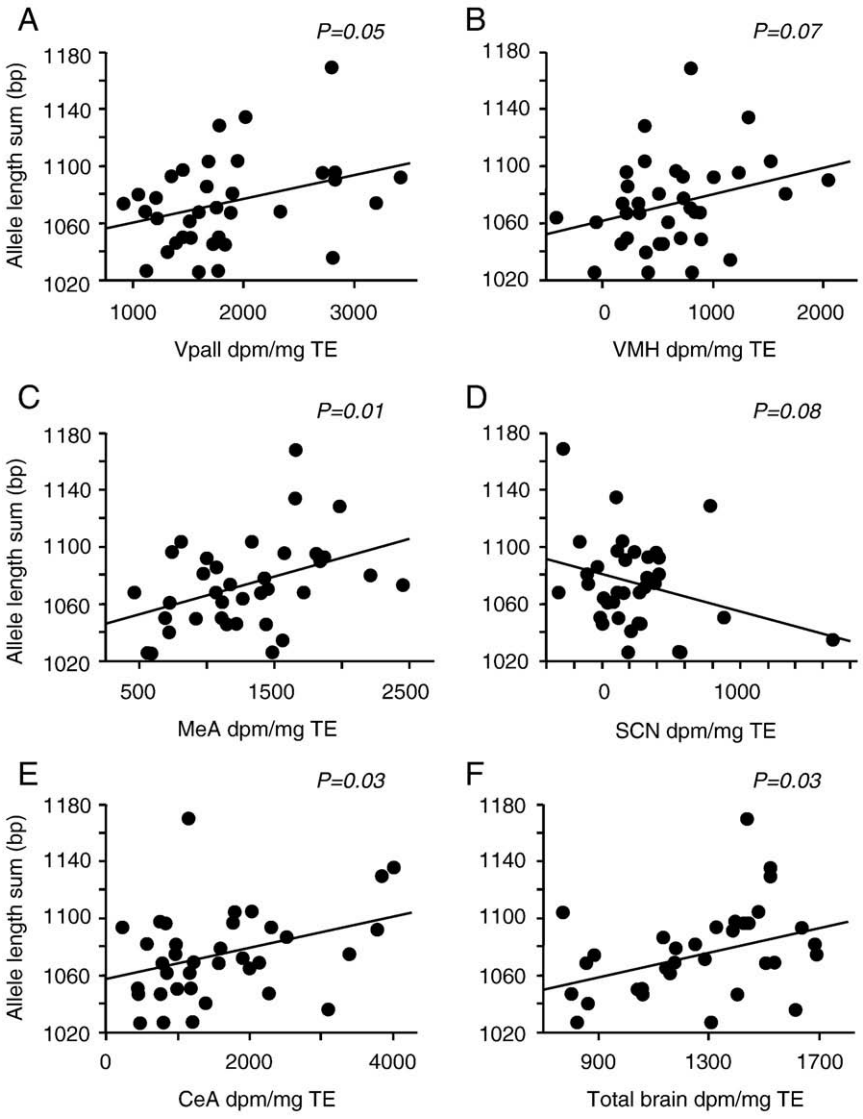

Fig. 3. Correlations between allele length (calculated as the sum of the number of base pairs [bp] for each allele) and V1aR binding in the (A) ventral pallidum (VPall), (B) ventromedial hypothalamus (VMH), (C) medial amygdala (MeA), (D) suprachiasmatic nucleus (SCN), (E) central amygdala (CeA), (F) paraventricular nucleus of the hypothalamus (PaV), (G) total brain binding, and (H) anterior hypothalamus (AH). V1aR binding represents ^{125}I-linear-AVP-specific binding disintegrations per minute (dpm) per mg in tissue equivalence (TE).

Length of the *avpr1a* microsatellite had no influence on male breeding success. 'Long' and 'short' males sired the same number of offspring *per capita* ($F_{(1,\ 20)}$=0.031; P=0.86; Fig. 5A). The timing of conception estimated by embryo size was similar for 'long' and 'short' males ($F_{(1,\ 9)}$=0.19; P=0.67; Fig. 5B). Moreover, summed allele length did not differ between males that sired offspring and those that did not ($F_{(1,\ 39)}$=1.22; P=0.28), nor did it correlate with the size of embryos (N=23, r=−0.02, P=0.94).

Monogamous patterns of male space use are characterized by small home ranges, which overlap few female home ranges (primarily just that of the mate) and few male home ranges (i.e. excluding potential usurpers). Our data show that there were no significant differences in the manner 'long' and 'short' genotype males used space. Regardless of genotype, male home ranges overlapped a similar number of females ($F_{(1,\ 19)}$=0.53; P=0.48; Fig. 5C) and males ($F_{(1,\ 19)}$=2.75; P=0.11; Fig. 5D), and allele length did not predict home range size ($F_{(1,\ 19)}$=0.27; P=0.61; Fig. 5E). Consistent with these results, summed allele length did not correlate with any measure of space use or monogamous behavior (Table 1). Even RER_{MAX}, arguably the most direct continuous measure of attachment, failed to correlate with allele lengths (Table 1, Fig. 5F).

Discussion

We explored the relationship between length polymorphism in a microsatellite in the *cis*-regulatory region of the prairie vole *avpr1a* gene, natural variation in neuronal V1aR expression, and field measures of male reproductive behavior. We found a positive relationship between allele length and V1aR expression in several brain regions, but no significant relationship between allele length and either behavior or fitness. These findings refute the hypothesis that alternative mating tactics adopted by male prairie voles are caused by individual differences in the *avpr1a* microsatellite allele lengths. The results are similar in some respects to prior data based on laboratory studies of prairie voles; the disparities, however, reveal a need to refine our interpretations of associations between microsatellite length, neuronal V1aR expression, and social behavior.

Microsatellite variation and V1aR expression

The cumulative findings of over a decade of research on the neurobiology of social attachment in voles provide a well-supported framework for a 'pairbonding neural circuit', to which three V1aR-

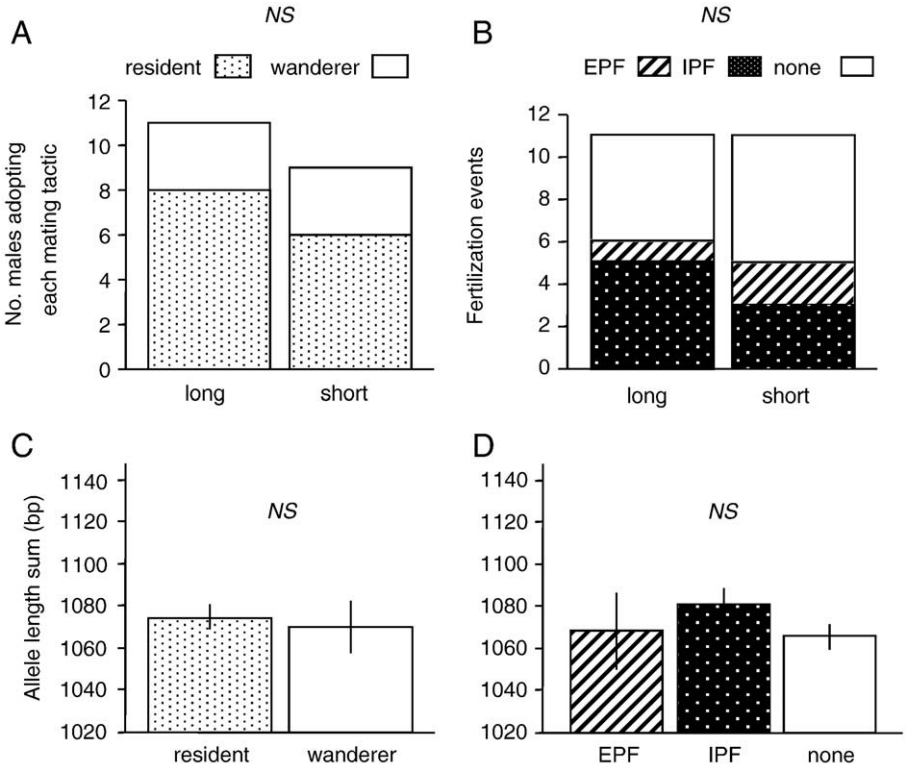

Fig. 4. (A) The number of paired resident (stippled) and single wanderer (white) males exhibiting a 'long' or 'short' genotype. (B) The number of 'long' and 'short' males that sired offspring exclusively with their paired mate (in-pair fertilization or IPF; stippled), sired offspring with a non-pair mate (extra-pair fertilization or EPF; striped), or did not sire offspring (white). Mean (±SE) allele length (calculated as the sum of the number of base pairs [bp] for each allele) of (C) resident and wanderer males and (D) males that sired offspring with a non-pair mate (EPF), sired offspring exclusively with their paired mate (IPF), or did not sire offspring (none).

expressing nuclei are integral: the ventral pallidum, lateral septum, and medial amygdala (Insel et al., 1994, Young et al., 2005). We found that in two of these regions, the ventral pallidum and medial amygdala, longer microsatellite alleles were associated with higher expression of V1aR (Figs. 2A, 3A, C). This pattern is consistent with our prediction that allele length should be positively correlated with a neural phenotype that promotes monogamy. Although we did not find a relationship between allele length and V1aR expression in the LS we note that, as in most neural areas we investigated, 'long' males tended to express more V1aR in the LS. While this trend is suggestive, the effect is weak (Fig. 2A, Table 1), and prior literature demonstrates that the relationship between V1aR in the LS and pairbonding is complex. Non-monogamous species of voles express more septal V1aR than monogamous species (Insel et al., 1994), and V1aR antagonists disrupt prairie vole pairbonding only at intermediate doses (Liu et al., 2001). The apparently modest influence of allele length in the lateral septum contrasts with other regions of the pairbonding circuit and underscores this complexity.

As in the VPall and MeA, we found a positive relationship between allele length and V1aR expression in the ventromedial hypothalamus (VMH; Figs. 2A, 3B). While it is unclear how microsatellite regulation of V1aR in the VMH relates to functions of the pairbonding circuit, it is worth noting that the MeA projects to the VMH (Kevetter and Winans, 1981; Sheehan et al., 2001). Although the VMH is best known for its role in female sexual behavior (e.g. Musatov et al., 2006; Pfaus et al., 1993), a recent study in prairie voles suggests that this structure may also be involved in the initial stages of social bond formation (Cushing et al., 2003). Similarly the central amygdala (CeA) showed a positive relationship between allele length and V1aR expression (Figs. 2A, 3E). Such an effect could mediate interactions between genotype, rearing environment, anxiety, aggression and social behavior (Elkabir et al., 1990; Francis et al 2002).

Of the 15 brain regions we examined, only the suprachiasmatic and paraventricular nuclei exhibited trends toward increased binding in short-allele males. Although neither effect was significant we note that, in contrast to all other regions investigated, V1aR in these two structures is involved primarily in basic physiological functions, such as the coordination of circadian rhythms (Reppert and Weaver, 2001), the regulation of thirst and hunger (Hoorneman and Buijs, 1982), and the maintenance of homeostasis (Swanson and Sawchenko, 1983). Considering these functional differences, as well as the cell-specific effects of allele length demonstrated by Hammock and Young (2004), regional variation in the relationship between allele length and V1aR abundance is not surprising.

Whole brain V1aR expression provides a gross snapshot of the effect of the microsatellite's influence on *avpr1a* expression. When averaged across all 15 brain regions, V1aR expression was significantly higher in long-allele males (Figs. 2A, 3F). This result is broadly consistent with a report by Hammock et al. (2005), in which 13 of 13 significant correlations between average allele length and V1aR binding were positive. This finding is also consistent with a large number of studies that indicate that microsatellite polymorphisms in *cis*-regulatory regions are most commonly associated with changes in transcription rate (reviewed in Li et al., 2004; Rockman and Wray 2002).

Microsatellite variation, behavior and reproductive success

Within prairie voles, differences in *avpr1a* microsatellite length have been associated with individual differences in social attachment, aggression and parental care—all central components of monogamous behavior (Hammock and Young, 2005; Hammock et al., 2005). In a field setting, however, we found no significant association between allele length and male space use or paternity. Long-allele males did,

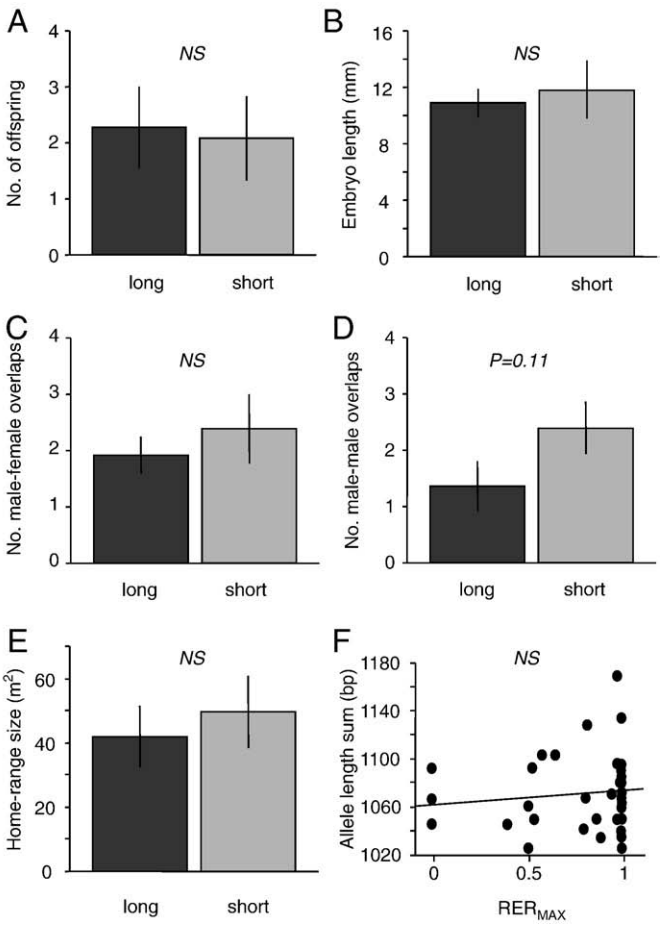

Fig. 5. (A) Mean (±SE) number of offspring sired by males exhibiting a 'long' (dark gray) or 'short' (light gray) genotype. (B) Mean (±SE) embryo size of offspring sired by males exhibiting a 'long' or 'short' genotype. (C) Mean (±SE) number of male–female home range overlaps produced by males exhibiting a 'long' or 'short' genotype. (D) Mean (±SE) number of male–male home range overlaps produced by males exhibiting a 'long' or 'short' genotype. (E) Mean (±SE) home range size for males exhibiting a 'long' or 'short' genotype. Home ranges were calculated using 75% minimum convex polygon estimates of core space usage. (F) Correlations between allele length (calculated as the sum of the number of base pairs [bp] for each allele) and maximal relative encounter rate (RER_{MAX}), a measure of attachment. RER_{MAX} = encounter rate between a given male and the female with whom he had the largest proportion of home range overlap, divided by the sum of the encounter rates for all females.

however, exhibit trends toward more monogamous behavior: their home ranges tended to be smaller on average and to overlap with fewer conspecifics. These patterns are generally attributed to increases in mate-guarding and mate-attachment and, in this respect, our data resemble findings indicating that long-allele males behave more like monogamous residents in partner preference trials in the laboratory (Hammock and Young, 2005). However, only allele length effects on male–male overlap approached significance (Fig. 5D, $P=0.11$; also see Table 1). Our prior data demonstrate that we are able to detect significant differences between residents and wanderer space use with these field measures (Ophir et al., 2008a, 2008b), suggesting that our negative behavioral findings are not simply due to variance arising from the semi-natural setting. If microsatellite allele length influences aspects of monogamous behavior in the field, its effects are weak.

Although we cannot rule out subtle influences of microsatellite length on monogamous behaviors, our data demonstrate that the alternative male mating tactics found in natural populations of prairie voles are not due to differences in allele length *per se*. This might seem surprising, given that allele length influences V1aR in the ventral pallidum and medial amygdala, important nodes in the 'pairbonding circuit'. It is consistent, however, with recent comparisons of brain and behavior in these subjects, which show that field variation in the reproductive tactic is not explained by V1aR variation in the medial amygdala (unpublished data), ventral pallidum or lateral septum (Ophir et al., 2008b). Taken together, our data suggest that microsatellite variation predicts differences in some neuronal phenotypes, but these effects do not produce substantial variation in our behavior measures.

The lack of association between *avpr1a* microsatellite length polymorphism and reproductive success suggests that allele length is not a strong determinant of male fitness (Figs. 4B, D, 5A). Elsewhere we report that paired animals exhibit much higher mating success (Ophir et al., 2008a); a strong influence of allele length on pairing and reproductive success would naturally lead to a depletion of microsatellite variability. The finding that allele length has no detectable impact on pairing or paternity could explain how such genetic diversity persists. Furthermore, significant population differentiation at this locus, in the absence of differences in behavior or V1aR expression (Ophir et al., 2007; this study), also suggests that geographic variation in allelic frequencies is due primarily to random processes of mutation and genetic drift. Lastly, the most common alleles were intermediate in length (Fig. 1). The absence of a strong skew in the length distribution argues against directional selection for long or short alleles; the absence of a clearly bi-modal distribution argues against disruptive selection for the maintenance of both.

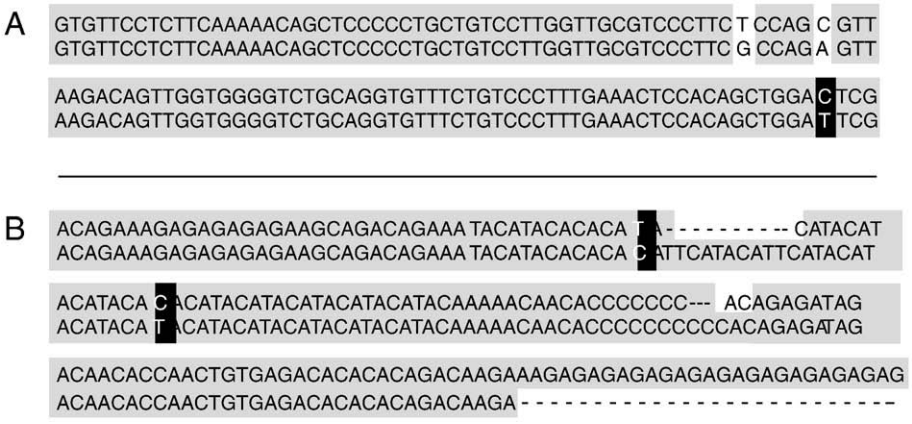

Fig. 6. Alignment depicting sequence and length polymorphism in two *avpr1a* alleles from Illinois prairie voles (GenBank accession no. AF069304, Young *et al.* 1999; S. M. Phelps, unpublished data). (A) Alignment of the less repetitive 5′ portion of the *avpr1a* locus reveals single nucleotide polymorphisms (black, white). (B) The same two alleles exhibit differences in repeat lengths across multiple motifs. In this case, the top allele is longer due to a GA expansion. Nevertheless, the shorter allele has expanded poly C and CATA repeats. Note that differing allele lengths are also markers for single nucleotide polymorphisms in neighboring sequence (A).

Table 2
Comparison of *avpr1a* microsatellite length in neural areas across studies

Brain structure		Hammock et al. 2005		Hammock and Young 2005		Current study	
		Short	Long	Short	Long	Short	Long
OB	EPl	low	high	low	high	ns	ns
	Gl	low	high	low (t)	high (t)		
	GrO	low (t)	high (t)	ns	ns		
	Mi/Pl	low	high	–	–		
AOB	EPlA/MiA	low	high	ns	ns	ns	ns
	GrA	low	high				
BST	LS	ns	ns	low	high	ns	ns
	VPall	ns	ns	ns	ns	low	high
	ADP	ns	ns	high	low	ns	ns
	dBST	ns	ns	high	low	ns	ns
	dBSTld	ns	ns	ns	ns		
	vBST	ns	ns	ns	ns		
	PCing	ns	ns	high	low	ns	ns
	DMH	low (t)	high (t)	high	low	–	–
	VMH	ns	ns	high	low	low	high
	BLA	low	high	high	low	–	–
	BMA	low	high	ns	ns	–	–
	CeA	low	high	high (t)	low (t)	low	high
	MeA	low	high	ns	ns	low	high
	VP Thal	ns	ns	–	–	ns	ns
	LD Thal	low	high	high (t)	low (t)	ns	ns
	MD ThalM	low	high	–	–	ns	ns
	MD ThalC/L	low (t)	high (t)	–	–		
	dPAG	ns	ns	high (t)	low (t)	–	–
	vPAG	ns	ns	high (t)	low (t)	–	–

Blue represents a negative relationship between allele length and V1aR expression; red represents positive relationships. Lighter colors with '(t)' indicate a non-significant statistical trend. Trends are defined as follows: Hammock et al. (2005), cases where one of the three correlation between *v1ar* length and V1aR binding was significant; Hammock and Young (2005), a significant difference was only found without correcting for multiple comparisons; current study, when the relationship between allele length and V1aR expression in a structure was significant ($P \leq 0.05$) in one of the two analyses (either long–short or summed allele length) we did not consider it a trend if the results from each comparison were consistent (i.e. CeA, VMH). Non-significant differences between long- and short-allele males are indicated by 'ns'; studies in which a particular neural area was not investigated or reported are indicated by '–'. We only report results from neural areas in which at least two of the three studies provided data; therefore some statistically significant differences reported in the three studies are excluded from the table. '{' indicates that components of a larger structure (e.g. EPlA/MiA and GrA) were considered together collectively (e.g. AOB). Abbreviations: OB, olfactory bulb; EPl, external plexiform layer of the OB; Gl, glomerular layer of the OB; GrO, granular layer of the OB; Mi/Pl, mitral cell and internal plexiform layers of the OB; AOB, accessory olfactory bulb; EPlA/MiA, external plexiform and mitral cell layers of the AOB; GrA, granular cell layer of the AOB; LS, lateral septum; VPall, ventral pallidum; ADP, anterodorsal preoptic nucleus; BST, bed nucleus of the stria terminalis; dBST, dorsal BST; dBSTld, lateral dorsal division of dBST; vBST, ventral BST; PCing, posterior cingulate/retrosplenial cortex; DMH, dorsomedial hypothalamus; VMH, ventromedial hypothalamus; BLA, basolateral amygdala; BMA, basomedial amygdala; CeA, central amygdala; MeA, medial amygdala; VP Thal, ventral posterior thalamus; LD Thal, laterodorsal thalamus; MD Thal, mediodorsal thalamus (M, medial; C/L central and lateral); dPAG, dorsal periaqueductal gray; vPAG, ventral periaqueductal gray. (For the interpretation of the references to colour in this table, the reader is referred to the web version of this article.)

Admittedly, our individual measures of mating success and population differentiation are incomplete metrics of selection at the *avpr1a* locus. When combined, however, these data suggest that the persistence of microsatellite length variation and its influence on neuronal phenotypes can be explained by their weak contributions to fitness.

Is length enough?

Both length-based and sequence-based transcriptional effects are common in *cis*-regulatory microsatellites; repeat expansion and contraction alter transcription factor binding affinities via changes in DNA conformation, and may delete or add specific binding sites (Kashi and King, 2006). The complexity of the prairie vole *avpr1a* microsatellite suggests that both types of effects may be important at this locus. The multiple repetitive domains, which include tetra-, di- and mononucleotide motifs, are disrupted by point mutations and interspersed with longer non-repetitive sequences (Hammock and Young, 2005; Young et al., 1999; Fig. 6). Thus, the probability of new mutations arising varies within the microsatellite, and alleles of equal length may differ at the nucleotide level. This complex pattern of sequence evolution and hidden sequence heterogeneity could have a profound influence on the relationship between allele length and transcriptional regulation. Indeed, *in vitro* studies of vole *avpr1a* microsatellites reveal that, while longer microsatellites often decrease transcription (Hammock and Young, 2004), long GA repeats actually increase transcription (Hammock and Young, 2005).

Determining whether *avpr1a* microsatellite nucleotide polymorphisms contribute to phenotypic variation *in vivo* awaits combined analysis of allele sequence, allele length, V1aR expression, and behavior. However, this hypothesis provides a plausible explanation for considerable across-study disparities in the relationship between allele length and both neuronal and behavioral phenotypes (Table 2). For example, two studies report positive associations between allele length and V1aR binding in olfactory structures; the same studies, however, found significant but opposing relationships between microsatellite length and social behaviors, and between length and V1aR expression in other structures (Hammock and Young, 2005; Hammock et al., 2005).

Other non-mutually exclusive interpretations for contrasting results across studies include epigenetic effects due to differences in rearing conditions, and epistasis between the *avpr1a* microsatellite and another site. For example, rearing influences were controlled by Hammock and Young (2005), but not in other studies. Thus, allelic influences on parental care could have epigenetic effects on brain and behavior that contribute to observed variation in offspring phenotypes (e.g. Francis et al., 2002, 2003). Likewise, epistatic effects are common in human *cis*-regulatory polymorphisms (Rockman and Wray, 2002), and phenotypic effects due to interactions between distinct repetitive regions within the same gene have been demonstrated in dogs (Fondon and Garner, 2004) and inferred in fish (Streelman and Kocher, 2002). The presence of a second microsatellite approximately 1600 bp upstream of the prairie vole *avpr1a* transcription start site (Young et al., 1999; S. M. Phelps, unpublished data) suggests that the potential for a functional interaction between the two sites warrants investigation.

Conclusions

The proposition that gene-associated microsatellites can provide a rapidly evolving source of functional genetic variation (King et al., 1997) is supported by a large number of studies, in organisms from bacteria to humans (Moxon et al., 1994; Rockman and Wray, 2002). Our study advances one particularly interesting case of the influence of a *cis*-regulatory microsatellite on neuronal and behavioral phenotypes in a highly social mammal. As in preceding reports, we found significant relationships between *avpr1a* microsatellite polymorphism and V1aR abundance in brain regions implicated in pairbonding and paternal care. Surprisingly, we found no significant relationships between allele lengths, male reproductive success, and multiple measures of monogamous behavior. Indeed, differences between studies indicate that, while allele length has been useful in revealing the functional consequences of *avpr1a* microsatellite variation, a singular focus on this character may overlook important sources of genetic variation. Analysis of the relation between sequence polymorphism, transcriptional regulation of V1aR, and V1aR-modulated behaviors promises to deepen our understanding of the contribution of genotype to behavioral diversity. It is already clear, however, that the integration of molecular neuroscience and behavioral ecology provides rich new perspectives on the complexities of brain and behavior.

Acknowledgments

We would like to thank Anna Bess Sorin for involvement in paternity analyses. We also thank Jerry Wolff, Jamie Gillooly and three anonymous reviewers for useful comments on this manuscript. This work was funded by an NSF grant (0316451) to SMP.

References

Bielsky, I.F., Hu, S.B., Szegda, K.L., Westphal, H., Young, L.J., 2004. Profound impairment in social recognition and reduction in anxiety-like behavior in vasopressin V1a receptor knockout mice. Neuropsychopharmacol 29, 483–493.

Cushing, B.S., Mogekwu, N., Le, W.-W., Hoffman, G.E., Carter, C.S., 2003. Cohabitation induced Fos immunoreactivity in the monogamous prairie vole. Brain Res. 965, 203–211.

Elkabir, D.R., Wyatt, M.E., Vellucci, S.V., Herbert, J., 1990. The effects of separate or combined infusions of corticotropin-releasing factor and vasopressin either intraventricularly or into the amygdala on aggressive and investigative behavior in the rat. Regul. Pept. 28, 199–214.

Ferris, C.F., Melloni, R.H., Koppel, G., Perry, K.W., Fuller, R.W., Deville, Y., 1997. Vasopression/Serotonin interactions in the anterior hypothalamus control aggressive behavior in golden hamsters. J. Neurosci. 17, 4331–4340.

Fondon III, J.W., Garner, H.R., 2004. Molecular origins of rapid and continuous morphological evolution. Proc. Natl. Acad. Sci. USA 101, 18058–18063.

Francis, D.D., Young, L.J., Meaney, M.J., Insel, T.R., 2002. Naturally occurring differences in maternal care are associated with the expression of oxytocin and vasopressin (V1a) receptors: gender differences. J. Neuroendocrinol. 14, 349–353.

Francis, D.D., Szegda, K., Campbell, G., Martin, W.D., Insel, T.R., 2003. Epigenetic sources of behavioral differences in mice. Nature Neurosci. 6, 445–446.

Getz, L.L., McGuire, B., Hofmann, J., Pizzuto, T., Frase, B., 1993. Social organization of the prairie vole (*Microtus ochrogaster*). J. Mammal. 74, 44–58.

Goudet, J., 1995. Fstat version 1.2: a computer program to calculate *F* statistics. J. Heredity 86, 485–486.

Hammock, E.A.D., Young, L.J., 2002. Variation in the vasopressin V1a receptor promoter and expression: implications for inter- and intraspecific variation in social behaviour. Eur. J. Neurosci. 16, 399–402.

Hammock, E.A.D., Young, L.J., 2004. Functional microsatellite polymorphism associated with divergent social structure in vole species. Mol. Biol. Evol. 21, 1057–1063.

Hammock, E.A.D., Young, L.J., 2005. Microsatellite instability generates diversity in brain and sociobehavioral traits. Science 308, 1630–1634.

Hammock, E.A.D., Lim, M.M., Nair, H.P., Young, L.J., 2005. Association of vasopressin 1a receptor levels with a regulator microsatellite and behavior. Genes Brain Behav. 4, 289–301.

Hoorneman, E.M.D., Buijs, R.M., 1982. Vasopressin fiber pathways in the rat brain following suprachiasmatic nucleus lesioning. Brain Res. 243, 235–241.

Insel, T.R., Wang, Z.X., Ferris, C.F., 1994. Patterns of brain vasopressin receptor distribution associated with social organization in microtine rodents. J. Neurosci. 14, 5381–5392.

Ishibashi, Y., Yoshinaga, Y., Saitoh, T., Abe, S., Iida, H., Yoshida, M.C., 1999. Polymorphic microsatellite DNA markers in the field vole *Microtus montebelli*. Mol. Ecol. 8, 157–168.

Kashi, Y., King, D.G., 2006. Simple sequence repeats as advantageous mutators in evolution. Trends Genet. 22, 253–259.

Kevetter, G.A., Winans, S.S., 1981. Connections of the corticomedial amygdala in the golden hamster: I. Efferents of the vomeronasal amygdala. J. Comp. Neurol. 197, 81–98.

King, D.G., Soller, M., Kashi, Y., 1997. Evolutionary tuning knobs. Endeavour 21, 36–40.

Li, Y.C., Korol, A.B., Fahima, T., Nevo, E., 2004. Microsatellites within genes: structure, function and evolution. Mol. Biol. Evol. 21, 991–1007.

Lim, M.M., Young, L.J., 2004. Vasopressin-dependent neural circuits underlying pair bond formation in the monogamous prairie vole. Neuroscience 125, 35–54.

Liu, Y., Curtis, J.T., Wang, Z.X., 2001. Vasopressin in the lateral septum regulates pair bond formation in male prairie voles (*Microtus ochrogaster*). Behav. Neurosci. 155, 910–919.

Mahady, S.J., Wolff, J.O., 2002. A field test of the Bruce effect in the monogamous prairie vole (*Microtus ochrogaster*). Behav. Ecol. Sociobiol. 52, 31–37.

Moxon, E.R., Rainey, P.B., Nowak, M.A., Lenski, R.E., 1994. Adaptive evolution of highly mutable loci in pathogenic bacteria. Curr. Biol. 4, 24–33.

Musatov, S., Chen, W., Pfaff, D.W., Kaplitt, M.G., Ogawa, S., 2006. RNAi-mediated silencing of estrogen receptor in the ventromedial nucleus of hypothalamus abolishes female sexual behaviors. Proc. Natl. Acad. Sci. USA 103, 10456–10460.

Ophir, A.G., Phelps, S.M., Sorin, A.B., Wolff, J.O., 2007. Morphological, genetic and behavioral comparisons of two prairie vole populations in the field and laboratory. J. Mammal. 88, 989–999.

Ophir, A.G., Phelps, S.M., Sorin, A.B., Wolff, J.O., 2008a. Social but not genetic monogamy is associated with greater breeding success in prairie voles. Anim. Behav. 75, 1143–1154.

Ophir, A.G., Wolff, J.O., Phelps, S.M., 2008b. Variation in neural V1aR predicts sexual fidelity and space use among prairie voles in semi-natural settings. Proc. Natl. Acad. Sci. USA 105, 1249–1254.

Pfaus, J.G., Kleopoulos, S.P., Mobbs, C.V., Gibbs, R.B., Pfaff, D.W., 1993. Sexual stimulation activates c-*fos* within estrogen-concentrating regions of the female rat forebrain. Brain Res. 624, 253–267.

Phelps, S.M., Young, L.J., 2003. Extraordinary diversity in vasopressin (V1a) receptor distributions among wild prairie voles (*Microtus ochrogaster*): Patterns of variation and covariation. J. Comp. Neurol. 466, 564–576.

Raymond, M., Rousset, F., 1995. genepop Version 1.2: population genetics software for exact tests and ecumenicism. J. Heredity 86, 248–249.

Reppert, S.M., Weaver, D.R., 2001. Molecular analysis of mammalian circadian rhythms. Ann. Rev. Physiol. 63, 647–676.

Rockman, M.V., Wray, G.A., 2002. Abundant raw material for *cis*-regulatory evolution in humans. Mol. Biol. Evol. 19, 1991–2004.

Schneider, S., Roessli, D., Excoffier, L., 2000. arlequin, a software program for population genetics data analysis Version 2.000. Genetics and Biometry Laboratory. University of Geneva, Switzerland.

Sheehan, T., Paul, M., Amaral, E., Numan, M.J., Numan, M., 2001. Evidence that the medal amygdala projects to the anterior/ventromedial hypothalamic nuclei to inhibit maternal behavior in rats. Neuroscience 106, 341–356.

Stewart, W.A., Piertney, S.B., Dallas, J.F., 1998. Isolation and characterization of highly polymorphic microsatellites in the water vole, *Arvicola terrestris*. Mol. Ecol. 7, 1247–1263.

Streelman, J.T., Kocher, T.D., 2002. Microsatellite variation associated with prolactin expression and growth of salt-challenged Tilapia. Physiol. Genomics 9, 1–4.

Swanson, L.W., Sawchenko, P.E., 1983. Hypothalamic integration: Organization of the paraventricular and supraoptic nuclei. Annu. Rev. Neurosci. 6, 269–324.

Taitt, M.J., Krebs, C.J., 1985. Population dynamics and cycles. In: Tamarin, R.H. (Ed.), Biology of new world *Microtus*. American Society of Mammalogists, Provo, Utah, pp. 567–620. Special publication No. 8.

Van de Zande, L., van Apeldoorn, R.C., Blijdenstein, A.F., De Jong, D., Van Delden, W., Bijlsma, R., 2000. Microsatellite analysis of population structure and genetic differentiation within and between populations of the root vole, *Microtus oeconomus*, in the Netherlands. Mol. Ecol. 9, 1651–1656.

Winslow, J.T., Hastings, N., Cater, C.S., Harbaugh, C.R., Insel, T.R., 1993. A role for central vasopressin in pair bonding in monogamous prairie voles. Nature 365, 545–548.

Young, L.J., 1999. Oxytocin and vasopressin receptors and species-typical social behaviors. Horm. Behav. 36, 212–221.

Young, L.J., Wang, Z., 2004. The neurobiology of pair bonding. Nature Neurosci. 7, 1048–1054.

Young, L.J., Winslow, J.T., Nilsen, R., Insel, T.R., 1997. Species differences in V1a receptor gene expression in monogamous and nonmonogamous voles: behavioral consequences. Behav. Neurosci. 111, 599–605.

Young, L.J., Nilsen, R., Waymire, K.G., MacGregor, G.R., Insel, T.R., 1999. Increased affiliative response to vasopressin in mice expressing the V1a receptor from a monogamous vole. Nature 400, 766–768.

Young, L.J., Young, A.Z.M., Hammock, E.A.D., 2005. Anatomy and neurochemistry of the pair bond. J. Comp. Neurol. 493, 51–57.