# Microsatellite Instability Generates Diversity in Brain and Sociobehavioral Traits

**Elizabeth A. D. Hammock and Larry J. Young***

**Repetitive microsatellites mutate at relatively high rates and may contribute to the rapid evolution of species-typical traits. We show that individual alleles of a repetitive polymorphic microsatellite in the 5′ region of the prairie vole *vasopressin 1a receptor* (*avpr1a*) gene modify gene expression in vitro. In vivo, we observe that this regulatory polymorphism predicts both individual differences in receptor distribution patterns and socio-behavioral traits. These data suggest that individual differences in gene expression patterns may be conferred via polymorphic microsatellites in the cis-regulatory regions of genes and may contribute to normal variation in behavioral traits.**

Social behavior evolves rapidly as evidenced by the diversity of species-typical social structures among closely related species; however, the underlying mechanisms of this rapid evolution are currently unknown. Evolution of species-typical behavioral traits requires behavioral diversity and a polymorphic genetic mechanism producing such diversity. The high levels of polymorphism in repetitive DNA sequences (microsatellites) make them useful as markers to distinguish among individuals. Expanded repeats in coding regions of genes are responsible for diseases such as Huntington's disease and the spinocerebellar ataxias (*1*). Recently, expansion and contraction in the coding regions of several developmental genes has been hypothesized to underlie the rapid and continuous evolution of snout morphology in domesticated dog breeds (*2*). Although there is not as much empirical evidence, variation in regulatory regions of genes likely plays a critical role in the generation of morphological variation by altering the timing and location of gene expression (*3*, *4*). Microsatellites in the cis-regulatory regions of genes may significantly enhance the rate of evolution of gene expression patterns and selectable phenotypic traits (*5*–*7*).

Rodents of the genus *Microtus* (voles) show dramatic species differences in social structure (*8*). Prairie voles form lifelong attachments with a mate, are biparental, and show high levels of social interest (*9*). In contrast, the closely related montane vole does not pair bond, the males do not contribute to parental care, and they appear socially indifferent (*10*). Species differences in the pattern of vasopressin 1a receptor (V1aR) expression in the brains of these species contribute to the species differences in social structure (*11*–*13*). The species-specific patterns of V1aR expression appear to be regulated by differences in a microsatellite in the 5′ regulatory region of the gene encoding V1aR (*avpr1a*). This microsatellite is highly expanded in prosocial prairie and pine voles, consisting of several repeat blocks interspersed with nonrepetitive sequences (Fig. 1, A and B), compared with a very short version in the asocial montane and meadow voles (*14*). The species differences in this microsatellite have functional consequences in cell culture: In transcription reporter assays, changes in the microsatellite locus modify luciferase reporter activity in a cell-type–dependent manner (*15*).

Because microsatellites offer a mechanism of continuous phenotypic variation, it follows that there should also be a microsatellite-phenotype relation within a species. Accordingly, within the prairie vole species, there is variation in social behavior across different geographical populations and even within the same population in communal pens (*16*, *17*). In addition, the length of the *avpr1a* microsatellite is variable [Fig. 1, B and C; (*15*, *18*)] as are V1aR distribution patterns across individuals within the prairie vole species (*19*), although levels of intraspecific variation are lower than the levels of interspecific variation in both genotype and phenotype (*19*, *20*). Whereas microsatellite length is as-

Department of Psychiatry and Behavioral Sciences, Center for Behavioral Neuroscience, Yerkes National Primate Research Center, Emory University, Atlanta, GA 30329, USA.

*To whom correspondence should be addressed. E-mail: Lyoun03@emory.edu; Edunn2@emory.edu

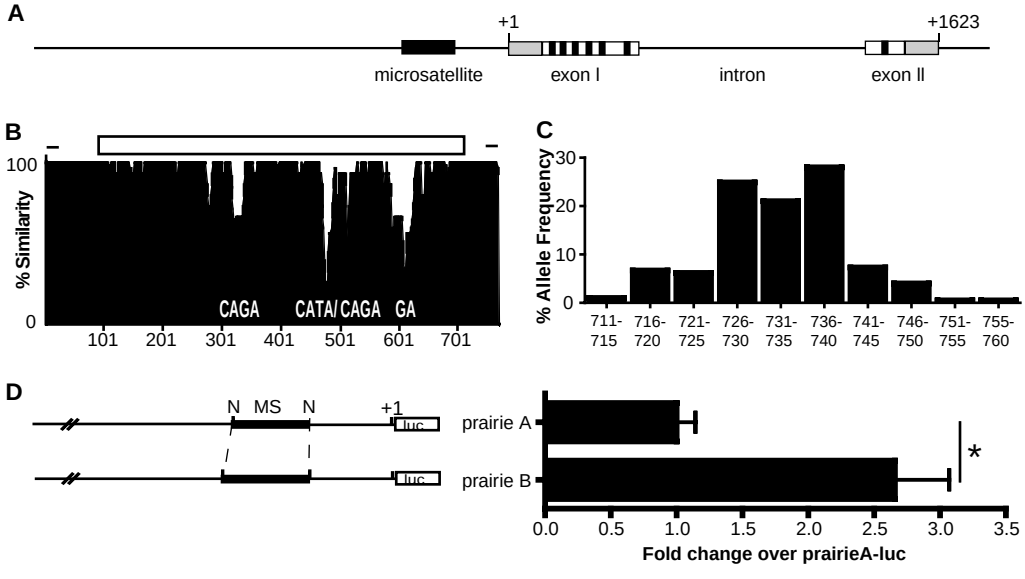



**Fig. 1.** The prairie vole *avpr1a* 5′ regulatory region contains a functional polymorphic microsatellite. (**A**) The prairie vole microsatellite is located ~500 bp upstream of the *avpr1a* transcription start site. (**B**) The prairie vole-specific microsatellite, indicated by the white box, is ~600 bp long. PCR primers used for genotyping, indicated by dashes, generate PCR products ranging from 710 to 760 bp in length. This alignment of various alleles from 9 prairie voles reveals that the 5′ end is relatively conserved among prairie voles, whereas the 3′ end is repetitive and highly polymorphic. The relative positions of various repeat motifs are indicated on the alignment. Expansion and contraction at these repeat motifs contributes to the length variation in microsatellite alleles. (**C**) A histogram of allele frequencies from our laboratory breeding colony of voles reveals high levels of length polymorphism. (**D**) Two different common alleles of this microsatellite differentially regulate gene expression in rat A7r5 cells. The length genotype of prairie A was 727 bp; the allele length of prairie B was 746 bp. Bars in (D) represent means + SEM ($n$ = 6 per experiment).

sociated with species differences in V1aR expression and social structure, we hypothesize that intraspecific variation in microsatellite length generates intraspecific variation in V1aR expression and, consequently, sociobehavioral traits.

To determine whether or not intraspecific variation in the microsatellite itself is sufficient to change gene expression, we created luciferase reporter constructs containing ~3.5 kb of prairie vole *avpr1a* 5′ regulatory region, including the microsatellite locus. Holding the rest of the 5′ regulatory region constant, we interchanged two different prairie vole alleles of this microsatellite to test them against each other for their ability to drive the luciferase reporter in A7r5 cells. The two alleles only differ in length by 19 base pairs (bp), primarily attributable to an expanded GA repeat in the longer allele. In three independent cell culture experiments, the longer allele had significantly increased levels of luciferase activity compared with the shorter allele, demonstrating that intraspecific variation in the microsatellite itself modifies gene expression (Fig. 1D, two-tailed Student's *t* test with Welch's correction, $P < 0.0001$). Although the long allele showed higher levels of expression in this particular cell line that we tested, other evidence indicates that the effect may be cell-type dependent.

We next created 25 breeding pairs of prairie voles based on the length of their *avpr1a* microsatellite polymorphism to generate testable offspring that were homozygous for either longer or shorter than the average allele length. Two out of three litter cohorts

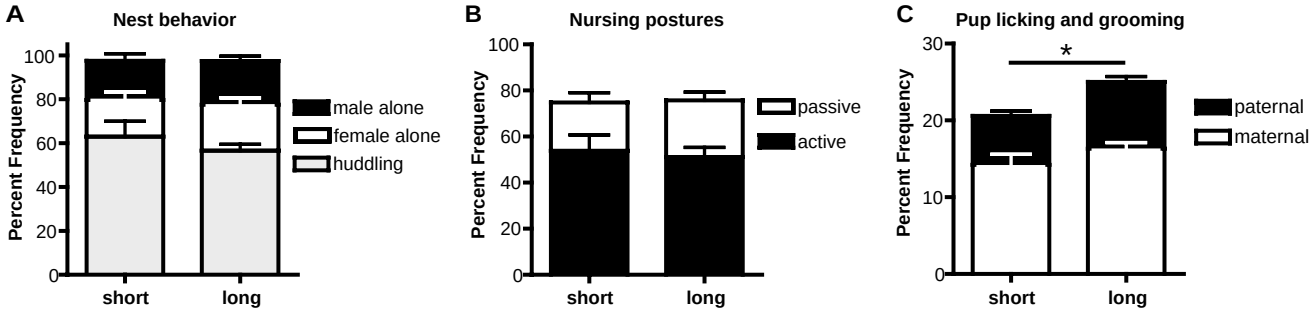
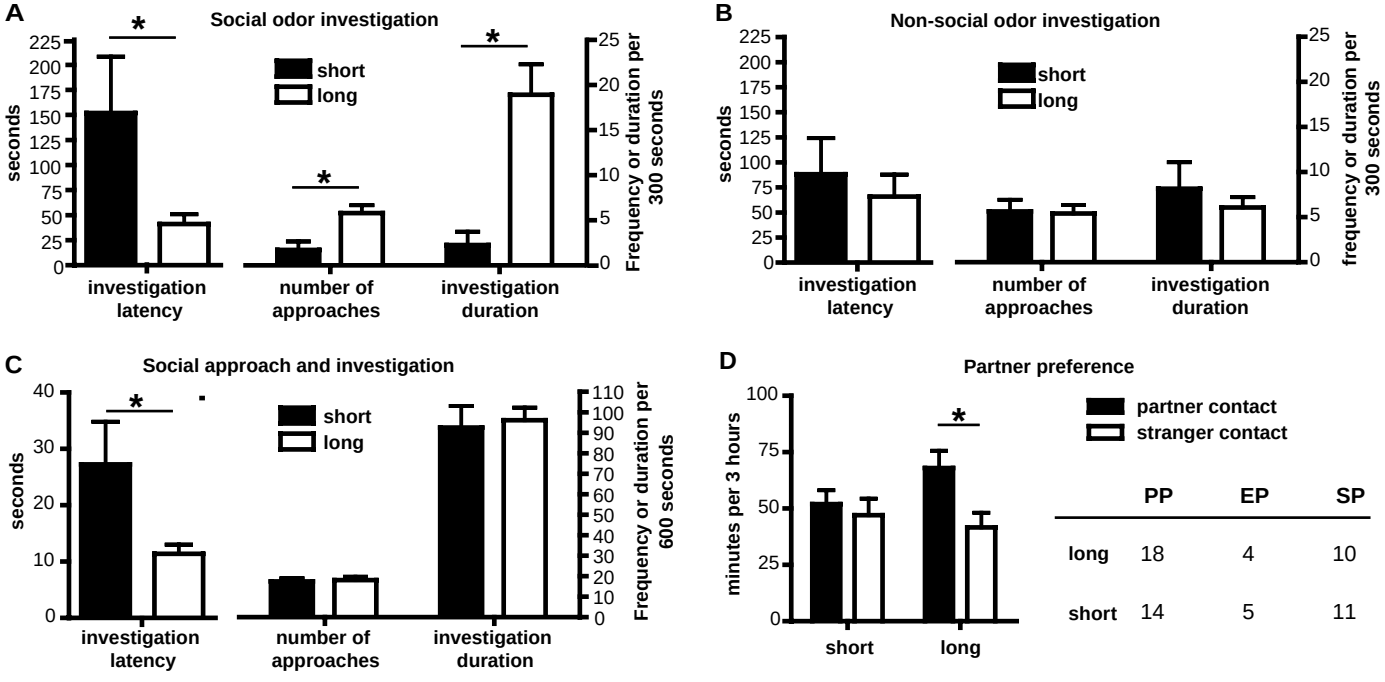



**Fig. 2.** Genotype differences in male but not female parental care. (**A**) Huddling rates and the frequency of each parent alone on the nest were not different between the two genotype groups. (**B**) Maternal nursing postures were not different between the two genotype groups. (**C**) Total rates of pup licking and grooming were higher in the long-allele group, and this was attributed to differences in grooming by the males, but not the females. Bars in (A, B, and C) represent means + SEM. $n = 6$ short allele pairs, $n = 11$ long allele pairs




| | PP | EP | SP |
|---|---|---|---|
| long | 18 | 4 | 10 |
| short | 14 | 5 | 11 |

**Fig. 3.** Long-allele males have a greater probability of social engagement and bonding behavior. (**A**) Long-allele males ($n = 12$) more quickly approached a novel social odor, approached the social odor more frequently, and spent a longer amount of time investigating the social odor than short-allele males ($n = 8$). (**B**) There were no genotype differences in the investigation of a nonsocial odor ($n = 12$ long, $n = 8$ short allele). (**C**) Long-allele males ($n = 49$; $n = 45$ short allele) more quickly approached a novel juvenile animal, although there were no genotype differences in total frequency of investigation or duration of investigation in this task. (**D**) Long-allele males ($n = 32$) displayed increased probability of preferences for a familiar partner female over a novel stranger female. Short-allele males ($n = 30$) did not display a partner preference. In the table, a partner preference "PP" is defined as a partner preference score of 66.67% or greater, where the partner preference score = (partner contact time)/(partner contact time + stranger contact time). "EP" is an equal preference for either partner or stranger (33.33 to 66.66%). "SP" is a preference for the stranger (less than 33.33%). Both groups showed strong preferences for either partner or stranger (PP+SP versus EP) compared with a random probability distribution, but only the long-allele group showed a significant preference for partner (PP versus EP+SP), compared with a random probability distribution. Bars in (A, B, C, and D) represent means + SEM.

were randomly cross-fostered within 6 to 8 hours after birth to reduce the potential confounding variable of rearing environment. There were no genotype differences in birth rates (Mann-Whitney, $P > 0.05$); however, short-allele breeder pairs had higher rates of pup mortality (12 out of 25, versus 3 out of 36, $\chi^2 = 10.83$, $P < 0.001$). This effect was only apparent with the first litter, as both genotype groups had high rates of survival after the pairs had become experienced parents. Because paternal care in prairie voles is influenced by vasopressin, as well as selective V1aR antagonists (*21*), we measured parental care during the first ten post-natal days. Twenty hours of observations revealed no genotype differences in the amount of time the pair were on the nest together or in the time each parent was alone on the nest (Fig. 2A, Mann-Whitney, $P > 0.05$). Additionally, there were no genotype differences in the amount of time the nest was left unoccupied by both parents simultaneously (Fig. 2A, Mann-Whitney, $P > 0.05$). There were also no genotype differences in nursing postures adopted by the females (Fig. 2B, Mann-Whitney, $P > 0.05$). In contrast, there were significant genotype differences in the frequency of pup licking and grooming (Fig. 2C, Mann-Whitney, $P < 0.05$). This genotype difference can be fully attributed to a higher grooming frequency of long-allele males compared with short-allele males (Mann-Whitney, $P < 0.01$), as there were no genotype differences in pup licking and grooming frequency by the females (Mann-Whitney, $P > 0.05$). These data coincide with the prevailing hypothesis in the literature that vasopressin systems contribute to male, not female, species-typical behaviors. The genotype differences in total licking and grooming (15 to 20%) are only slightly less than the 20 to 30% differences in grooming rates in high versus low licking and grooming rat dams, which have well-established consequences for the receiving offspring (*22*).

Vasopressin and the V1aR have been implicated in social recognition and investigation processes in a variety of species (*23*). We had preliminary correlative evidence (*20*) from a random sample of 20 animals from our regular laboratory breeding colony to suggest that the microsatellite would highly predict levels of V1aR in the olfactory bulb, and that V1aR levels in the olfactory bulb would be associated with social behavior. It can be difficult to control the experiment-wise variability in social behavior tasks due to variation contributed by the stimulus animal. Therefore, as an initial test of social behavior, we tested the $F_1$ males for their behavior toward novel social odors. We placed each male in an empty arena for a 2-min acclimation period and then added a small cartridge filled with soiled bedding from the cage of an unrelated female of similar age. Long-allele males displayed a shorter latency to first approach, and higher frequency and longer duration of investigation compared with short-allele males (Fig. 3A, Mann-Whitney, $P < 0.05$). This effect appears to be specific to social odors, as there were no genotype differences in response to a banana-like odor, amyl-acetate (Fig. 3B, Mann-Whitney, $P > 0.05$).

To determine whether the behavioral differences observed in the social odor task would translate into differences in prosocial behavior toward another individual, a juvenile prairie vole (PND15 to 18) was placed in the test subject's home cage for 10 min, and the reaction of the test subject was recorded. As with the social odorant test just described, long-allele animals began investigating the juvenile with a reduced latency (Fig. 3C, Mann-Whitney, $P < 0.05$). There were no genotype differences in huddling or attack behavior, or in the frequency or duration of investigation measures as seen in the social odor task. Regardless, the genetic differences in approach latency have consequences for the probability of social contact in ethologically relevant contexts.

The partner preference test has been the hallmark assay for demonstrating a role for

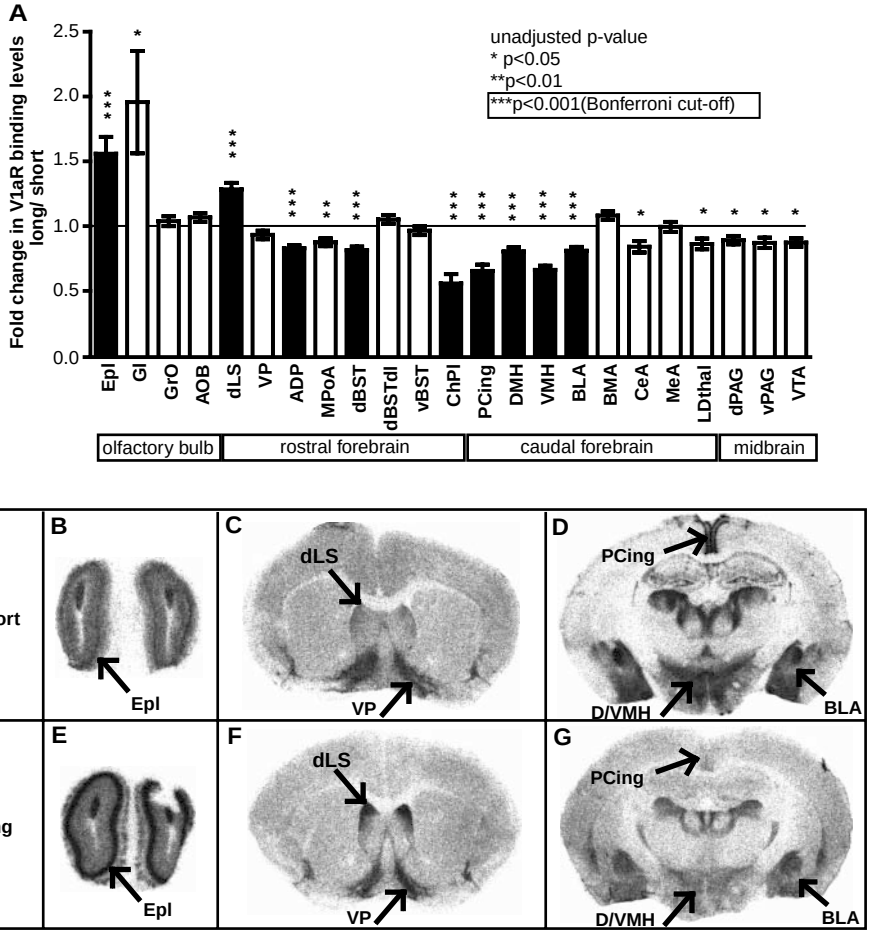



**Fig. 4.** Microsatellite length predicts robust differences in the distribution patterns of V1aR. (**A**) Compared with short-allele males ($n = 45$; line at unity), long-allele males ($n = 49$) have higher levels of V1aR in some brain regions and lower levels of V1aR in others. For each brain region, binding values were normalized to average values for the short-allele group. Bars represent means ± SEM of the long-allele males. (**B** to **D**) Examples of autoradiographs from short-allele males, showing lower levels of V1aR in the external plexiform layer of the olfactory bulb (B) and dorsal lateral septum (C), no differences in the ventral pallidum (C), and higher V1aR levels in the posterior cingulate cortex, hypothalamus, and amygdala (D). Matched sections of sample autoradiographs from long-allele males are displayed in (**E** to **G**). Abbreviations: ADP, anterior dorsal preoptic nucleus; AOB, accessory olfactory bulb; BLA, basolateral amygdala; BMA, basomedial amygdala; CeA, central amygdala; ChPl, choroid plexus; dBST, dorsal bed nucleus of the stria terminalis; dBSTdl, dorsolateral dBST; dLS, dorsal lateral septum; DMH, dorsomedial hypothalamus; dPAG, dorsal periaqueductal gray; D/VMH, DMH and VMH; EPl, external plexiform layer; Gl, glomerular layer; GrO, granular layer; LDthal, laterodorsal thalamus; MeA, medial amygdala; MPoA, medial preoptic area; PCing, posterior cingulate cortex; vBST, ventral BST; VMH, ventromedial hypothalamus; VP, ventral pallidum; vPAG, ventral PAG; VTA, ventral tegmental area.

vasopressin and V1aR in pair bonding. We paired the test males with sexually receptive females for an overnight cohabitation. We used a truncated (18-hour) cohabitation period in an effort to increase the variability in the development of partner preferences. In the 3-hour partner preference test, long- and short-allele males spent equal amounts of time in total social contact (student's $t$ test, $P > 0.05$). However, long-allele males spent more time with their partner compared with the stranger, whereas short allele-males did not (Fig. 3D; two-way ANOVA, preference and genotype as factors, planned $t$ test for partner time versus stranger time $P < 0.05$ in long-allele males, $P > 0.05$ in short-allele males). Criteria for a partner preference are met when the test animal spends twice as much social contact time with the partner compared with the stranger. As a group, long-allele males displayed partner preferences (Fig. 3D, $\chi^2 = 7.56$, $P < 0.05$), whereas short-allele animals did not ($\chi^2 = 2.4$, $P > 0.05$), when compared with a random distribution of preferences. Because there were no genotype differences in total social contact time, the genotype differences in partner preference behavior could be due to differences in some aspect of social discrimination of the two females. Perhaps

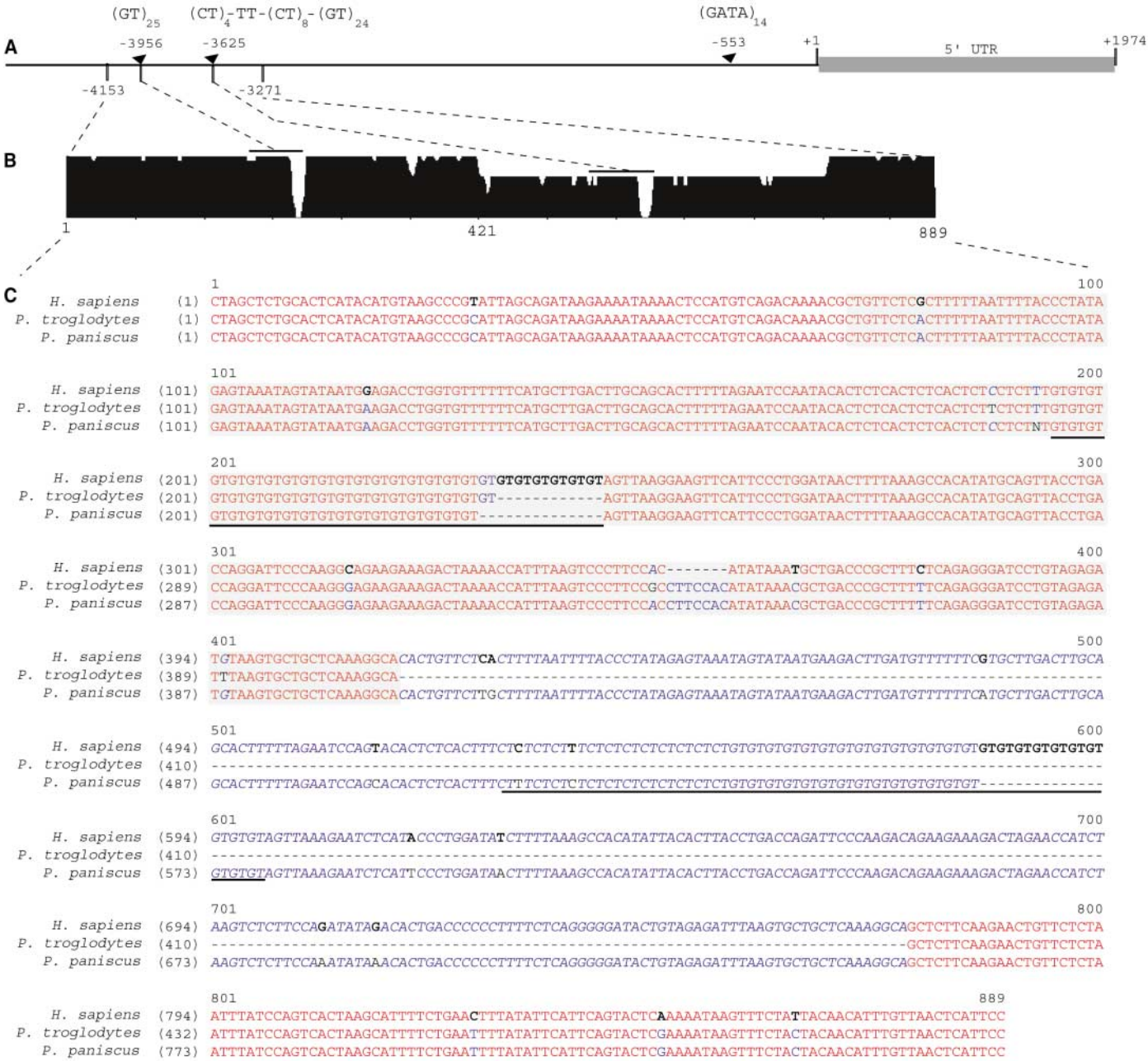



**Fig. 5.** Humans (*H. sapiens*) and bonobos (*P. paniscus*) share a cryptic microsatellite element in the 5′ regulatory region of *AVPR1A*, which is absent in the chimpanzee (*P. troglodytes*). The schematic (**A**) illustrates the position of 5′ microsatellite elements in relation to the annotated transcription start site (+1) of the human *AVPR1A* gene (start codon at +1974). Human, chimpanzee, and bonobo similarity plot (**B**) at this region reveals high levels of homology around the –3956 bp microsatellite, whereas the microsatellite at –3625 bp is not as conserved. The reduction in similarity around the –3625 bp microsatellite is due to the complete absence of ~360 bp in chimpanzees (**C**). Underscores indicate the positions of the two repeat regions. Red letters indicate sequence that is conserved across all three species. Blue sequence represents conservation in two out of three species; blue italicized sequence represents sequence that is shared between human and bonobo, but not chimpanzee. Bold black letters highlight sequence that is unique to humans. The bonobo/human-specific region appears to be a duplication of the preceding sequence, indicated by the shaded gray box.

short-allele males need a longer cohabitation period to learn to distinguish partner from stranger.

To control for the possibility of a confounding effect of V1aR on anxiety, we tested the animals in the elevated plus maze and the open field test. Although there were subtle genotype differences in each of these tests (see supporting online material), neither test revealed genotype differences on the classic measures of trait anxiety: namely, time in the open arms of the elevated plus maze and time in the center of the open field test. Therefore, although there may be subtle genotype differences in some measure of emotionality, there were clearly no genotype differences in the classic measures of trait anxiety to sufficiently explain the observed differences in social behavior.

The genotype differences in social behavior are associated with robust genotype differences in V1aR binding in the brains of the $F_1$ males (Fig. 4). Several brain regions showed up to 50% changes in the quantity of V1aR binding. In some brain regions, a long allele conferred a higher level of receptor binding (olfactory bulb, lateral septum), and in other regions a long allele conferred a lower level of receptor binding (amygdala, hypothalamus, posterior cingulate cortex). Microsatellites may generate phenotypic diversity by acting in a cell-type dependent manner, i.e., altering the pattern of gene expression across brain regions, rather than globally altering total levels of expression. This could generate heritable behavioral diversity by altering the neural circuits that are engaged in behavioral contexts that cause the central release of vasopressin.

Several of the brain regions with genotype differences in V1aR levels have previously been implicated in the behaviors described above. For example, V1aR activation in the lateral septum has been associated with both increased rates of pup licking and grooming, as well as increased partner preference formation (*21*, *24*). Our long-allele males showed higher levels of V1aR in the lateral septum, higher rates of pup licking and grooming, and higher levels of partner preference formation. The long-allele males also showed higher levels of V1aR in the external plexiform layer of the olfactory bulb. Lesions of the olfactory bulb inhibit partner preference formation and social approach behaviors in prairie voles (*25*, *26*). Also, pharmacological manipulation of vasopressin systems in both the olfactory bulb and lateral septum modulate social recognition in rats and mice (*27–29*). Therefore, genotype differences in V1aR binding in the lateral septum and olfactory bulb are likely candidates for serving as the neural substrates underlying the observed genotype differences in social behavior. Variation in the lateral septum and olfactory bulb probably influence the requisite social recognition component of pair bonding.

Previous comparative (*11*, *12*), pharmacological (*30*), and viral vector gene-transfer (*13*, *31*) experiments have demonstrated that species differences in V1aR levels in the ventral pallidum contribute to species differences in the development of partner preference formation in voles. Interestingly, V1aR levels in the ventral pallidum, which are associated with species differences in social structure, were not associated with genotype within the prairie vole species. Perhaps V1aR binding levels in the ventral pallidum are now genetically fixed by conservation of the 5′ end of the prairie-specific microsatellite, but the hypervariable 3′ end produces variation in other brain regions, such as the lateral septum, olfactory bulb, and hypothalamus, within this species.

Although we have established a clear functional role for the microsatellite in cell culture, the possibility still exists that some of the variation in V1aR binding patterns and behavior may be due to linkage disequilibrium with the microsatellite. However, the in vitro evidence in this manuscript combined with ample experimental evidence from other reports implicating V1aR in social behavior indicate that the microsatellite is likely a causal mechanism of behavioral trait variation through alterations in V1aR distribution.

We theorize that microsatellites in the regulatory regions of the *avpr1a* gene confer this locus with high levels of evolvability, which in itself may be a target of selection (*32*). Interestingly, four polymorphic microsatellites surround the human *avpr1a* gene (*33*). Two independent reports have indicated modest association of microsatellite alleles at the –3625 bp locus with autism (*34*, *35*), which is a disease of profound social deficit. Considering that variation at this locus may have important implications for our own species-typical social behavior, we compared the publicly available *avpr1a* gene sequences of chimpanzees [*Pan troglodytes* (*36*)] and humans (*37*) and found that 360 bp in and around this microsatellite locus was deleted in chimpanzees, although the flanking regions were >96% conserved. In contrast, the same locus in the *avpr1a* gene in the bonobo (*Pan paniscus*), which is known for its socio-sexual reciprocity and bonding (*38*), has high homology with the human microsatellite (Fig. 5). Perhaps in primate species, as in vole species, both inter- and intraspecific variation in regulatory microsatellites of the *avpr1a* gene can give rise to behavioral variation via altered regulation of the distribution of this gene product across individuals.

## References and Notes


1. H. Y. Zoghbi, H. T. Orr, *Annu. Rev. Neurosci.* **23**, 217 (2000).
2. J. W. Fondon III, H. R. Garner, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 18058 (2004).
3. G. A. Wray *et al.*, *Mol. Biol. Evol.* **20**, 1377 (2003).
4. N. Gompel, B. Prud'homme, P. J. Wittkopp, V. A. Kassner, S. B. Carroll, *Nature* **433**, 481 (2005).
5. D. G. King, *Science* **263**, 595 (1994).
6. Y. Kashi, D. King, M. Soller, *Trends Genet.* **13**, 74 (1997).
7. Y. C. Li, A. B. Korol, T. Fahima, E. Nevo, *Mol. Biol. Evol.* **21**, 991 (2004).
8. L. E. Shapiro, D. A. Dewsbury, *J. Comp. Psychol.* **104**, 268 (1990).
9. L. L. Getz, C. S. Carter, L. Gavish, *Behav. Ecol. Sociobiol.* **8**, 189 (1981).
10. F. J. Jannett, *J. Mammol.* **63**, 495 (1982).
11. T. R. Insel, Z. X. Wang, C. F. Ferris, *J. Neurosci.* **14**, 5381 (1994).
12. L. J. Young, J. T. Winslow, R. Nilsen, T. R. Insel, *Behav. Neurosci.* **111**, 599 (1997).
13. M. M. Lim *et al.*, *Nature* **429**, 754 (2004).
14. L. J. Young, R. Nilsen, K. G. Waymire, G. R. MacGregor, T. R. Insel, *Nature* **400**, 766 (1999).
15. E. A. Hammock, L. J. Young, *Mol. Biol. Evol.* **21**, 1057 (2004).
16. J. A. Thomas, E. C. Birney, *Behav. Ecol. Sociobiol.* **5**, 171 (1979).
17. R. L. Roberts, J. R. Williams, A. K. Wang, C. S. Carter, *Anim. Behav.* **55**, 1131 (1998).
18. E. A. Hammock, L. J. Young, *Eur. J. Neurosci.* **16**, 399 (2002).
19. S. M. Phelps, L. J. Young, *J. Comp. Neurol.* **466**, 564 (2003).
20. E. Hammock, M. Lim, H. Nair, L. Young, *Genes Brain Behav.*, in press.
21. Z. Wang, C. F. Ferris, G. J. De Vries, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 400 (1994).
22. D. Francis, J. Diorio, D. Liu, M. J. Meaney, *Science* **286**, 1155 (1999).
23. I. F. Bielsky, L. J. Young, *Peptides* **25**, 1565 (2004).
24. Y. Liu, J. T. Curtis, Z. Wang, *Behav. Neurosci.* **115**, 910 (2001).
25. J. R. Williams, B. M. Slotnick, B. W. Kirkpatrick, C. S. Carter, *Physiol. Behav.* **52**, 635 (1992).
26. B. Kirkpatrick, J. R. Williams, B. M. Slotnick, C. S. Carter, *Physiol. Behav.* **55**, 885 (1994).
27. I. F. Bielsky, S. B. Hu, K. L. Szegda, H. Westphal, L. J. Young, *Neuropsychopharmacology* **29**, 483 (2004).
28. D. E. Dluzen, S. Muraoka, M. Engelmann, R. Landgraf, *Peptides* **19**, 999 (1998).
29. M. Engelmann, R. Landgraf, *Physiol. Behav.* **55**, 145 (1994).
30. M. M. Lim, L. J. Young, *Neuroscience* **125**, 35 (2004).
31. L. J. Pitkow *et al.*, *J. Neurosci.* **21**, 7392 (2001).
32. M. Kirschner, J. Gerhart, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 8420 (1998).
33. M. Thibonnier *et al.*, *J. Mol. Cell. Cardiol.* **32**, 557 (2000).
34. S. J. Kim *et al.*, *Mol. Psychiatry* **7**, 503 (2002).
35. T. H. Wassink *et al.*, *Mol Psychiatry* **9**, 986 (2004).
36. GenBank accession number NW_114825.
37. GenBank accession number AF208541.
38. F. B. M. de Waal, *Behaviour* **106**, 183 (1988).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**Supporting Online Material**

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Materials and Methods
Fig. S1


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