The genetic basis of parental care evolution in monogamous mice

Andres Bendesky1,2,3, Young-Mi Kwon1,2, Jean-Marc Lassance1,2,3, Caitlin L. Lewarch1,3, Shenzin Yao1,3, Brant K. Peterson1,2,†, Meng Xiao He†, Catherine Dulac1,3,5 & Hopi E. Hoekstra1,2,3,4,6

Parental care is essential for the survival of mammals, yet the mechanisms underlying its evolution remain largely unknown. Here we show that two sister species of mice, Peromyscus polionotus and Peromyscus maniculatus, have large and heritable differences in parental behaviour. Using quantitative genetics, we identify 12 genomic regions that affect parental care, 8 of which have sex-specific effects, suggesting that parental care can evolve independently in males and females. Furthermore, some regions affect parental care broadly, whereas others affect specific behaviours, such as nest building. Of the genes linked to differences in nest–building behaviour, vasopressin is differentially expressed in the hypothalamus of the two species, with increased levels associated with less nest building. Using pharmacology in Peromyscus and chemogenetics in Mus, we show that vasopressin inhibits nest building but not other parental behaviours. Together, our results indicate that variation in an ancient neuropeptide contributes to interspecific differences in parental care.

In mammals, parental care is critical for the survival of young. Parental behaviour, however, is not uniform and instead varies in form and magnitude among individuals, between sexes, and across species. A particularly variable feature of parental behaviour is the care for young by their fathers, which occurs more often and to a larger extent in monogamous species than promiscuous ones.

Parental care, performed by mothers and some fathers, encompasses an array of behaviours including retrieving, huddling, nursing, and grooming pups along with building a nest, which is often initiated or enhanced by the birth of a litter. The rich diversity of parental behaviours raises several questions about the evolution of parental care, particularly in monogamous mammals: how does the genetic architecture differ between males and females? Does evolution act on genetic loci that affect multiple components of parental behaviour in concert or do particular components have independent genetic underpinnings? What specific genes are involved? How does variation in these genes act within neuronal circuits to alter behaviour?

Comparative analyses of species with divergent social systems have offered important insights into the genetic and neurobiological basis of natural variation in social behaviour. Studies of monogamous and promiscuous voles defined a prominent role for the vasopressin and oxytocin pathways in affiliative behaviours, showing, for example, that differences in the spatial distribution of the vasopressin 1a receptor in the male brain are associated with changes in pair-bonding behaviour.4,5,6 Oxytocin and vasopressin are known to regulate many aspects of parental care,4–7, but how genetic variation in these pathways contributes to natural differences in parental care is poorly understood.

To study the genetic basis of parental care, we focused on mice of the genus Peromyscus, in which both social and genetic monogamy has evolved independently at least twice (Fig. 1a). One of these lineages and its sister species, the monogamous old-field mouse (P. polionotus subgrisius)11,12 (Fig. 1a, b). These species are at opposite ends of a monogamy–promiscuity spectrum, yet—in contrast to the classic vole species—they can interbreed in the laboratory. The remarkable divergence in behaviour between such closely related species offers a unique opportunity to define the genomic architecture of a mammalian social behaviour and to identify pathways and genes that contribute to the evolution of parental behaviour.

Monogamous Peromyscus are more parental

First, we measured components of both maternal and paternal behaviour (Supplementary Videos 1–8) and found that, in general, P. polionotus fathers provided care similar to mothers, whereas P. maniculatus fathers provided little parental care. Specifically, P. polionotus fathers built nests, licked pups, and huddled pups to the same extent as P. polionotus mothers and to a larger extent than both P. maniculatus fathers and mothers, but retrieved fewer pups than P. polionotus mothers (Fig. 1c–f). P. maniculatus fathers did as little nest building and pup licking as P. maniculatus mothers but huddled and retrieved pups significantly less than P. maniculatus mothers (Fig. 1c–f). Mothers of the two species also differed: P. polionotus provided more care than P. maniculatus in all behaviours but pup retrieval (Fig. 1c–f). Overall, we observed large differences in parental behaviour, and the differences were most pronounced in fathers.

To test whether these interspecific and between-sex differences in parental behaviour persisted in less-controlled conditions, we videotaped parents in their home cage for 3 consecutive days starting at the birth of a litter. Results were consistent with the shorter behavioural assays: P. polionotus fathers were more paternal than P. maniculatus fathers, whereas mothers of the two species were similar in their parental care (Extended Data Fig. 1). Together, both the acute and multi-day behavioural patterns aligned with the highly divergent mating systems of these two species.
Parental behaviour in *Peromyscus* is heritable

To determine whether interspecific differences in parental behaviour are influenced by the parental care received as pups, we cross-fostered *P. polionotus* and *P. maniculatus* pups with parents from the other species (Fig. 2a). The parental behaviour of cross-fostered animals, once they became parents themselves, was indistinguishable from the parental care of non-cross-fostered animals (Fig. 2b–e). There were two exceptions: *P. maniculatus* males raised by *P. polionotus* parents licked and retrieved their own pups less than *P. maniculatus* males raised by their own parents. In summary, the care that pups receive does not explain species differences that emerge in adulthood, consistent with a strong heritable component to parental behaviour.

Differences in parental care also may occur because *P. polionotus* pups demand more care than *P. maniculatus* pups. To test this possibility, we compared the parental behaviour of animals towards their own pups with the behaviour towards pups from the other species. In general, fathers and mothers of both species behaved indistinguishably towards their own pups and pups of the other species (Extended Data Fig. 2 and Supplementary Discussion), indicating that the higher levels of parental behaviour in *P. polionotus* are not due simply to an increased demand for care by their pups.

Genetic architecture of parental care

Encouraged by the large and plausibly heritable differences in parental behaviour between *P. polionotus* and *P. maniculatus*, we conducted an interspecies cross to identify genetic components that modulate parental behaviour. We first crossed *P. maniculatus* females to *P. polionotus* males to generate (F1) hybrids. Next, we intercrossed these F1 hybrids to generate 769 F2 hybrids (419 males and 350 females) and measured parental behaviour towards their F3 pups (Fig. 3a). The distribution of each component of parental care among the F2 mice encompassed the distributions of both species (Fig. 3b–e). On the basis of the largely unimodal distributions of parental behaviours among the F2 hybrids, which resembled *P. maniculatus* more closely than *P. polionotus*, the more extensive parental care of *P. polionotus* probably involves more than one genetic locus.

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**Figure 1** | Parental behaviours of monogamous and promiscuous *Peromyscus* mice. a. Cladogram of selected *Peromyscus* species, including known monogamous species (adapted from ref. 8). b. Typical *P. maniculatus* and *P. polionotus* parents in the laboratory. c–f. Parental behaviours of *P. maniculatus* and *P. polionotus*. Each circle represents the median behaviour of an animal tested three times (c–e) or the fraction of pups retrieved over the three tests (f). Box plots indicate median, interquartile range, and 10th–90th percentiles. Kruskal–Wallis followed by Dunn’s test (c) and two-way analysis of variance (ANOVA) results (d–f): significant effects of species (sp), sex (s), or sex-by-species interaction (×) at *P* < 0.01.

**Figure 2** | Effect of cross-fostering on parental behaviour. a. Design of the cross-fostering experiment between *P. maniculatus* and *P. polionotus*. b–e. Parental behaviour of animals raised by their own parents (white) or by parents of the other *Peromyscus* species (grey). Each circle represents the median behaviour of an animal tested three times. Box plots indicate median, interquartile range, and 10th–90th percentiles. *P* < 0.05; **P < 0.01; NS, not significant by two-sided Mann–Whitney *U*-test with Bonferroni correction.

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directions in the two sexes (Extended Data Fig. 4 and Supplementary Discussion). In addition, the strength of correlation between genotype and parental behaviour was often very different between the sexes (Fig. 4b), suggesting that some genetic variants have a stronger effect in one sex than the other. Together, these results indicate that the genetic architecture of parental behaviour differs between males and females.

Several QTLs had effects on specific parental behaviours, whereas others affected more than one behaviour. For example, one QTL on chromosome 4 and one on chromosome 9 were linked to nest building in both males and females, but were not associated with any other behaviours (Fig. 4b, Extended Data Fig. 3 and Supplementary Discussion), consistent with the low phenotypic correlation between nest building and the other parental behaviours (Fig. 4a). By contrast, we found two QTLs that affected several aspects of paternal behaviour: a chromosome 12 QTL affected huddling, licking, and retrieving of pups, and a chromosome 5 QTL affected huddling and latency to handle pups (Fig. 4b and Extended Data Fig. 3). These data raise the possibility that there are genes that have specific behavioural effects and others that can affect parental behaviour more broadly.

**A QTL for parental nest building**

We next focused on a QTL on chromosome 4 that was associated with parental nest building and had the strongest effect on behaviour of all QTLs (Extended Data Fig. 4). Nest building is a core component of the parental behaviour of vertebrates, which, in *Peromyscus*, increases significantly when animals become parents (Extended Data Fig. 5a). Moreover, monogamous *P. polionotus* build more elaborate nests than promiscuous *P. maniculatus* during an acute parental assay, and *P. polionotus* pairs maintain more elaborate nests than *P. maniculatus* for at least 2 weeks after the birth of their pups (Extended Data Fig. 5b). The chromosome 4 QTL was not detected when the nest building of the same animals was measured before mating (data not shown), implying that this QTL is important in a parental context. These results indicate that the chromosome 4 QTL affects an interspecific difference in nesting behaviour that is enhanced by, and maintained during, parenthood.

To identify genes mediating interspecific differences in parental nest building, we first determined which of the genes in the chromosome 4 QTL differed in their protein sequence. Of the 498 genes encompassed by both the male and female QTL regions, 34 had interspecific differences (Supplementary Data File 1), but only 4 were predicted to affect protein function (Supplementary Data File 1). These included *ALK*, *PIK3C2G*, *CCL1*, and *RAB27A*. None were strong candidates for affecting nest building.

**Sex–specific genetics of parental care**

Of the 12 QTLs, 8 showed evidence of having different effects in the two sexes as determined by a QTL analysis with sex as a covariate (Fig. 4b). Genotypes at some QTLs preferentially affected the behaviour of one sex, and at other QTLs they affected behaviour in opposite

**Figure 3 | Distribution of parental behaviours in each species and their interspecific hybrids.** a, Genetic cross design. Female *P. maniculatus* were mated to *P. polionotus* males to find the cross (see Methods); the behaviour of females and males of each species is shown here for comparison. b–e, Violin plots show the distribution of behaviours. Each dot represents the mean behaviour of an animal over three trials.

Next, we tested for correlations among behaviours in the F2 progeny and analysed the sexes independently (Fig. 4a). We found that times spent huddling and licking were positively correlated in both males and females (Spearman’s correlation $r_s = 0.88$, 0.66, respectively), and these two behaviours were also strongly correlated with pup retrieval (huddling–retrieving $r_s = 0.60$ in males, 0.51 in females; licking–retrieving $r_s = 0.52$ in males, 0.45 in females). In males, huddling and licking were also correlated with how soon they handled the pup after the assay started ($r_s = 0.72$ for both). By contrast, in both males and females, nest-building behaviour had much weaker correlations with the other parental behaviours ($r_s$ ranged from 0.23 to 0.43 in males, from 0.22 to 0.31 in females). These results suggest that some genetic loci affect multiple parental behaviours, whereas nest building is more genetically independent from the other behaviours measured.

To identify specific genetic regions that mediate the differences in parental behaviour between *P. polionotus* and *P. maniculatus*, we performed quantitative trait locus (QTL) analysis. To start, we assembled the *P. maniculatus* genome into chromosomes. To genotype the F2 animals, we combined double-digest restriction–site–associated DNA sequencing (ddRAD-seq) with multiplexed shotgun genotyping (MSG). Using this strategy, we genotyped the 769 F2 mice at 406,611 DNA sequencing (ddRAD-seq) with multiplexed shotgun genotyping (MSG). Using this strategy, we genotyped the 769 F2 mice at 406,611

**Vasopressin inhibits nest building**

The gene for arginine vasopressin (*Avp*), an important modulator of social behaviours, including maternal care, stood out as a top candidate for variation in nest building. It is found within the large-effect nest-building QTL, highly expressed (among the 4% most highly expressed genes in the hypothalamus), robustly differentially expressed between the two species in both sexes (2.8-fold higher levels in
Specifically manipulate the activity of vasopressin neurons. Consistent QTL containing the vasopressin gene for nest building (Fig. 5c). Remarkably, vasopressin administration did not affect any similar nonapeptide oxytocin did not have a measurable effect on this inhibition of nest building was specific to vasopressin, as the highly inverse relationship between vasopressin levels and nest expression (Fig. 5a and Extended Data Figs 7b and 8). These results are consistent with differences in vasopressin protein levels in the PVN of these species. Moreover, while vasopressin is known to promote anxiety in rodents, our genetic analyses of anxiety-related behaviour are consistent with differences in vasopressin protein levels in the PVN of these species. Consistent with the largely polygenic nature of variation in parental nest-building behaviour in Peromyscus, we documented large, heritable differences in parental behaviour and then dissected the genetic architecture of this behaviour variation. Consistent with the largely polygenic nature of variation in behaviour, we found that the recent evolution of increased parental care in the monogamous Peromyscus involved many regions spread across the genome. Most of these regions have sex-specific effects, suggesting that variation in male and female parental care has a different genetic basis, even if many of the behaviours appear similar between the sexes. Some brain sexual dimorphisms are thought to compensate for other biological differences between the sexes (like pregnancy and parturition only in females), ensuring that males and females behave similarly. Our results provide genetic evidence of this phenomenon: monogamous males and females evolve through different genetic routes to achieve similarly high levels of parental care.

Figure 4 | The genetic architecture of parental behaviour. a, Correlation matrix of parental behaviours in F2 hybrids. b, The linkage (lod score) of each behaviour to each marker in each sex and the sex-by-genotype interaction (grey). Boxes delineate the Bayes 0.95 credible interval of QTL of genome-wide significance (α = 0.05). Dots denote the allele at

P. maniculatus than P. polionotus; moderated t-statistic, false discovery rate (FDR)-adjusted P = 5 × 10⁻⁵, and showed allele-specific expression (Fig. 5a and Extended Data Figs 7b and 8). These results are consistent with differences in vasopressin protein levels in the PVN of these species. Moreover, while vasopressin is known to promote anxiety in rodents, our genetic analyses of anxiety-related behaviour in the same animals argue that effects of vasopressin on parental nest-building behaviour in Peromyscus are not mediated simply through changes in anxiety (Extended Data Fig. 9 and Supplementary Discussion). Thus, vasopressin emerged as a strong candidate to test for a role in variation in parental nest-building behaviour in Peromyscus.

We next performed pharmacological experiments to determine whether vasopressin regulates nest building in Peromyscus. Consistent with the inverse relationship between vasopressin levels and nest building in both Mus musculus and Peromyscus (this study), intracerebroventricular administration of vasopressin into parents of the high nest-building P. polionotus inhibited nest building (Fig. 5c). The inhibition of nest building was specific to vasopressin, as the highly similar nonapeptide oxytocin did not have a measurable effect on this behaviour. Remarkably, vasopressin administration did not affect any other parental behaviours measured, in line with the specificity of the QTL containing the vasopressin gene for nest building (Fig. 5c).

To confirm that vasopressin can suppress nest building, we performed chemogenetic experiments in M. musculus, where we could specifically manipulate the activity of vasopressin neurons. Consistent with our expectations, inhibition of vasopressin neurons in the PVN, the only vasopressin nucleus previously implicated in nest building (by lesion studies), increased nest building, whereas excitation decreased it (Extended Data Fig. 10 and Supplementary Discussion). Together, our results indicate that vasopressin has an inhibitory effect on parental nest-building behaviour as shown in P. polionotus and confirmed in M. musculus.

Discussion

Here, using a pair of sister species that lie at opposite ends of the monogamy–promiscuity spectrum, we documented large, heritable differences in parental behaviour and then dissected the genetic architecture of this behaviour variation. Consistent with the largely polygenic nature of variation in behaviour, we found that the recent evolution of increased parental care in the monogamous Peromyscus involved many regions spread across the genome. Most of these regions have sex-specific effects, suggesting that variation in male and female parental care has a different genetic basis, even if many of the behaviours appear similar between the sexes. Some brain sexual dimorphisms are thought to compensate for other biological differences between the sexes (like pregnancy and parturition only in females), ensuring that males and females behave similarly. Our results provide genetic evidence of this phenomenon: monogamous males and females evolve through different genetic routes to achieve similarly high levels of parental care.
hence less pleiotropic, than mutations in the respective ligands34,35. Because altering their expression pattern or levels can be more modular, receptors are preferred targets of evolutionary change in behaviour such as vasopressin and nest building.

Reproductive output can provide novel molecular handles for multiple levels of this hierarchy. Thus, the genetic architecture of parental behaviour broadly, and others that are specific to a particular parenting type, probably control the execution of each component. However, it is unknown how this hierarchical organization is manifested at the genetic level. Here we found genetic loci that affect parenting the species with higher expression. Circles represent each individual. No significant differences between sexes for any gene. c. Parental behaviours of P. polionotus after vehicle, 100 ng vasopressin (AVP), or 100 ng oxytocin (OXT) intracerebroventricular administration. Each individual (grey) is connected by lines. Red and blue circles with whiskers indicate mean ± s.e.m. *P < 0.05; **P < 0.01; NS, not significant by Friedman followed by Dunn’s test compared with vehicle.

Ethological observations and theory30,31 as well as neurobiological experiments31,32 indicate that behaviours are often hierarchically organized such that an internal state (such as hunger) can motivate multiple specific behaviours related to that state (such as searching for and then consuming food). This organization is further manifested in the brain: some brain areas and neuron types control the execution of specific behaviours. For example, galanin neurons in the medial preoptic area of the hypothalamus are essential for multiple components of parental care33, and more discrete neuronal types probably control the execution of each component. However, it is unknown how this hierarchical organization is manifested at the genetic level. Here we found genetic loci that affect parenting behaviour broadly, and others that are specific to a particular parenting behaviour sequence. Thus, the genetic architecture of parental behaviour mirrors its neuronal organization, and our results begin to provide novel molecular handles for multiple levels of this hierarchy, such as vasopressin and nest building.

It has long been seen that neurotransmitter and neuromodulator receptors are preferred targets of evolutionary change in behaviour because altering their expression pattern or levels can be more modular, hence less pleiotropic, than mutations in the respective ligands34,35. Here we uncovered a case where differences in the levels of the vasopressin peptide itself have probably contributed to interspecific variation in parental behaviour, arguing that, at least in some instances, genetic polymorphism affecting neuromodulators can contribute to behavioural evolution.

Our genetic dissection of parenting—from natural behaviour to candidate gene—opens exciting new avenues of research for the neuro-biological and circuit-based understanding of a complex social behaviour. In particular, discovering molecular and neural mechanisms by which genes with sex-specific effects act to modulate behaviours common to both sexes, and how highly pleiotropic genes such as vasopressin may change only specific aspects of behaviour, represent important next steps in understanding how behaviours and the brain evolve.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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1. Lukas, D. & Clutton-Brock, T. H. The evolution of social monogamy in mammals. Science 341, 526–530 (2013).
2. Lim, M. M. et al. Enhanced partner preference in a promiscuous species by manipulating the expression of a single gene. Nature 429, 754–757 (2004).
Okhovat, M., Berrio, A., Wallace, G., Ophir, A. G. & Phelps, S. M. Sexual fidelity trade-offs promote regulatory variation in the prairie vole brain. Science 350, 1371–1374 (2015).

Wang, Z., Ferris, C. F. & De Vries, G. J. Role of septal vasopressin innervation in paternal behavior in prairie voles (Microtus ochrogaster). Proc. Natl Acad. Sci. USA 91, 400–404 (1994).

Bosch, O. J. & Neumann, I. D. Both oxytocin and vasopressin are mediators of maternal care and aggression in rodents from central release to sites of action. Horm. Behav. 61, 293–303 (2012).

Dulac, C., O’Connell, L. A. & Wu, Z. Neural control of maternal and paternal behaviors. Science 345, 765–770 (2014).

Scott, N., Prigge, M., Yzhar, O. & Kimchi, T. A sexually dimorphic hypothalamic circuit controls maternal care and oxytocin secretion. Nature 525, 519–522 (2015).

Turner, L. M. et al. Monogamy evolves through multiple mechanisms: evidence from V1AR in deer mice. Mol. Biol. Evol. 27, 1269–1278 (2010).

Birdsall, D. A. & Nash, D. Occurrence of successful multiple insemination of females in natural populations of deer mice (Peromyscus maniculatus). Evolution 27, 106–110 (1973).

Dewsbury, D. A. Agonistic reproduction of deer mice (Peromyscus maniculatus) in a semi-natural enclosure. Behaviour 91, 1–23 (1984).

Dewsberry, D. A. & Loveccky, D. V. Copulatory behavior of old-field mice (Peromyscus polionotus) from different natural populations. Behav. Genet. 4, 347–355 (1974).

Foltz, D. W. Genetic evidence for long-term monogamy in a small rodent, Peromyscus polionotus. Am. Nat. 117, 665–675 (1981).

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S. & Hoekstra, H. E. Double digest RASeq: an inexpensive method for de novo SNP discovery and genotyping in model and nonmodel species. PLoS ONE 7, e37135 (2012).

Andolfatto, P. et al. Multiplexed shotgun genotyping for rapid and efficient genetic mapping. Genome Res. 21, 610–617 (2011).

Royle, N. J., Smiseth, P. T. & Kolliker, M. The Evolution of Parental Care (Oxford Univ. Press, 2012).

Choi, Y., Sims, G. E., Murphy, S., Miller, J. R. & Chan, A. P. Predicting the functional effect of amino acid substitutions and indels. PLoS ONE 7, e46688 (2012).

Lagoutte, E. et al. Oxidation of hydrogen sulfide remains a priority in mammalian cells and causes reverse electron transfer in colonocytes. Biochim. Biophys. Acta Bioenerg. 1797, 1500–1511 (2010).

Seth, R. B., Sun, L., Es, C.-K. & Chen, Z. J. Identification and characterization of MADS-a motif antiviral signaling protein that activates NF-κB and IRF 3 Cell 122, 669–682 (2005).

Renella, R. et al. Codanin-1 mutations in congenital dyserythropoietic anemia type 1 affect HPL1 localization in erythroblasts. Blood 117, 6928–6938 (2011).

Lindfors, P. H., Lindahl, M., Rossi, J., Saarma, M. & Airaksinen, M. S. Ablation of persephin receptor glial cell line-derived neurotrophic factor family receptor α4 impairs thyroid calcitonin production in young mice. Endocrinology 147, 2237–2244 (2006).

Numan, M. Medial preoptic area and maternal behavior in the female rat. Proc. Natl Acad. Sci. USA 91, 400–404 (1994).

Seth, R. B., Sun, L., Ea, C.-K. & Chen, Z. J. Identification and characterization of MADS-a motif antiviral signaling protein that activates NF-κB and IRF 3 Cell 122, 669–682 (2005).

Insel, T. R. & Harbaugh, C. R. Lesions of the hypothalamic paraventricular nucleus disrupt the initiation of maternal behavior. Physiol. Behav. 45, 1033–1041 (1989).

Insel, T. R. The challenge of translation in social neuroscience: a review of oxytocin, vasopressin, and affiliative behavior. NeuroN 65, 768–779 (2010).

Krawczak, M., Yamamoto, Y., Hoffman, G. E. & Cushing, B. S. Estrogen receptor α and vasopressin in the paraventricular nucleus of the hypothalms in Peromyscus. Brain Res. 1032, 154–161 (2005).

Neumann, I. D. & Landgraf, R. Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. Trends Neurosci. 35, 649–659 (2012).

Bult, A., van der Zee, E. A., Compaijn, J. C. & Lynch, C. B. Differences in the number of arginine-vasopressin-immunoreactive neurons exist in the suprachiasmatic nuclei of house mice selected for differences in nest-building behavior. Brain Res. 578, 335–338 (1992).

Bendesky, A. S. & Bergmann, C. L. Genetic contributions to behavioural diversity at the gene–environment interface. Nat. Rev. Genet. 12, 809–820 (2011).

De Vries, G. J. Sex differences in adult and developing brains: compensation, compensation, compensation. Endocrinology 145, 1063–1068 (2004).

Timbergen, N. The hierarchical organization of nervous mechanisms underlying instinctive behaviour. Symp. Soc. Exp. Biol. 4, 305–312 (1950).

Kennedy, A. et al. Internal states and behavioral decision-making: toward an integration of emotion and cognition. Cold Spring Harb. Symp. Quant. Biol. 79, 199–210 (2014).

Devidze, N., Lee, A. W., Zhou, J. & Pfaff, D. W. CNS arousal mechanisms bearing on and other biologically regulated behaviors. Physiol. Behav. 88, 283–293 (2006).

Wu, Z., Autry, A. E., Bergan, J. F., Watabe-Uchida, M. & Dulac, C. G. Galanin neurons in the medial preoptic area govern parental behaviour. Nature 509, 325–330 (2014).

Insel, T. R., Gilhard, R. & Shapiro, L. E. The comparative distribution of forebrain receptors for neurohypophysial peptides in monogamous and polygamous mice. Neuroscience 43, 623–630 (1991).

Donaldson, Z. R. & Young, L. J. Oxytocin, vasopressin, and the neurogenetics of sociality. Science 322, 900–904 (2008).

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Author Contributions A.B. and H.E.H. conceived and designed the study, A.B. and Y.-M.K. collected and analysed behavioural data, A.B., Y.-M.K., and C.L.L. generated ddRAD-seq libraries. B.K.P. wrote code to map ddRAD-seq data. B.K.P. and A.B. wrote code to track animal behaviour from videos. A.B. and M.X.H. made a chromosome-level map of the Peromyscus genome. A.B. performed genetic mapping, pharmacology, and chemogenetics experiments. A.B. and J.-M.L. collected and analysed the RNA sequencing data. C.L.L. blind-scored behavioural assays. S.Y. made the Ave-Cre transgenic mice. A.B., Y.-M.K., J.-M.L., C.D., and H.E.H. analysed and interpreted results. A.B. and H.E.H. wrote the paper with input from all authors.

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METHODS

Except where stated, no statistical methods were used to predetermine sample size, the experiments were not randomized, and the investigators were not blinded to allocation during experiments and outcome assessment.

Animal husbandry. We established colonies of *P. maniculatus bairdii* (strain BW) and *P. polionotus subgriseus* (strain PO) at Harvard University from animals originally obtained from the Peromyscus Stock Center at the University of South Carolina. We housed animals in barrier, specific-pathogen-free conditions with 16 h light–8 h dark at 22 °C in individually ventilated cages (total cage size ≥ 12° long × 6.5° high; Allentown, Allentown, New Jersey) with quarter-inch Bed-o-cob bedding (The Andersons, Maumee, Ohio). Breeding animals and their litters were fed irradiated PicoLab Mouse Diet 20 5058 (LabDiet, St. Louis, Missouri) ad libitum and had free access to water. We weaned animals at 23 days of age into cages with at most four other members of the same strain and sex. After weaning, we fed irradiated LabDiet Prolabs Isopro RMH 3000 5P75 (LabDiet) ad libitum with free access to water and provided them with nesting material (Nestlet, Ancare, Bellmore, New York) and a polycarbonate translucent red hut.

Animal experimentation protocols were approved by the Harvard University Faculty of Arts and Sciences Institutional Animal Care and Use Committee.

Parental behaviour assay. We tested animals for parental behaviour when their pups were 4, 5, and 6 days old. Parental behaviour was tested with the first litter unless otherwise noted. Testing was performed between zeitgeber times 6 and 15. We transferred animals to a testing room adjacent to the housing room. The female, pups, nest, red hut, and food hopper were transferred to a new cage and placed 30–300 cm from the male cage, undisturbed while the male was being tested.

To begin each assay (time 0), we gave ~0.625 g of compacted cotton nesting material (Ancare, Bellmore, New York) to the male within 1 min of being transferred to the test cage. At 30 min, we placed one of the animal’s own pups inside the cage (in the centre of the cage if the male had built a nest in a corner or was sitting in a corner or in a corner if the animal had built a nest in the centre). We then scored the latency to approach the pup, to handle (move) the pup with the front paws, to lick the pup, to huddle over the pup (by covering at least 50% of the pup’s body with the parent’s body), and to retrieve the pup (pick up the pup with the mouth and displace it from its original position). We also scored the total time licking the pup and huddling over the pup until minute 50, when we scored nest quality according to the scale described below. At minutes 50 and 52 (20 and 22 min after adding the pup), we added another of the father’s own pups to the cage (following the same placement guidelines as above and at least 5 cm from pups already in the cage) and scored the time to retrieve the pup. If there were fewer than three pups in the litter, we used one of the pups again (removing it at minutes 50 and 52), waiting 10–15 s before adding it back to the cage to measure retrieval.

We tested female mice in a similar fashion, after the male, in the new cage where she had been placed at the beginning of the assay.

The experimenter scored each assay ~200 cm from the cage by observing the cage and a monitor with a video feed from the side of the cage opposite to the experimenter. This double view facilitated scoring of all behaviours, particularly huddling and licking. In Figs 1 and 2, we used the median of the three tests for each behavioural parameter. To increase genetic mapping resolution by avoiding ties in the F1 mice, however, we used the mean of the three tests.

Nest quality score. We used the following scoring system to quantify nest-building behaviour: 0, nesting material is not shredded; 1, all of the nesting material is shredded but is scattered; 2, nesting material is shredded and gathered in a flat platform; 3, nesting material is shredded and has enough height to cover the entire animal; 4, nest covers the entire animal including a complete roof. Nests that were between two of the above categories received an intermediate score in increments of 0.1.

Home cage long-term behavioural observations. We set up breeding cages as described above in the Animal husbandry section. Two days before their first litter, we attached three cameras (Microsoft LifeCam HD-5000, with the infrared cut filter removed, adapted with a 180° 0.25× fisheye lens, and a Neewer 720 nm long-pass filter over the fisheye lens) to the cage. Two cameras were inside the cage, above the food hopper, and one camera was outside the cage, on one side, focused on the nest. We illuminated the cage with a strip of infrared light-emitting diodes attached to the outside of the cage, near the top. To provide a better view of the parents and pups, we removed part of the nest, leaving enough nesting material for a nest with walls but not enough for a roof. The cameras were connected to a computer that recorded continuously with a custom Python script from before birth to 3 days after birth.

We scored the following behaviours on the video recordings, sampling 5 min from every hour at 1 h intervals: amount of time in and outside the nest, time licking pups, time huddling over the pups, time building nest, time retrieving pups, time grooming their mate.

Cross-fostering experiments. Within 24 h of birth, we exchanged pups from one species (*P. polionotus* or *P. maniculatus*) with pups of the other species that were also born within 24 h. In no case was there any obvious rejection of the pups. We weaned cross-fostered pups at 23 days of age with pups of their biological species in cages of five animals of the same sex, in the same way as non-cross-fostered animals. At 60–90 days old, we paired cross-fostered animals with an animal of their own biological species (cross-fostered or not) and tested parental behaviours as described above. Numbers of litters from which cross-fostered and fostered animals were derived: *P. polionotus* mothers, 8; fathers, 9; *P. maniculatus* mothers, 9; fathers, 7.

Cross design. To generate a genetically heterogeneous F1 hybrid population, we first mated four *P. maniculatus* females to four *P. polionotus* males. We were limited to only a single cross direction because of excessive fetal growth and the resultant death of mothers when *P. polionotus* females are mated with *P. maniculatus* males46. Next, 16 F1-sib pairs were intercrossed to generate 769 F2 hybrids. These F2 individuals have autosomes that are a recombinant mix of the two species as well as the *P. maniculatus* mitochondria, and males carry the *P. polionotus* Y chromosome.

ddRAD-seq. We performed ddRAD-seq as described in ref. 14, with some modifications. In brief, we extracted DNA from a liver sample using an AutoGenprep 965 (Autogen). Next, we digested DNA with MluCI and NlaIII enzymes (New England Biolabs), ligated fragments to biotinylated barcoded adapters, and combined them in pools of ≤48 samples. Using a Pippin Prep (Sage Science), we selected fragments of 216–276 base pairs (bp) and purified biotinylated fragments with streptavidin beads, then PCR amplified each pool using a different Illumina multiplex read index for ten cycles using Phusion (New England Biosciences). We evaluated the quality of the final libraries in a 2200 TapeStation (Agilent Technologies) and quantified them fluorimetrically with a Qubit 2.0 (Thermo Fisher Scientific). We sequenced 384 individuals per lane of an Illumina HiSeq 2500 with HiSeq version 4 chemistry in high output mode and paired-ends (2 × 125 bp) to a median depth of 7.8 × 10^6 reads per individual. We then processed Illumina fastq files into separate files on the basis of Illumina multiplex read indices. We merged and trimmed overlapping paired-ends to remove adaptor sequence readthrough and then further de-multiplexed on the basis of the custom barcode.

Overlapping reads were mapped to the *P. maniculatus bairdii* (strain BW) genome reference (Pman_1.0, GenBank assembly accession: GCA_000500345.1) using BWA 0.7.5a (ref. 37) and then re-mapped using Stampy 1.0.18 (ref. 38). Using GATK 2.7-2, we re-aligned reads and called genotypes using UnifiedGenotyper45. The haplotypes of the Illumina reads are the result of a multiple-reference mapping, so we then used variants obtained from multiple reads to determine the genotypes. We then used VCFtools 0.1.11 (http://vcftools.sourceforge.net/) to set the minor allele frequency threshold to 0.005 and then generate genotype and allele calls.

Chromosome-level map of *P. maniculatus* genome. The *P. maniculatus bairdii* (BW) draft reference genome (Pman_1.0, GenBank assembly accession: GCA_000500345.1) has a total sequence length of 2,630,541,020 bp and is assembled into 30,921 scaffolds with a N50 of 3,760,915 bp. To anchor scaffolds into chromosomes, we used genetic linkage data, which reflect the ordering and relative orientation of scaffolds in each linkage group. The procedure involved the following steps.

1. To minimize conflicts in genetic linkage arising from translocations, inversions, and other structural variants that could arise in an interspecific cross, we used genetic information from an intraspecific cross between *P. maniculatus bairdii* (BW) and *P. maniculatus rubidus* (NUB) from Pennsylvania (E. Kingsley, unpublished observations). We genotyped 403 F2 individuals with ddRAD-seq, using EcoRI–NlaIII fragments of 216–276 bp. After quality filtering (quality by depth (QD) > 5, genotype quality (GQ) > 20, removing markers with identical genotypes in all F2 mice, and markers with genotypes in fewer than 220 F2 mice), we obtained 1,779 high-confidence markers.

2. A genetic map was constructed from these 1,779 markers using R/qtl and marker ordering was optimized following guidelines from http://www.rqtl.org/tutorials/geneticmaps.pdf. This produced a map with 23 autosomal linkage groups as compared to the 22 autosomal chromosomes of *P. maniculatus bairdii* (BW) and *P. maniculatus rubidus* (NUB) as described in the mclust package ref. 42. The X chromosome markers were identified via model-based clustering that used the heterozygote frequencies of markers in males and in females, using the Mclust function in the mclust package in R42. We assigned 130 markers to the X chromosome and determined their order as we did for the autosomes.

3. We ordered the 557 scaffolds represented by these 1,779 markers on the basis of the relative position of the markers and oriented them whenever there was more than one marker per scaffold. Spacing between scaffolds was based on the median genetic to physical distance correspondence of 1.4 centimorgans per megabase calculated with the scaffolds with more than one marker per scaffold.

4. This chromosomal map was used for MSG genotyping of the *P. maniculatus × P. polionotus* F2 hybrids. The recombination fraction plots were examined in detail using plotRF (R/qtl) and iplotRF (R/qtlcharts43), and scaffolds were moved to a place with higher linkage and reoriented, whenever possible. This generated a new map that was used for a new MSG run and further
improvement of the scaffold coordinates. This cycle was repeated 13 times, when no further improvement was seen. At this point, the 557 scaffolds encompassed 1,872,038,949 bp, or 71% of the total sequence in the P. _maniculatus_ 1.0 assembly.

5. To add additional scaffolds (and thus include a larger fraction of the genome) to our chromosome-level map, we genotyped 614 _P. maniculatus_ × _P. polionotus_ F₂ hybrids using high-confidence GATK genotypes (filtered depth of coverage at the sample level, DP > 7) directly (as opposed to genotype probabilities from MSG), from ddRAD libraries sequenced at higher coverage. We then mapped these markers into the existing genetic map using Haley–Knott regression. This procedure allowed us to add 1,166 scaffolds to the map.

6. Scaffold coordinates (chromosomal location and orientation) were optimized as described in step 4, eight times, when no further improvement was seen.

7. We assigned chromosome names to the linkage groups in our map on the basis of the localization of known genes in _Peromyscus_ chromosomes in our linkage groups. Chromosomes 16 and 21 from ref. 44 are represented by a single linkage group in our map, and there was no obvious position to split this linkage group, as there was strong linkage among markers throughout. On the basis of new data from fluorescence hybridization experiments (M. Felder and R. O’Neill, personal communication), we find that chromosome 8a (from ref. 44) actually corresponds to chromosome 16, 8b (from ref. 44) is chromosome 8, and in agreement with our linkage results, chromosomes 16 and 21 (from ref. 44) should both be included in chromosome 21. The chromosome names reported here reflect this updated nomenclature.

Our map includes 1,723 scaffolds, which encompass 2,444,934,125 bp (93% of the total sequence in _P. _maniculatus_ 1.0), in 23 autosomes and the X chromosome (Supplementary Data File 2). Genetic linkage data suggest that 63 scaffolds are erroneous chimaeras, since different parts of those scaffolds map to different chromosomes or different parts of a single chromosome. MSG, to identify variants fixed between the _P. maniculatus_ and _P. polionotus_ founders of our intercross, we performed ddRAD-seq in eight founders of the cross (four per species). We used Picard (http://broadinstitute.github.io/picard/) to merge the reads of the four animals of each species into one pseudo-individual of each species and then used GATK 2.7-2 UnifiedGenotyper to genotype the two pseudo-individuals. Variants called at GQ > 15 that were opposite homozygotes in the two pseudo-individuals amounted to a preliminary set of 961,137 fixed variants. To eliminate variants that were not fixed, we genotyped 614 of the F₂ mice in additional HiSeq lanes (as described above) to a median depth of 1 × 10⁶ reads per individual. Only one-quarter of the F₂ mice were expected to be homozygous at a given variant, so we removed the 126,629 variants that were homozygous in all F₂ mice genotyped at that position with a DP > 7. This left us with a set of 836,508 fixed variants.

Next, we performed MSG as described in ref. 15, with some modifications. From Samtools49 mpileups of the GATK RealignedReads BAMs, we extracted the fixed variants and kept only those that were separated by at least 1 kilobase (kb), thus ensuring they originated from independent reads. We formatted these filtered mpileups to be compatible with the hidden Markov model (fit-hmm.R) step of MSG and started the MSG pipeline at that step. Before running fit-hmm, the nqrank normalized values in F₂ mice using 1−10⁻5 log10 as implemented in fitql from R/qtl.

**Comparative transcriptomics.** To estimate mRNA abundance in whole hypothalamus of _P. maniculatus_ and _P. polionotus_, we conducted an RNA sequencing (RNA-seq) experiment. Specifically, immediately after a parental behaviour assay on the sixth day after the birth of their litter, we euthanized five male and five female parents by CO₂ inhalation for 2 min and collected hypothalami according to ref. 49. We immersed the tissue in ice-cold TRIzol reagent (Thermo Fisher, Waltham, Massachusetts) and disrupted the tissues with a motorized pestle before storage at −80 °C until processing. Hypothalamus samples from males and females from both species were processed together from RNA extraction to sequencing to alleviate batch effects. Total RNA was extracted using Direct-96 RNA (Zymo Research, Irvine, California) and used as input to isolate messenger RNA with a NEB magnetic mRNA isolation kit (New England Biolabs, Ipswich, Massachusetts). We constructed complementary DNA (cDNA) libraries using the NEBNext Ultra Directional RNA Library Prep Kit for Illumina (NEB, Ipswich, Massachusetts). Multiplexed libraries were sequenced on an Illumina HiSeq 2500 (Illumina, San Diego, California) with HiSeq version 4 chemistry in high output mode and paired-ends (2 × 125 bp). Libraries from males and females from both species were sequenced in the same lanes to alleviate batch effects. The average depth per sample was 46.1 million reads. We removed low-quality and adaptor bases from raw reads using TrimGalore v0.4.0 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) and mapped the cleaned reads to the genome using the STAR RNA-Seq aligner version 2.4.2a in two-pass mode50. We obtained the _P. maniculatus_ genome sequence and annotation from the Peromyscus Genome Project (P. _maniculatus_ 1.0, GenBank accession number GCA_000500345.1). We created a _P. polionotus_ genome and annotation by incorporating single nucleotide polymorphism (SNPs) and indels into the _P. maniculatus_ P. _maniculatus_ 1.0 reference using the AlleleSeq package51 and the UCSC liftOver tool. Briefly, _P. polionotus_ genomic DNA reads (GenBank BioProject accession number PRJNA535993) were aligned to the _P. manisculus_ 1.0 reference using Stampy52 version 1.0.21 with a substitution rate = 0.04. The resulting alignment files were pre-processed according to the GATK Best Practices. We used HaplotypeCaller to perform variant calling as implemented in GATK 3.3-0 and filtered out variants with non-reference allele frequency <0.50.

We estimated expression using STAR alignments in transcriptomic coordinates and the RSE package53, and calculated differential expression using the limma package following voom transformation of the estimated counts54,55. Raw read counts for genes with at least one count per million in at least three samples (15,729 genes) were used as inputs to the limma/voom pipeline, and scale normalization of the RNA-seq read counts was performed using the TMM normalization method52. The Benjamini and Hochberg method56 was used for adjusting for multiple testing, and we report values of expression at an FDR of 5% for the species factor in a linear model that included species, sex, and sex × species as factors.

**Allele-specific expression analysis.** We crossed two _P. maniculatus_ females to two _P. polionotus_ males to produce F₁ hybrids. Then, we dissected the hypothalami of six female and six male adult F₁ offspring. We used the same procedure described above to sequence RNA libraries. Then we aligned the quality and adaptor trimmed reads to a hybrid diploid _P. maniculatus_ × _P. polionotus_ genome using STAR version 2.4.2a (ref. 50). For each gene, we selected variants common to all isoforms and kept only the variants that were homozygous for opposite alleles in the two species. Of the 23 genes that were differentially expressed between _P. maniculatus_ and _P. polionotus_ and present in the chromosome 4 nest-building QTL, 15 had variants that allowed us to distinguish the species of origin of the transcripts in the F₁ hybrids. We counted the number of reads containing the _P. maniculatus_ allele or the _P. polionotus_ allele in the F₁ hybrids. Using one to three variants per gene represented by different reads, we fitted a linear model for each gene in the R package (lm(ln(maniculatus mapping reads)−lnpolionotus mapping reads)) ~ sex), where ~ represents linear predictor, and identified genes with significant differences in allele-specific expression at an FDR of 5%.

To perform allele-specific expression analysis of _Avp_ by droplet-digital PCR (ddPCR), we extracted RNA from whole brain or from specific brain regions. We extracted RNA from whole brain RNA using TRIzol and chloroform, cleaned the RNA using Qiagen RNeasy columns, and made cDNA with Quanta qScript cDNA Supermix followed by DNaseig digestion. To microdissect individual vasopressin-producing nuclei, we froze freshly dissected brains in Tissue-Tek OCT compound and then mounted brains in cryostat, sectioned coronally until we reached the most posterior part of the anterior commissure, and finally made 1 mm diameter, ~1.5 mm thick punches with the aid of a stereo microscope (Extended Data Fig. 8a). We quickly froze these punches in an RNAqueous-Micro Total RNA Isolation Kit (Ambion) lysis solution on dry ice, and extracted RNA.
RNA following the manufacturer's protocol. Finally, we made cDNA followed by DNasel digestion using Superscript VILO (Invitrogen).

We used a BioRad QX200 Droplet Generator to create droplets, BioRad S1000 for PCR, a QX200 Reader to read droplets, and BioRad dPCR Supermix for probes (No UDT), all according to the manufacturer's instructions.

We used a TaqMan SNP Genotyping assay (Thermo Fisher) targeting the Avp SNP (P. maniculatus = C, P. polionotus = T) at position NW_006501288.1:14,146,329:1, C3TAATGGCCGGAGAATTGCT; primer 2, CAGCCCGCTCAAGNAGCA; reverse primer, AGGCCGCTGCTACTGTTCC.

Protein-coding variant analysis. For variant calling using the hypothalamus RNA-seq data, we mapped the cleaned reads from P. maniculatus and P. polionotus to the Pmanu.1.0 reference genome using STAR RNA-Seq aligner version 2.4.2a in two-pass mode and applying a mapping quality (MAPQ) of 60 for uniquely mapped reads. The resulting alignment files were pre-processed according to the GATK Best Practices recommendations for calling variants on RNA-seq data. Briefly, duplicate reads were marked using Picard (version 1.115) and reads spanning introns were split into exon segments using the SplitNCigarReads function of GATK. Next, we used HaplotypeCaller to perform variant calling as implemented in GATK 3.3-0 on each sample separately using the GVCF mode. Joint genotyping was subsequently performed using all gvcf files simultaneously, allowing a maximum of 12 alternate alleles. We used SnpEff version 4.3 (ref. 57) to annotate and predict the effects of variants on genes.

From the SnpEff-annotated vcf, we determined the genes with fixed differences between the species. A total of 21,316 protein-coding genes out of 22,720 in the annotation had variants that passed our quality filters (GATK’s Best Practices: QD >2; FS <30; DP >4), indicating we could determine variation in 94% of genes in the genome using hypothalamus RNA-seq. To be considered a fixed difference, variants had to be called in at least three individuals of the ten per species, be homozygous in all called individuals, and have different alleles in the two species; these variants were all confirmed with DNA sequences of one independent P. maniculatus and one P. polionotus animal. To identify variants that affected protein sequences, we used SnpEff to select variants with MODERATE or HIGH putative impact. To evaluate the potential impact of fixed variants on protein function, we used the standalone version of the PROVEAN algorithm (version 1.1.5)32 and the NCBI nr database (downloaded April 2015).

Elevated plus maze assay. We designed an elevated plus maze that included four arms (31 cm length × 5 cm width), two closed arms with walls 15 cm high, and the maze floor raised 55 cm above the ground. We first used 50- to 60-day-old P. maniculatus and P. polionotus mice that were housed with same-sex animals. Testing was performed between zeitgeber times 8 and 15 with the lights on. We transferred animals in their home cage into the behaviour testing room, where they were removed from the cage—reduced the number of times

3¾ × l min

1⅞ × l using a MyNeurolab syringe pump under light (1.5% isoflurane) −1, KCl 0.37 g l −1, pH 7.35) via a C315IAS-5 33-gauge hydrogel. We covered the cage with a clear Plexiglass lid and recorded the animal

46. Cande, J., Andolfatto, P., Prud’homme, B., Stern, D. L. & Gompel, N. Evolution of

39. Van der Auwera, G. A.

37. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25, 1754–1760 (2009).

38. Lunter, G. & Goodson, M. Stampy: a statistical algorithm for sensitive and fast mapping of illumina sequence reads. Genome Res. 21, 935–939 (2011).

39. Van der Auwera, G. A. et al. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Curr. Protoc. Bioinformatics 11, 11.10.1–11.10.33 (2013).

40. Painter, T. S. A comparative study of the chromosomes of mammals. Am. Nat. 39, 385–409 (1925).

41. Greenbaum, I. F. et al. Cytogenetic nomenclature of deer mice, Peromyscus (Rodentia); review and review of the standardized karyotype. Report of the Committee for the Standardization of Chromosomes of Peromyscus. Cytogenet. Cell Genet. 66, 181–195 (1994).

42. Fraley, C. & Raftery, A. McClust version 4 for R: normal mixture modeling for model-based clustering, classification, and density estimation (Department of Statistics, University of Washington, 2012).

43. Broman, K. W. & R/qplot: interactive graphics for quantitative trait locus mapping. Genetics 199, 359–361 (2015).

44. Kenney-Hunt, J. et al. A genetic map of Peromyscus with chromosomal assignment of linkage groups (a Peromyscus genetic map). Mamm. Genome 25, 160–179 (2014).

45. Li, H. et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25, 2078–2079 (2009).

46. Cande, J., Andolfatto, P., Frud’homme, B., Slem, D. L. & Gompel, N. Evolution of multiple additive loci caused divergence between Drosophila yakuba and D. santomea in wing rowing during male courtship. PLoS ONE 7, e43888 (2012).
47. Lynch, M. & Walsh, B. Genetics and Analysis of Quantitative Traits 469–476 (Sinauer, 1998).
48. Broman, K. W., Wu, H., Sen, S. & Churchill, G. A. R/qtl: QTL mapping in experimental crosses. Bioinformatics 19, 889–890 (2003).
49. Zapala, M. A. et al. Adult mouse brain gene expression patterns bear an embryologic imprint. Proc. Natl Acad. Sci. USA 102, 10357–10362 (2005).
50. Dobin, A. et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29, 15–21 (2013).
51. Rozowsky, J. et al. AlleleSeq: analysis of allele-specific expression and binding in a network framework. Mol. Syst. Biol. 7, 522 (2011).
52. Li, B. & Dewey, C. N. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12, 323 (2011).
53. Ritchie, M. E. et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 43, e47 (2015).
54. Law, C. W., Chen, Y., Shi, W. & Smyth, G. K. voom: precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol. 15, R29 (2014).
55. Robinson, M. D. & Oshlack, A. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol. 11, R25 (2010).
56. Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. B 57, 289–300 (1995).
57. Cingolani, P. et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff. Fly 6, 80–92 (2012).
58. Clark, R. G., Jones, P. M. & Robinson, I. C. A. F. Clearance of vasopressin from cerebrospinal fluid to blood in chronically cannulated Brattleboro rats. Neuroendocrinology 37, 242–247 (1983).
59. Diamant, M. & De Wied, D. Differential effects of centrally injected AVP on heart rate, core temperature, and behavior in rats. Am. J. Physiol. 264, R51–R61 (1993).
60. Pedersen, C. A., Ascher, J. A., Monroe, Y. L. & Prange, A. J., Jr. Oxytocin induces maternal behavior in virgin female rats. Science 216, 648–650 (1982).
61. Fahrbach, S. E., Morrell, J. I. & Pfaff, D. W. Oxytocin induction of short-latency maternal behavior in nulliparous, estrogen-primed female rats. Horm. Behav. 18, 267–286 (1984).
62. Winslow, J. T., Hastings, N., Carter, C. S., Harbaugh, C. R. & Insel, T. R. A role for central vasopressin in pair bonding in monogamous prairie voles. Nature 365, 545–548 (1993).
63. Kessler, M. S., Bosch, O. J., Bunck, M., Landgraf, R. & Neumann, I. D. Maternal care differs in mice bred for high vs. low trait anxiety: impact of brain vasopressin and cross-fostering. Soc. Neurosci. 6, 156–168 (2011).
64. Bosch, O. J. & Neumann, I. D. Brain vasopressin is an important regulator of maternal behavior independent of dams’ trait anxiety. Proc. Natl Acad. Sci. USA 105, 17139–17144 (2008).
65. Kuroda, K. O., Tachikawa, K., Yoshida, S., Tsuneoka, Y. & Numan, M. Neuromolecular basis of parental behavior in laboratory mice and rats: with special emphasis on technical issues of using mouse genetics. Prog. Neuropsychopharmacol. Biol. Psychiatry 35, 1205–1231 (2011).
66. Xu, X. et al. Modular genetic control of sexually dimorphic behaviors. Cell 148, 596–607 (2012).
Extended Data Figure 1 | Parental behaviours in undisturbed home cages for 3 days after the birth of a litter. The fraction of time an animal was engaged in each behaviour averaged across 5-min samples for each hour, for the 16 h of light and 8 h of dark parts of the day separately. Horizontal lines denote the mean. *P < 0.05; NS, not significant by Mann–Whitney U-test.
Extended Data Figure 2 | Parental behaviours towards own pups and heterospecific pups. The behaviour of parents was measured across 4 consecutive days, alternating the pup species each day (randomizing the pup that was given on day 1). Grey lines connect an individual's behaviour. Blue and red lines denote the median for fathers and mothers, respectively. *P < 0.05; **P < 0.01; NS, not significant by paired t-test or Wilcoxon signed-rank test (nest quality).
Extended Data Figure 3 | QTL mapping of parental behaviours.
Non-parametric interval mapping of (a) six parental behaviours in males ($n = 419$) and females ($n = 350$), and (b) the subset of F2 animals that performed a behaviour (that is, excluding the animals that did not huddle or lick their pups for the duration of all three trials). Sample sizes for huddling were as follows: males, 259; females, 300; for licking: males, 319; females, 313. c, Haley–Knott regression on nqrank normalized values of all F2 individuals, using sex as a covariate. Plots show the difference in lod scores for the scan with sex as an interactive and as an additive covariate minus the scan with sex as an additive covariate alone. The artificial narrow peaks at the ends of chromosomes result from lack of genotype imputation by MSG at chromosome ends (nest quality, chromosome 22; latency to approach, chromosome 9; latency to handle, chromosome 14). a–c, Dashed lines denote the $P = 0.05$ genome-wide significance level determined by 1,000 permutations.
Extended Data Figure 4 | QTL effect plots. Phenotype means ($\pm$ s.e.m.) against genotypes at peak QTL markers reported in Fig. 4 and Extended Data Fig. 3. Above each graph is the chromosomal position of the QTL peak. *Significant QTL-by-sex interaction. The per cent variance explained is given for each QTL and is underlined if the QTL was significant in a QTL analysis of that sex (Extended Data Fig. 3a, b).
Extended Data Figure 5 | Behaviour of sexually naive and parental animals. a, Sexually naive animals were tested with 4- to 6-day-old conspecific pups, and parents with their own 4- to 6-day-old pups. Box plots indicate median, interquartile range, and 10th–90th percentiles. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; NS, not significant by Mann–Whitney U-test or Fisher’s exact test (retrieving and infanticide).
b, Nest quality (mean ± s.e.m., and each pair in magenta circles for P. maniculatus and green squares for P. polionotus) in the 2 weeks after the birth of a litter. Existing nests were removed from the parents’ cage at the time of weaning and 5 g of new cotton nesting material (Nestlet) was provided. Litters were born 1–4 days after weaning the previous litter, and nest quality was evaluated once a day in the home cage, where both mother and father were present. ****P < 0.0001 effect of species in a two-way ANOVA including species and time as factors. There was no significant effect of time or species-by-time interaction.
Extended Data Figure 6 | RNA-seq analysis of *P. maniculatus* and *P. polionotus* hypothalamus. a, Clustering dendrogram of RNA-seq samples by Euclidian distances of transcript expression levels. Samples cluster by species but not by sex. b, Strength of differential expression between species. Each circle represents a gene and is colour-coded in magenta or green if its differential expression was significant at a 5% FDR. c, Relationship between average gene expression level and differential expression between species. In both b and c, genes that have been associated with parental care in previous studies (by physiological/pharmacological studies or induced mutations) are labelled; the gene is also boxed if located inside parental behaviour QTLs identified in this study.
Extended Data Figure 7 | Expression analysis of genes in the chromosome 4 nest-building QTL. a, Twenty-three genes that are differentially expressed in the hypothalamus at 5% FDR between *P. maniculatus* and *P. polionotus*, sorted by FDR-adjusted *P* value. There were no significant differences between sexes for any gene. For each gene, its average expression level in each species and sex is shown on the left, the fold-difference in expression between the species in the middle, and the FDR-adjusted *P* value on the right. b, Allele-specific expression of the 15 genes from a for which interspecific genetic variation allows this analysis. Mean fraction of reads (±s.e.m.) matching the *P. maniculatus* allele from RNA-seq of the hypothalamus of 12 male and 12 female *P. maniculatus × P. polionotus* F1 hybrids. There were no significant differences between males and females, so the sexes were combined for the plot. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 by a linear model measuring allelic bias for each gene: ln(allelic bias) = α + β × sex + ε.
Extended Data Figure 8 | Allele-specific expression of Avp in different brain regions. a, Immunofluorescence staining of vasopressin in a coronal section of a *P. maniculatus* male brain, showing the main vasopressin-producing nuclei. Red circles illustrate the 1 mm diameter (1.5 mm thick) circular punches used to microdissect these nuclei. b, c, Number of Avp transcripts (b) and allele-specific expression of Avp (c) in each of the microdissected regions in *P. maniculatus × P. polionotus* F1 hybrid animals, measured by droplet-digital PCR; horizontal line at the mean. **P < 0.01; ***P < 0.001 by ANOVA with Bonferroni correction. SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; PVN, paraventricular nucleus of the hypothalamus; BNST, bed nucleus of the stria terminalis.
Extended Data Figure 9 | Relationship between anxiety and nest building. a, Fraction of time in the open arms of an elevated plus maze. Line at the mean. ****P < 0.0001 for difference between species by two-way ANOVA with sex and species as factors. No significant effect of sex or sex-by-species interaction. b, Spearman’s correlation coefficients among F2 mice between fraction of time in the open arms and parental behaviours. Handling and approach are promptness to perform those behaviours. c, Linkage (lod score) to chromosome 4 of nest-building behaviour and fraction of time in open arms. Males and females combined since there are no major differences in the lod scores between sexes. Red line denotes the location of Avp. Dashed lines denote the P = 0.05 genome-wide significance level determined by 1,000 permutations of each trait (n = 769).
Extended Data Figure 10 | Chemogenetic experiments on vasopressin neurons of M. musculus. a, Generation of Avp-Cre BAC-transgenic M. musculus. Top: schematic diagram illustrating the targeting of the IRES-Cre cassette immediately after the Avp stop codon. Bottom: immunofluorescence histology of vasopressin (AVP) and Cre in the paraventricular nuclei (PVN) of the hypothalamus of an Avp-Cre BAC-transgenic M. musculus. Scale bar, 100 μm. b, A recombinant adenovirus (rAAV) containing a Cre-dependent DREADD was injected into the PVN of Avp-Cre transgenic M. musculus. c, d, Nest-building behaviour for 1 h after intraperitoneal injection with 0.9% NaCl or with the DREADD agonist clozapine-N-oxide (CNO) at 10 mg per kg. In c, animals expressed the inhibitory Gi-DREADD and in d, the excitatory Gq-DREADD. Males (with blue symbols at the mean ± s.e.m.) are on the left and females (red) are on the right in each panel. Statistical significance determined by repeated-measures ANOVA for quadratic trend.