Supplemental Materials

Supplemental Figure 1.

A)

PND8 Pup Directed Care

B)

PND15 Pup Directed Care
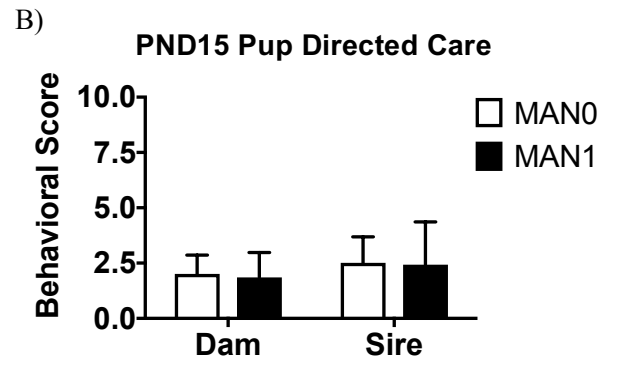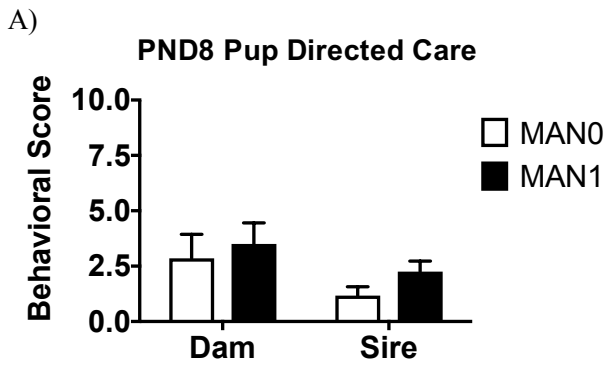
Supplemental Figure 2.

A)

Human: >hg38_dna range=chr3:8769078-8769148

GTCTCAATCCCGAGAAGTCACTTTACAATCTCTCGGAACACCCCGAGCTCCTCCAGGAACAAGGAGTGCGA
　　　　　　　-901　　　　　　　　　　　　　-924　　　　　-934

Mouse: >mm10_dna range=chr6:112490620-112490691

ACCTTCGTCCAAGCTGTCACTTTTCAGTCTCTCTCGGTACACCCTGAGCACCTCCCGGAACCAGAGACGTGA
　　　　　　　　　　　　　　　　　　　　-924

Rat: >rn6_dna range=chr4:144416943-144417009

ATCTTCGTCCAAGCTTTTCAGGCTCTCAGAACATCCTGAGCACCTCCTCCGGGAACCAGAGGTGCGA

Prairie vole*

GTAGTCGTCCGAACTGTCACTTTTCAGTCCCTCGGAGTACCCTGAGCGATTTCGGGAACCAGAAGTGTGA
　　　　　　　-901　　　　　　　　　　　　　-924　　　　　　　-934_2　-934_1

B)

```
                                       -901
        Human  gtctcaat-----cccgagaagt------------------c-----------------------
  Prairie vole  gtagtcgt-----ccgaa-ctgt------------------c-----------------------
        Mouse  accttcgt-----ccaag-ctgt------------------c-----------------------
          Rat  atcttcgt-----ccaag------------------------------------------------
                                        -924        -934
        Human  ---actt----tac-aa--tctc----tcggaa--caccccgagctcctc-----ca---ggaacaa---
  Prairie vole  ---actt----ttc-ag--tccc----tcggag--taccctgagcgattt-----cg---ggaacca---
        Mouse  ---actt----ttc-agtctctc----tcggta--caccctgagcacctc-----cc---ggaacca---
          Rat  ----ctt----ttc-ag--gctc----tcagaa--catcctgagcacctc-----ctccgggaacca---


        Human  ---ggagtg-c-ga
  Prairie vole  ---gaagtg-t-ga
        Mouse  ---gagacg-t-ga
          Rat  ---gaggtg-c-ga
```

C)

```
Statistics: (shuffled [200]) MLE statistics: Lambda= 0.2081;  K=0.4636
 statistics sampled from 1 (1) to 200 sequences
Algorithm: Smith-Waterman (SSE2, Michael Farrar 2006) (7.2 Nov 2010)
Parameters: DNA matrix (5:-4), open/ext: -12/-4
 Scan time:  0.000
```

```
The best scores are:                                          s-w bits E(10000)
Vole                                              (  70) [f]   157 48.3 1.5e-11
```

>>>Human, 71 nt vs TMP.q2 library

```
>>Vole                                                       (70 nt)
 s-w opt: 157   Z-score: 239.9  bits: 48.3 E(10000): 1.5e-11
Smith-Waterman score: 157; 72.6% identity (72.6% similar) in 62 nt overlap (10-71:9-70)
Sequence Lookup   General re-search   Pairwise alignment
              10        20        30        40        50        60        70
Human  GTCTCAATCCCGAGAAGTCACTTTACAATCTCTCGGAACACCCCGAGCTCCTCCAGGAACAAGGAGTGCGA
          ::::   ::::::::: :: :: ::::::  :::: ::::   : : ::::: :: :::: ::
Vole    GTAGTCGTCCGAACTGTCACTTTTCAGTCCCTCGGAGTACCCTGAGCGATTTCGGGAACCAGAAGTGTGA
              10        20        30        40        50        60        70
```
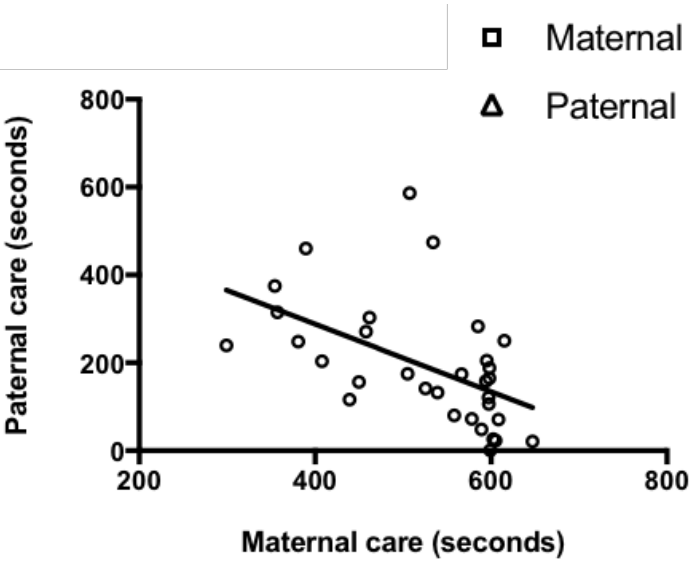
Supplemental Figure 3.
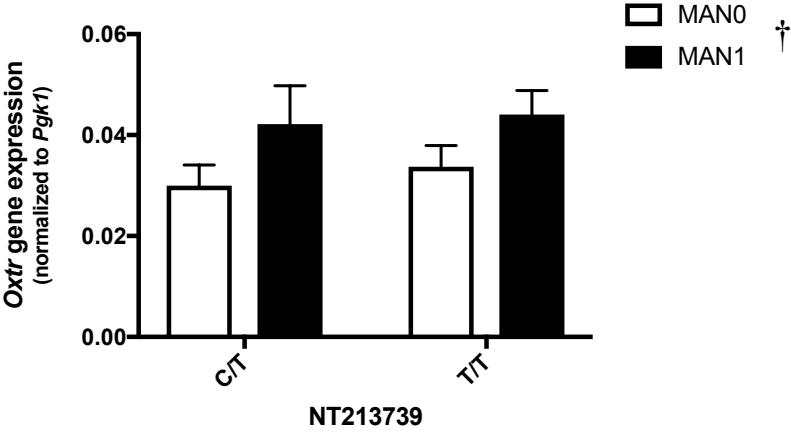
Supplemental Figure 4.

Supplemental Table 1.

	1	2	3	4	5	6	7	8
1. Blood -934_1	1.00							
2. Blood -934_2	0.95	1.00						
3. Blood -924	0.96	0.95	1.00					
4. Blood -901	0.86	0.88	0.92	1.00				
5. Nacc -934_1	0.68	0.70	0.59	0.53	1.00			
6. NAcc -934_2	0.60	0.68	0.49	0.42	0.90	1.00		
7. NAcc -924	0.71	0.75	0.65	0.57	0.91	0.88	1.00	
8. NAcc -901	0.76	0.80	0.71	0.65	0.83	0.81	0.89	1.00

Supplemental Figure and Table Legends

Supplemental Figure 1. Differences in parental care in MAN model are transient. Parental care of offspring following early manipulation did not differ between MAN0 and MAN1 groups on **(A)** PND 8 or **(B)** PND 15.

Supplemental Figure 2. A portion of the human MT2 region in *OXTR* is conserved in prairie voles. **(A)** Genome position and sequences of similar *OXTR* regulatory region in humans and rodents. Prairie vole sequence from GenBank: DP001214.1 **(B)** Partial output of UCSC Multiz alignment highlighting conserved CpG sites of putative *OXTR* regulatory region in multiple species. **(C)** Output from PRSS found at http://fasta.bioch.virginia.edu/.

Supplemental Figure 3. Polymorphism NT213739 does not impact *Oxtr* gene expression in this sample. A polymorphism (NT213739) in the prairie vole *Oxtr* gene was previously reported (King et al, 2016) to impact transcription of the gene. We did not identify C/C homozygotes in the sample we studied and there was no significant difference in transcription between C/T (MAN0, n=5; MAN1, n=4) and T/T (MAN0, n=9; MAN1, n=8) genotypes in our sample. (2-way Handling Condition x SNP ANOVA, † main effect of handling, $F_{(1,22)}$ =4.559, p=0.0441; no effect of SNP, $F_{(1,22)}$ =0.2909, p=0.5950; error bars indicate standard error).

Supplemental Figure 4. Relationship between maternal and paternal behavior. Naturally varying levels of maternal and paternal care are inversely related.

Supplemental Table 1. Correlation of DNA methylation levels between *Oxtr* CpG sites. Methylation values are strongly correlated between CpG sites both within and across tissue types.