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Gene Targeting Approaches to Neuroendocrinology: Oxytocin, Maternal Behavior, and Affiliation

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Transgenic technology affords exciting new opportunities in the field of behavioral neuroendocrinology. We have extended our research into the behavioral function of oxytocin in maternal and social behavior using two transgenic approaches: (i) targeted deletion of the oxytocin gene in mice and (ii) augmented oxytocin receptor expression in the brain. Mice genetically deficient in oxytocin can mate, give birth, and display normal maternal behavior; however, milk ejection and certain aspects of social behavior are affected. Comparative studies of oxytocin receptors have led to the observation that species differences in social organization are associated with differences in receptor distribution. Specifically, monogamous prairie voles and nonmonogamous, asocial montane voles exhibit different patterns of OT receptor expression in the brain. Transgenic mice have been created with a reporter gene driven by the prairie vole oxytocin receptor gene promoter. Analysis of the expression pattern suggests that it should be possible to manipulate receptor expression in the vole brain in order to examine the effects of receptor distribution on behavior.

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Oxytocin (OT) has been implicated in several aspects of reproduction, including sexual behavior (Caldwell, Prange, and Pederson, 1986; Witt and Insel, 1991), induction of labor (Fuchs, Fuchs, Husslein, and Soloff, 1982), milk ejection (Smith, 1989), and maternal behavior (Van Leengoed, Kerker, and Swanson, 1987; Pedersen and Prange, 1979; Pederson, Caldwell, Walker, Ayers, and Mason, 1994). Oxytocin appears also to play a role in social behaviors, such as separation distress and affiliation (Insel, 1992; Insel and Winslow, 1991; Witt, Winslow, and Insel, 1992), as well as species-specific social behaviors, such as pair bonding in monogamous species (Insel, 1992; Insel and Hulihan, 1995). Oxytocin is a nonapeptide produced primarily in the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus (Gainer and Wray, 1994). Although OT was originally characterized as a neurohypophyseal hormone, it is now clear that OT fibers project throughout the brain and that OT functions as a neurotransmitter. The central effects of OT are mediated by a seven-transmembrane domain, G-protein-coupled receptor (Kimura, Tanizawa, Mori, Brownstein, and Okayama, 1992) that is localized in discrete brain nuclei (Barberis and Tribollet, 1996). One of the most interesting features of brain oxytocin pathways is the species diversity in the neuroanatomical distribution of the receptor (Insel, Young, Witt, and Crews, 1993). This plasticity in receptor distribution suggests that OT function may vary among species and may be related to species differences in social behavior (Insel and Shapiro, 1992).

Transgenic technology now provides powerful tools for investigating the role of neuropeptides in controlling behaviors. We have begun using two strategies to investigate the function of OT in reproductive and social behaviors in rodents. First, we are characterizing

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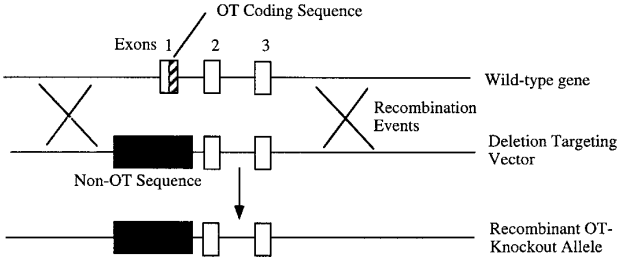

FIG. 1. Schematic illustrating the approach for creating an OT knockout allele. Embryonic stems cells were electroporated with the deletion targeting DNA sequence. Homologous recombination of the targeting sequence with the OT gene resulted in a recombinant allele lacking the DNA sequences coding for the OT peptide, which are located on the first exon. Cell lines with the correct recombination event were injected into blastocysts in order to create chimeras. Chimeras with germ-line transmission were used to create the strain of OT ''knockout'' mice. (Reproduced by permission from Nishimori *et al.,* 1996. Copyright 1996 National Academy of Sciences, U.S.A.).

the behavioral phenotype of a strain of mice that has been genetically engineered to lack the DNA sequence encoding the OT peptide. Second, we are beginning experiments which should ultimately allow the manipulation of the species-typical pattern of expression of oxytocin receptors in a targeted manner. Each of these studies are discussed below.

OT KNOCKOUT MICE

The oxytocin gene is composed of three exons which encode the oxytocin-neurophysin precursor peptide (Hara, Battey, and Gainer, 1990; Ivell and Richter, 1984). Using a targeted deletion vector, an OT knockout mouse was created by deleting the first exon of the OT precursor gene by homologous recombination in embryonic stem cells (Fig. 1) (Nishimori, Young, Guo, Wang, Insel, and Matzuk, 1996). This resulted in a mutant allele which completely lacks the nucleotide sequences encoding the OT peptide. As expected, no OT mRNA can be detected in the brain of homozygous knockout mice, while arginine vasopressin (AVP) mRNA appears to be unaffected (Fig. 2). The effect of the knockout allele on OT immunoreactivity in the PVN and SON is illustrated in Fig. 3. The density of OT-ir staining is reduced in heterozygotes compared to wild-types and is absent in homozygote knockouts. This graded, genotype-dependent decrement in OT peptide provides a useful opportunity to study the behavioral and physiological effects of modest as well as complete deficiencies in the OT system.

One of the most striking features of the OT and AVP system is the variability in the neuroanatomical distribution of the receptor system, both during development (Shapiro and Insel, 1989; Snijdewint, 1989) and among species (Insel *et al.,* 1993). We predicted that if OT projections during development contribute to the final distribution of OT receptors in the adult brain, the OT knockout mouse should show an altered distribution of OT receptors. Surprisingly, both the distribution and the concentration of OT receptors are unaffected in the OT knockout mouse (Fig. 4), suggesting that presynaptic OT does not contribute to the neuroanatomical distribution of receptors during development and thus is unlikely to account for the species differences in receptor distribution.

What about the behavior of the OT knockout mice? As OT is thought to be crucial for several functions necessary for reproduction, we would predict that animals genetically deficient in OT would not mate readily, be unable to deliver, fail to lactate, and be deficient in maternal behavior. However, OT knockout mice mate, have normal gestation periods, build nests, and deliver pups normally (Nishimori *et al.,* 1996). Pups from OT knockout mothers die within 24 hr due to the mother's inability to eject milk. Exogenous OT rescues milk ejection, allowing the pups to develop normally (Nishimori *et al.,* 1996). In order to measure maternal behavior quantitatively, behavioral tests were performed on the morning of parturition. Two pups were removed from the nest and placed in opposite corners of the mother's home cage and the behavior of the mother was videotaped for 30 min. The results reveal no differences in latency to retrieve pups into the nest, amount of time spent in the nest, and time spent grooming the pups, comparing mothers with and without OT (Fig. 5).

Initially we felt that it was possible that a related neuropeptide, such as vasopressin, might be interacting with the OT receptor to permit maternal behavior in the knockout mice. To rule out this possibility, virgin OT knockout mice were fitted with micro-osmotic pumps (Alzet, Model 1007D) which were used to centrally infuse either an oxytocin receptor antagonist ($[d(CH_2)_5,Tyr(Me)^2,Thr^4,Tyr\text{-}NH_2^9]OVT$) at a constant rate of 75 ng/hr, or artificial CSF for 5 days. Maternal behavior was assessed as described above using foster pups on Days 1, 3, and 5 of treatment. On the last day, the brains were harvested and receptor autoradiography was used to determine the degree of receptor blockade. This treatment effectively blocked 68% of the OT receptors in the lateral septum compared to CSF-infused controls; however, there was no difference in maternal behavior. Female mice lacking the gene for OT and with pharmacological blockade of the OT receptor appear to exhibit normal maternal behavior. These data further

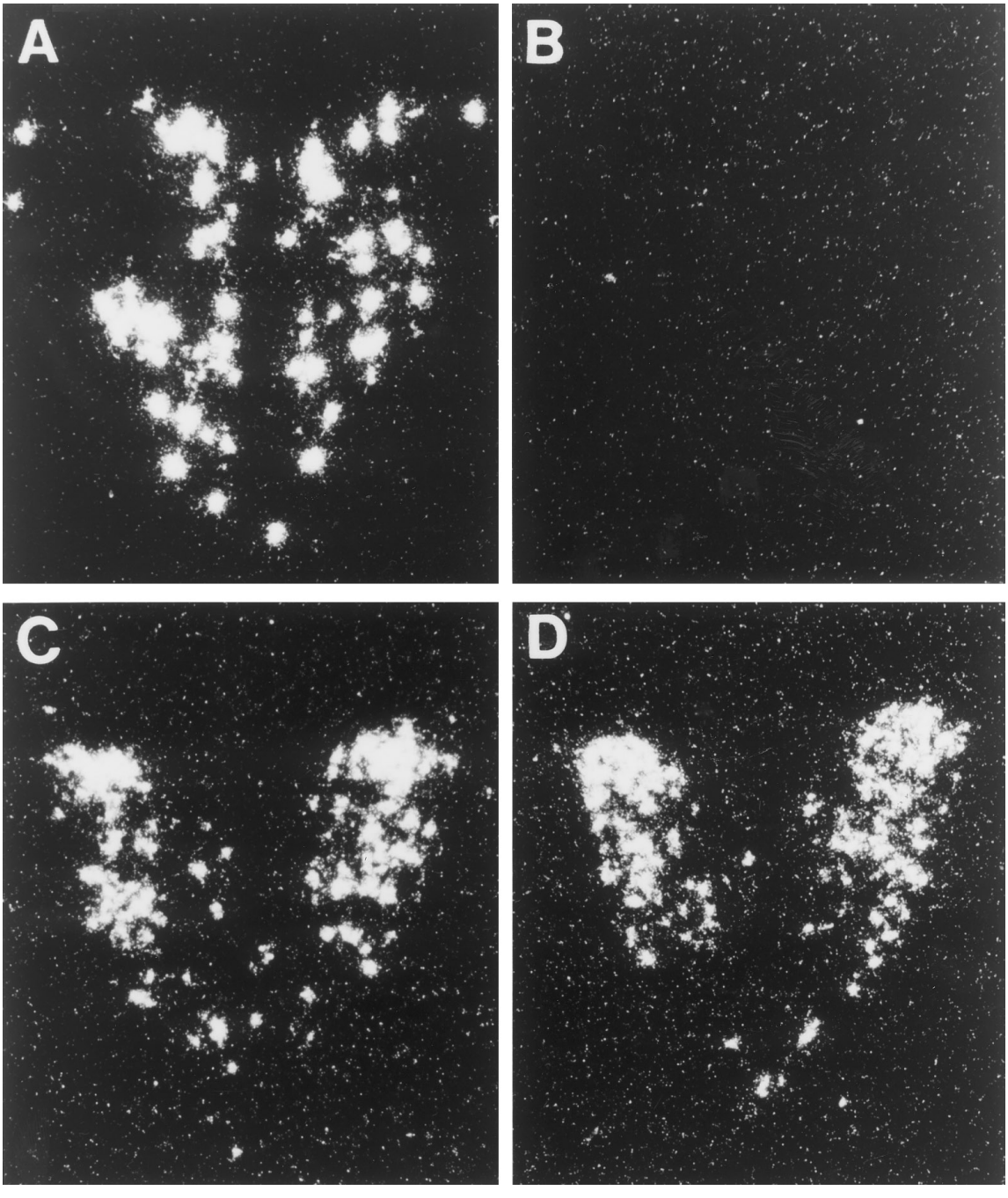

FIG. 2. OT (A, B) and arginine vasopressin (AVP) (C, D) mRNA in the paraventricular nucleus (PVN) of heterozygous (A, C) and homozygous (B, D) OT knockout mice. No OT mRNA is detected in the brain of homozygous mice. The knockout allele does not affect AVP gene expression. (Reproduced by permission from Nishimori *et al.*, 1996. Copyright 1996 National Academy of Sciences, U.S.A.).

support the notion that neither OT nor the OT receptor is necessary for the normal expression of maternal behavior in mice. However, proof of this hypothesis awaits the development of an OT receptor knockout mouse. For example, it is possible that the OT receptor could be activated by a ligand-independent mechanism as has been recently described for the progesterone receptor (Mani, Allen, Clark, Blaustein, and O'Malley, 1994), although no evidence for such a mechanism in OT receptors has been reported.

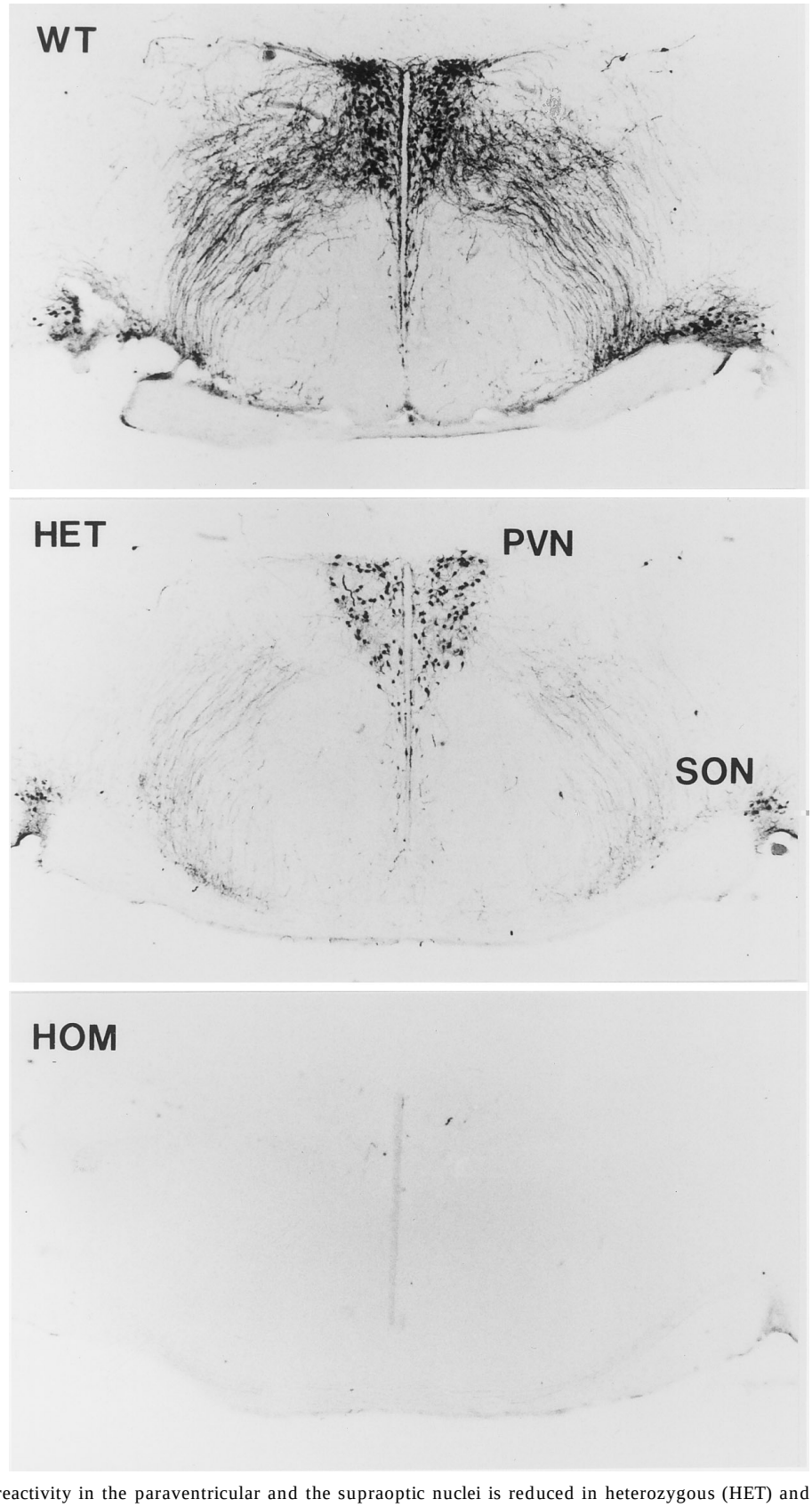

FIG. 3. OT immunoreactivity in the paraventricular and the supraoptic nuclei is reduced in heterozygous (HET) and absent in homozygous (HOM) mice compared to wildtype (WT) siblings. Note the reduction in fiber staining in the heterozygote verses homozygote brains.

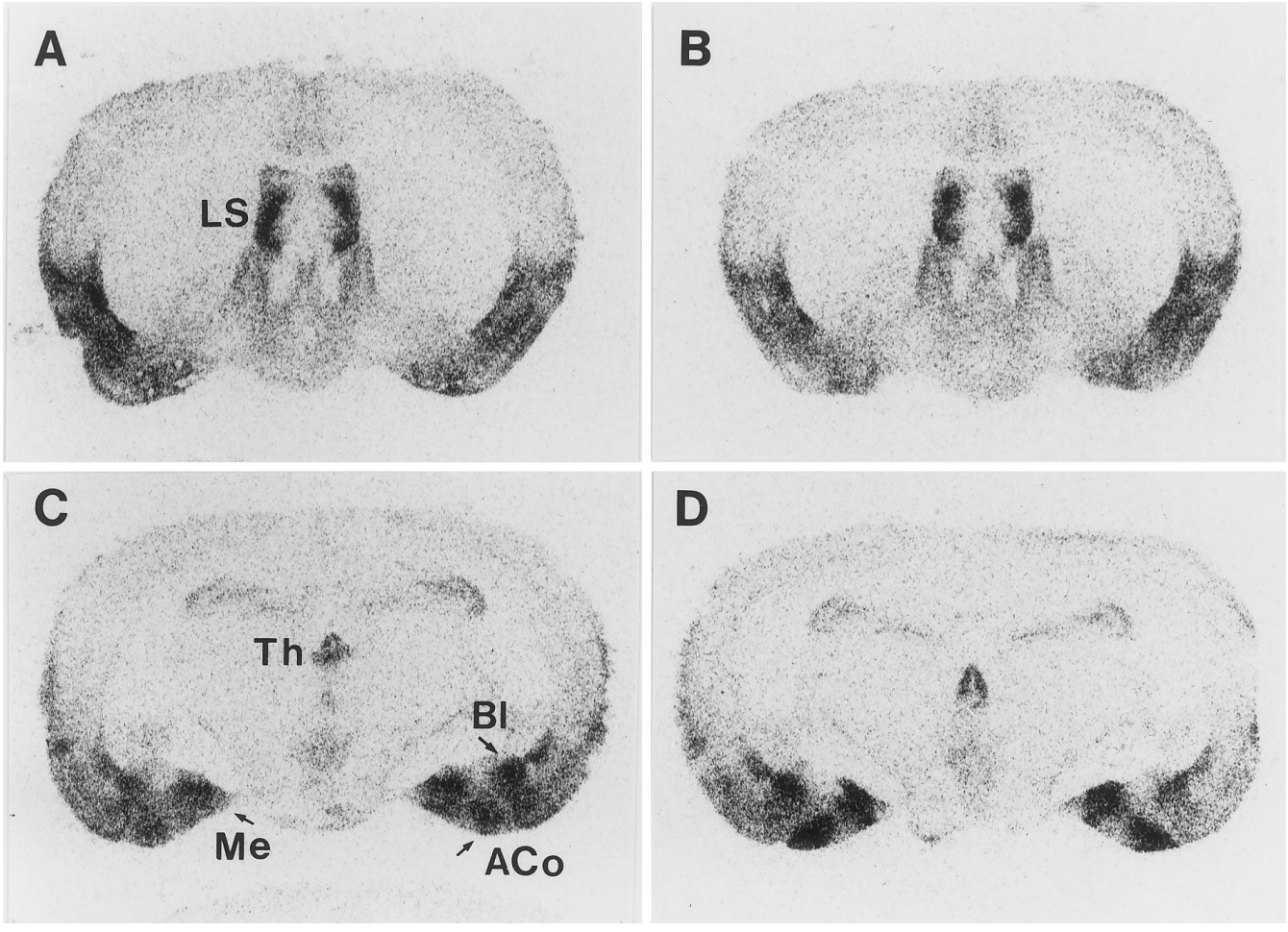

FIG. 4. OT receptor autoradiography in heterozygote (A, C) and homozygote (B, D) OT knockout mice brains. No differences in receptor distribution or concentration of OT receptors were detected. ACo, anterior cortical amygdala; BL, basolateral amygdala; LS, lateral septum; Me, medial amygdala; Th, thalamus. (Reproduced by permission from Nishimori *et al.*, 1996. Copyright 1996 National Academy of Sciences, U.S.A.).

We have also begun to characterize other social behaviors of the OT knockout mice (Table 1). In a resident intruder paradigm, an ovariectomized, wildtype female was placed in the home cage of an adult male and the behavioral interactions were recorded. Wildtype males typically spend much of the time in olfactory investigation of the intruder and show little aggression. Heterozygote and homozygote males show decreased olfactory investigation and increased aggressive behavior. The levels of social investigation parallel, and the levels of aggression inversely parallel the OT immunoreactivity in each genotype.

Oxytocin has also been implicated in the ultrasonic isolation calls emitted by infant rodents when separated from the mother and littermates (Insel and Winslow, 1991). Oxytocin injected into the brain reduces the frequency of these calls in a dose-dependent fashion (Insel and Winslow, 1991). We measured separation distress in OT knockout pups and found a significant decrease in distress calls in heterozygote and homozygote pups relative to wildtype pups. Again the change in behavior parallels the levels of OT immunoreactivity in each genotype. One interpretation of these paradoxical data is that animals deficient in OT fail to form social attachments early in life, and are therefore not distressed by the separation (Shapiro and Insel, 1990). An interesting parallel for these data comes from a comparison of social and asocial vole species. Prairie voles are highly social animals, spending over 50% of the time in side-by-side contact with conspecifics, while montane voles are virtually asocial. Isolation elicits a strong ultrasonic vocalization response in prairie vole pups, but little response in montane vole pups. It has been hypothesized that species differences in OT pathways may be associ-

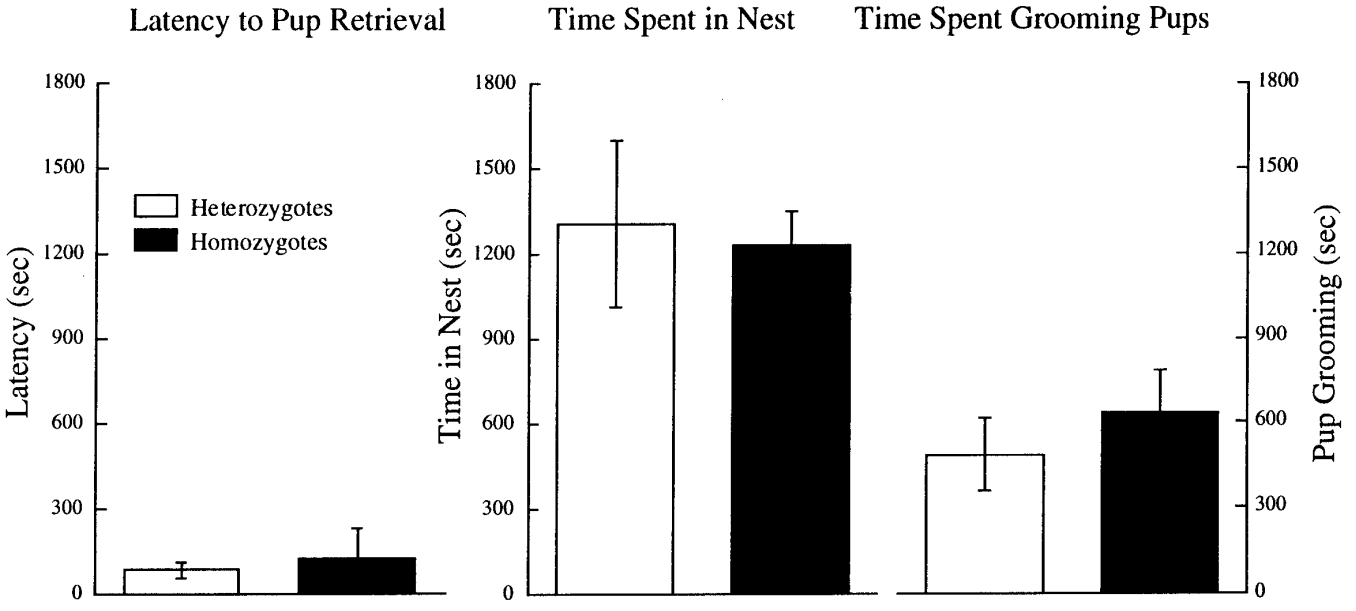

FIG. 5. Maternal behavior in heterozygous and homozygous mice. On the morning of parturition, two pups were removed from the nest and placed in opposite corners of the cage. The latency of pup retrieval, time spent in the nest, and time spent grooming pups were recorded during a 30-min session. No deficiencies in maternal behavior were observed.

ated with differences in social behavior in voles. The effects of the OT knockout appear to support this hypothesis and demonstrate the role of OT in the normal expression of social behavior.

IS MATERNAL BEHAVIOR INDEPENDENT OF OXYTOCIN?

The lack of a defect in maternal behavior in the OT knockout mouse suggests that the underlying neural circuits controlling maternal behavior are independent of oxytocin. However, this interpretation appears to contradict several studies in the rat (and in sheep) which report a role for oxytocin in the induction of maternal behavior. Virgin rats do not show maternal behavior, but rather they ignore pups and sometimes exhibit infanticide. Just prior to parturition, there is a rapid, dramatic shift in motivation from a lack of interest to a driven, relentless pursuit of nest-building, retrieval, licking, grouping of pups, and protection of pups. Central infusions of oxytocin in virgin rat facilitates this shift toward maternal behavior (Pedersen and Prange, 1979), whereas oxytocin antiserum (Pederson, Caldwell, Fort, and Prange, 1985) and oxytocin receptor antagonists (Van Leengoed *et al.*, 1987) infused into the brain block the induction of maternal behavior. However, once maternal behavior is established, oxytocin blockade has no effect. In fact, established maternal behavior is unaffected by lesions of the PVN (Numan and Corodimas, 1985). These data suggest that OT, released during parturition, acts as a switch to *initiate* the behavior and has little role in the maintenance of the behavior.

TABLE 1
Social Behavior in OT Knockout Mice

	Genotype		
Behavior	Wildtype	Heterozygote	Homozygote
Maternal behavior	+++	+++	+++
Olfactory investigation	+++	++	−
Aggression	−	+	+++
Infant separation distress	+++	++	−

In contrast to the rat (and wild house mice (McCarthy, 1990)), the strain of laboratory mice used to create the OT knockout , as well as other strains of laboratory mice are spontaneously maternal (L. Young, unpublished observation; Gandelman, 1973). Virgin females exhibit full maternal behavior immediately upon their first exposure to a pup. Since there is no shift in maternal behavior which occurs at parturition in laboratory mice, it is not surprising that OT-deficient mice show normal maternal behavior.

A species comparison of the distribution and regulation of oxytocin receptors in the brain suggests a potential mechanism for the species difference in OT function (Fig.

6). The pattern of OT receptors in the brain varies among each of the species studied (Insel *et al.*, 1993). For example, rats have high concentrations of OT receptors in the bed nucleus of the stria terminalis (BnST), with little binding in the lateral septum (LS). The concentration of OT receptors in the BnST increases significantly at parturition (Insel, 1990), leading to the hypothesis that this region is important for the OT induction of maternal behavior (Insel and Shapiro, 1992). In contrast, mice have little OT binding in the BnST and high concentrations in the LS. In addition, the OT receptors in the ventromedial nucleus of the hypothalamus (VMH) of the rat are increased by gonadal steroids (Johnson, Coirini, Insel, and McEwen, 1991), a process that has been proposed to be crucial for the induction of lordosis behavior in rats (Schumacher, Coirini, Pfaff, and McEwen, 1990). In contrast, gonadal steroids have the opposite effect in the mouse, decreasing OT receptor density in the VMH (Insel *et al.*, 1993).

The disparity in OT-sensitive brain structures among species suggests that we must be careful in making generalizations regarding the relationship between OT and specific behaviors. This, in addition to the behavioral results of the OT knockout mice, demonstrates that the role of oxytocin in the regulation of social behaviors must be considered on a species-by-species basis. Therefore, the development of primate models will be necessary to elucidate the behavioral functions of OT in the primate and human brain.

INTERPRETING BEHAVIORAL DATA OF KNOCKOUTS

Knockout mice clearly provide excellent opportunities to investigate the neuroendocrine mechanisms underlying behavior. However, there are several considerations which must be kept in mind while interpreting behavioral data of knockouts, especially in knockouts which disrupt hormone or neuropeptide function (Table 2). In certain situations, behavioral analysis of knockout mice may fail to produce the expected results based on previous research. These ''false negative'' results may be the result of (i) embryonic exposure of the knockout to maternal hormone, (ii) compensatory mechanisms such as receptor cross-reactivity, or (iii) species differences in mechanisms controlling behavior.

TABLE 2
Considerations in Evaluating the Behavioral Effects of Knockouts

Source for false negative effects	Potential solutions
Embryonic exposure	Vary maternal genotype
Compensatory mechanisms	Target receptors
Species differences	Comparative studies
Source for false positive effects	**Potential solutions**
Indirect effects	Replacement therapy
Developmental effects	Inducible gene approach

In many cases, wildtype offspring and heterozygous and homozygous mutant offspring for behavioral studies are produced by mating heterozygous pairs. This strategy permits the use of siblings which share identical gestational and rearing experiences, but differ only in genotype. While this is a good strategy to control for maternal influences, it potentially allows for embryonic exposure to maternal hormones. An interesting example of a false negative phenotype attributed to prenatal exposure is the transforming growth factor-β1 (TGF-β1) knockout mice (Letterio, Geiser, Kulkarni, Roche, Sporn, and Roberts, 1994). Although the null mutation should be lethal, knockout pups are indistinguishable from wildtype siblings. Careful examination revealed that TGF-β1 from maternal sources during development rescued the phenotype. Examining homozygote offspring from homozygote mothers should eliminate this problem. Maternal behavior is present in OT knockout mice derived from both homozygous and heterozygous mothers. The issue of compensating mechanisms, such as receptor cross-reactivity, is particularly important. For example, a null mutation of a peptide gene may result in an increase in synthesis of related peptides which could activate the receptor. For this reason, receptor knockouts may be more informative than hormone or neurotransmitter knockouts. Finally, the issue of species differences in the physiology of behavior should be addressed whenever unanticipated results are obtained. Much of the information on the molecular

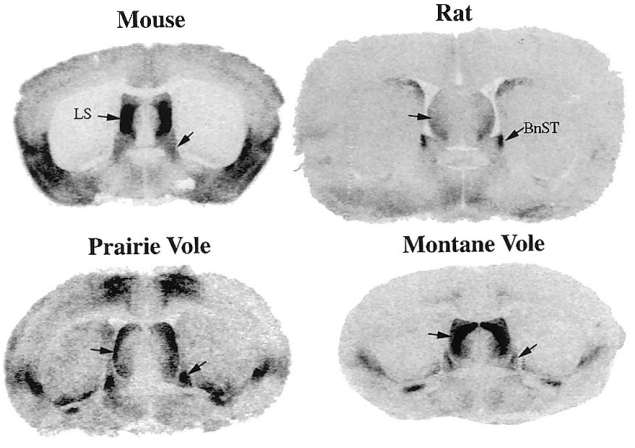

FIG. 6. Receptor autoradiograms illustrating the species diversity in OT receptors in the brain of female mice, rats, prairie voles, and montane voles. Note the inverse pattern of OT receptors in the lateral septum (LS) and bed nucleus of the stria terminalis (BnST) in rats and mice.

mechanisms controlling behavior is derived from studies in rats, which may differ significantly from laboratory mice.

Behavioral defects in knockout mice may also be observed which are not due to the immediate absence of the gene product under study. ''False positive'' behavioral defects could be due to (i) indirect effects of the mutation (e.g., changes in water balance), or (ii) developmental defects resulting from the mutation. When possible, replacement therapy should be used to verify that the behavioral defects are actually due to the lack of gene product. The potential for developmental effects of null mutants makes it difficult to determine the acute effects of the knockout. For example the Brattleboro rat, which is a natural vasopressin null mutant, suffers from several growth and brain development defects as well as diabetes insipidus, making it difficult to use as a behavioral model. These problems may be overcome in the future using inducible knockout strategies which are currently being developed. A strategy proposed by Lucas and Hen (1995) involves crossing a knockout mouse, which was created using a deletion vector encoding a tetracycline-controlled transcription factor, with a transgenic mouse created with a functional copy of the gene of interest with tetracycline-controlled *cis* regulatory elements linked to the promoter. The double-mutant offspring from the cross will express the transcription factor (from the deletion vector) which will activate normal expression of the transgene throughout development. However, administration of tetracycline will inhibit the transcription factor, thereby blocking the expression to the transgene, resulting in an ''induced'' knockout.

RECEPTOR TARGETING

A second transgenic approach for investigating the role of oxytocin in social behavior involves manipulating receptor expression. As discussed above, species differences in oxytocin-mediated behavior may be related to species differences in oxytocin receptor distribution. Comparative studies in voles support this hypothesis. Prairie voles (*Microtus ochrogaster*) are highly social and display high levels of affiliative behavior (prairie voles spend more than 50% of the time in side-by-side contact). In the field and in the laboratory, prairie voles nest in mated pairs, both mother and father contribute to the rearing of the offspring, and mated pairs form long-lasting social bonds with each other (Getz, McGuire, Pizzuto, Hofmann, and Frase, 1993). In contrast to prairie voles, montane voles (*M. montanus*) are asocial, are rarely in physical contact with other conspecifics, nest in isolation, and do not form social bonds between mates (Jannett, 1980, 1982). These species differences in social behavior are striking because these species are closely related and exhibit relatively few differences in nonsocial behaviors, such as locomotor activity or responses to novelty.

What physiological differences between prairie voles and the nonmonogamous montane voles could account for the differences in social behavior? Pair bonding in females of the monogamous prairie voles appears to be mediated by OT (Williams, Insel, Harbaugh, and Carter, 1994). Although few differences in OT-producing cells or fibers have been found (Wang, Zhou, Hulihan, and Insel, 1996), the distribution of OT receptors in the brain is very different between the species (Insel and Shapiro, 1992). This striking difference in OT receptor binding patterns led to the hypothesis that differences in OT pathways may be associated with species differences in social organization. Comparison of OT receptor patterns in other monogamous and nonmonogamous voles species revealed a correlation between receptor binding patterns and social organization (Insel and Shapiro, 1992).

Species differences in binding patterns in voles could be due to (i) species differences in posttranscriptional processing of the gene product (such as neuronal transport), or to (ii) differences in regional gene expression in the brain. Using *in situ* hybridization, we determined that the pattern of OT receptor binding was similar to the pattern of OT mRNA expression in the brain in each species (Young, Huot, Nilsen, Wang, and Insel, 1996). Therefore species differences in receptor distribution are due to species differences in region-specific gene expression.

Since region-specific gene expression is controlled by *cis* regulatory elements generally located in the 5′ flanking region of genes, we cloned and partially sequenced the 5′ flanking region of the prairie vole and montane vole OT receptor genes (Young *et al.*, 1996). To determine whether sequences in the 5′ flanking region of the vole OT receptor gene are capable of correctly targeting specific regions, 5000 bp of the 5′ flanking region of the prairie vole OTR receptor gene was placed upstream of the reporter gene, β-galactosidase. PCR, utilizing Pfu DNA polymerase rather than *Taq* polymerase (Pfu has a lower error rate than *Taq* polymerase), was used to amplify the promoter sequence from the cloned gene. The 3′ PCR primer contained the translation start site (ATG) of the prairie vole OT receptor gene and both primers were designed with *Kpn*I restriction sites to facilitate cloning. This fragment was gel purified and cloned into the *Kpn*I site in front of the bacterial β-galactosidase gene with the OT receptor

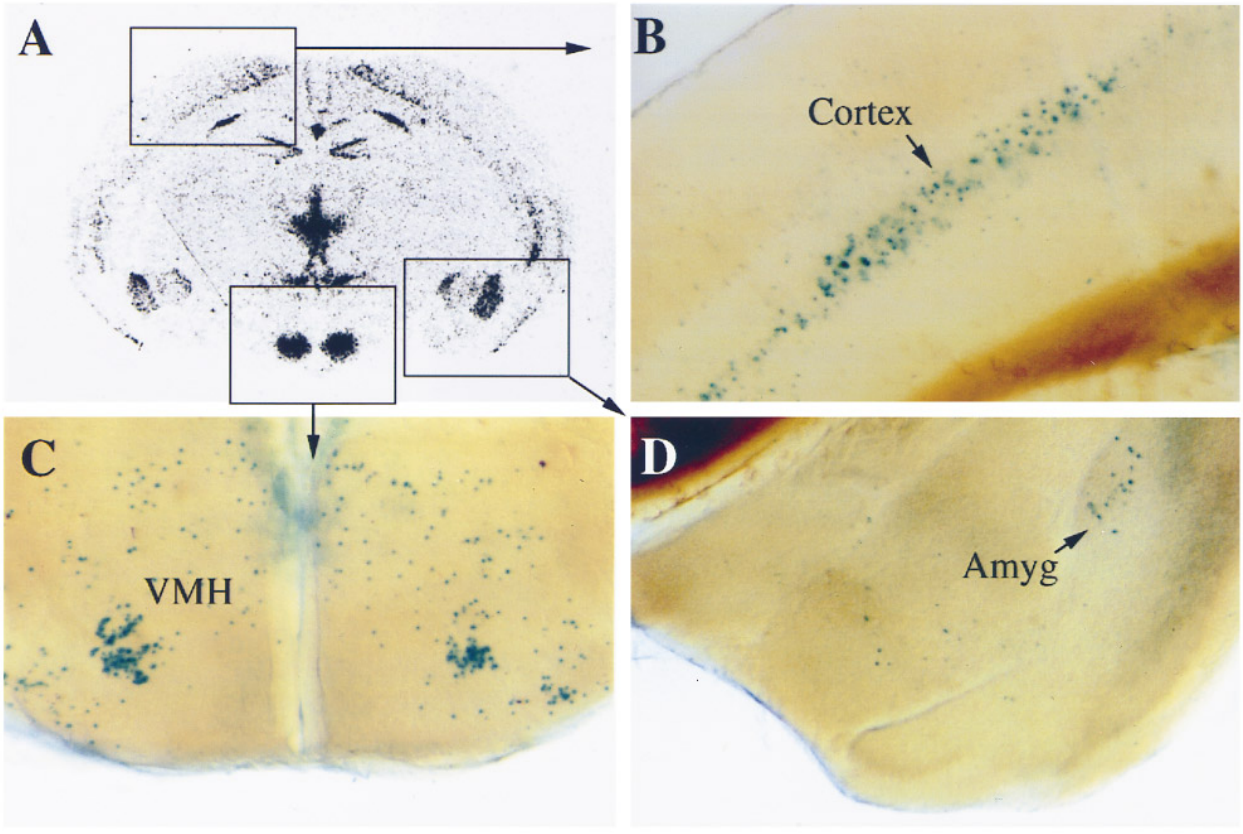

FIG. 7. The normal pattern of oxytocin receptor mRNA in the prairie vole brain is shown in A. A 5000-bp stretch of the prairie vole oxytocin receptor drives a similar pattern of transgene expression in the transgenic mouse brain. β-galactosidase expression in the OT receptor/β-galactosidase transgenic mice is detected in the cortex (B), ventromedial nucleus of the hypothalamus (VMH, C), and amygdala (D).

start site in the correct reading frame with respect to the β-galactosidase gene. We then created transgenic mice carrying this construct. Histological processing of the tissue results in a deep blue staining in cells which express the transgene. This 5000-bp fragment of the OTR gene resulted in targeted gene expression in the cortex, lateral septum, VMH, and amygdala, each of which normally expresses OT receptor in the prairie vole brain (Fig. 7). Independently derived lines express the transgene in slightly different patterns, probably due to different integration sites in the genome.

Now that it is possible to target gene expression fairly reliably to brain regions involved in OT function, it should be possible to manipulate receptor distribution and concentration in a controlled manner. We are now constructing transgenes containing 5000 bp of the prairie vole OT receptor promoter described above, spliced to OT receptor cDNAs to create functional OT receptor minigenes. These constructs should drive the expression of a functional OT receptor in a pattern similar to the endogenous OT receptor of the prairie vole. If the neuroanatomical distribution of OT receptor is associated with social behavior, then modifying the receptor distribution in a given species might modulate behavior. As mentioned, montane voles express very low levels of social behavior. We intend to use the OT receptor minigene, containing the prairie vole 5′ flanking region, to augment OT receptor expression in the asocial montane vole. If successful, it should be possible to demonstrate whether a relationship exists between social behavior and receptor gene expression. In addition, this approach should make it possible to identify specific brain regions involved in specific social behaviors by comparing changes in social behavior in transgenic montane voles with changes in brain OT receptor expression.

CONCLUSION

Transgenic technology has added to the battery of techniques available for investigating the physiological

function of oxytocin. The oxytocin knockout mice have provided surprising results regarding the neuroendocrine regulation of maternal behavior, emphasizing the importance of species diversity in OT function. The data demonstrate that the neural circuits underlying maternal behavior are independent of oxytocin; however, in some species, OT released at parturition may act as a switch to activate these circuits. This appears to be the case in rats as well as sheep. It is interesting to speculate whether the rat or mouse is a more appropriate model for maternal behavior in the human female. Nevertheless, the OT knockout mice demonstrate the significance of OT modulation of social and attachment behaviors.

The ability to manipulate OT receptor gene expression using target transgene expression may also provide a powerful tool for exploring the relationship between receptor distribution and OT mediated behaviors. The great diversity in receptor localization and regulation, even among closely related species, suggests that the plasticity in receptor gene expression may play a significant role in the control and the evolution of species-specific social behaviors. The ability to alter receptor expression of a species in a single generation using a transgenic approach offers an exciting possibility to manipulate social behavior in a species and to better understand the neural substrates controlling these behaviors.

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REFERENCES

Barberis, C., and Tribollet, E. (1996). Vasopressin and oxytocin receptors in the central nervous system. *Crit. Rev. Neurobiol.* **10,** 119–154.

Caldwell, J. D., Prange, A. F., and Pederson, C. A. (1986). Oxytocin facilitates the sexual receptivity of estrogen-treated female rats. *Neuropeptides* **7,** 175–189.

Fuchs, A. R., Fuchs, F., Husslein, P., and Soloff, M. S. (1982). Oxytocin receptors and human parturition: A dual role for oxytocin in the initiation of labor. *Science* **215,** 1396–1398.

Gainer, H., and Wray, W. (1994). Cellular and molecular biology of oxytocin and vasopressin. *In* E. Knobil and J. D. Neill (Eds.), *The Physiology of Reproduction,* pp. 1099–1129. Raven Press, New York.

Gandelman, R. (1973). The ontogeny of maternal responsiveness in female Rockland-Swiss albino mice. *Horm. Behav.* **4,** 257–268.

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

Hara, Y., Battey, J., and Gainer, H. (1990). Structure of mouse vasopressin and oxytocin receptor genes. *Mol. Brain Res.* **8,** 319–324.

Insel, T. (1992). Oxytocin: A neuropeptide for affiliation—Evidence from behavioral, receptor autoradiographic, and comparative studies. *Psychoneuroendocrinology* **17,** 3–33.

Insel, T. R. (1990). Regional changes in brain oxytocin receptor postpartum: Time-course and relationship to maternal behavior. *J. Neuroendocrinol.* **2,** 539–545.

Insel, T. R., and Hulihan, T. (1995). A gender-specific mechanism for pair bonding: Oxytocin and partner preference formation in monogamous voles. *Behav. Neurosci.* **109,** 782–789.

Insel, T. R., and Shapiro, L. E. (1992). Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proc. Natl. Acad. Sci. USA* **89,** 5981–5985.

Insel, T. R., and Winslow, J. T. (1991). Central administration of oxytocin modulates the infant rats response to social isolation. *Eur. J. Pharmocol.* **203,** 149–152.

Insel, T. R., Young, L., Witt, D. M., and Crews, D. (1993). Gonadal steroids have paradoxical effects on brain oxytocin receptor. *J. Neuroendocrinol.* **5,** 619–628.

Ivell, R., and Richter, D. (1984). Structure and comparison of the oxytocin and vasopressin genes from rat. *Proc. Natl. Acad. Sci. USA* **81,** 2006–2010.

Jannett, F. J. (1980). Social dynamics of the montane vole *Microtus montanus*, as a paradigm. *The Biologist* **62,** 3–19.

Jannett, F. J. (1982). Nesting patterns of adult voles, *Microtus montanus. J. Mammol.* **63,** 495–498.

Johnson, A. E., Coirini, H., Insel, T. R., and McEwen, B. S. (1991). The regulation of oxytocin receptor binding in the ventromedial hypothalamic nucleus by testosterone and its metabolites. *Endocrinol.* **128,** 891–896.

Kimura, T., Tanizawa, O., Mori, K., Brownstein, M. J., and Okayama, K. (1992). Structure and expression of a human oxytocin receptor. *Nature* **356,** 526–529.

Letterio, J. J., Geiser, A. G., Kulkarni, A. B., Roche, N. S., Sporn, M. B., and Roberts, A. B. (1994). Maternal rescue of transforming growth factor-β1 null mice. *Science* **264,** 1936–1938.

Lucas, J. J., and Hen, R. (1995). New players in the 5-HT receptor field: Genes and knockouts. *Trends Pharmacol. Sci.* **16,** 246–252.

Mani, S. K., Allen, J. M. C., Clark, J. H., Blaustein, J. D., and O'Malley, B. W. (1994). Convergent pathways for steroid hormone- and neurotransmitter-induced rat sexual behavior. *Science* **265,** 1246–1249.

McCarthy, M. M. (1990). Oxytocin inhibits infanticide in house mice (*Mus domesticus*). *Horm. Behav.* **24,** 365–375.

Nishimori, K., Young, L. J., Guo, Q., Wang, Z., Insel, T. R., and Matzuk, M. M. (1996). Oxytocin is required for nursing but is not essential for partuition or reproductive behavior. *Proc. Natl. Acad. Sci. USA* **93,** 11699–11704.

Numan, M., and Corodimas, K. P. (1985). The effect of paraventricular hypothalamic lesions on maternal behavior in rats. *Physiol. Behav.* **35,** 417–425.

Pedersen, C. A., and Prange, A. J. (1979). Induction of maternal behavior in virgin rats after intracerebroventricular administration of oxytocin. *Proc. Natl. Acad. Sci. USA* **76,** 6661–6665.

Pederson, C. A., Caldwell, J. D., Fort, S. A., and Prange, A. J. (1985). Oxytocin antiserum delays onset of ovarian steroid-induced maternal behaviour. *Neuropeptides* **6,** 175–182.

Pederson, C. A., Caldwell, J. D., Walker, C., Ayers, G., and Mason, G. A. (1994). Oxytocin activates the postpartum onset of rat maternal behavior in the ventral tegmental and medial preoptic area. *Behav. Neurosci.* **108,** 1163–1171.

Schumacher, M., Coirini, H., Pfaff, D. W., and McEwen, B. S. (1990).

Behavioral effects of progesterone associated with rapid modulation of oxytocin receptors. *Science* **250,** 691–694.

Shapiro, L. E., and Insel, T. R. (1989). Ontogeny of oxytocin receptors in rat forebrain: A quantitative study. *Synapse* **4,** 259–266.

Smith, M. S. (1989). Lactation. *In* H. D. Patton, A. F. Fuchs, B. Hille, A. M. Scher, and R. S. Steiner (Eds.), *Textbook of Physiology*, Vol. 2, pp. 1408–1421. Saunders, Philadelphia.

Snijdewint, F. G. M., Van Leeuwen, F. W., and Boer, G. J. (1989). Ontogeny of vasopressin and oxytocin binding sites in the brain of wistar and brattleboro rats as demonstrated by lightmicroscopical autoradiography. *J. Chem. Neuroanat.* **2,** 3–17.

Van Leengoed, E., Kerker, E., and Swanson, H. H. (1987). Inhibition of postpartum maternal behaviour in the rat by injecting an oxytocin antagonist into the cerebral ventricles. *J. Endocrinol.* **112,** 275–282.

Wang, Z., Zhou, L., Hulihan, T. J., and Insel, T. R. (1996). Immunoreactivity of central vasopressin and oxytocin pathways in microtine rodents: A quantitative comparative study. *J. Comp. Neurol.* **366,** 726–737.

Williams, J. R., Insel, T. R., Harbaugh, C. R., and Carter, C. S. (1994). Oxytocin administered centrally facilitates formation of a partner preference in prairie voles (*Microtus ochrogaster*). *J. Neuroendocrin.* **6,** 247–250.

Witt, D. M., and Insel, T. R. (1991). A selective oxytocin antagonist attenuates progesterone facilitation of female sexual behavior. *Endocrinology* **128,** 3269–3276.

Witt, D. M., Winslow, J. T., and Insel, T. R. (1992). Enhanced social interactions in rats following chronic, centrally infused oxytocin. *Pharmacol. Biochem. Behav.* **43,** 855–861.

Young, L. J., Huot, B., Nilsen, R., Wang, Z., and Insel, T. R. (1996). Species differences in central oxytocin receptor gene expression: Comparative analysis of promoter sequences. *J. Neuroendocrinol.* **8,** 777–783.