THE JOURNAL OF COMPARATIVE NEUROLOGY 433:499–514 (2001)

Expression and Estrogen Regulation of Brain-Derived Neurotrophic Factor Gene and Protein in the Forebrain of Female Prairie Voles

YAN LIU,[1] CHRISTIE D. FOWLER,[1] LARRY J. YOUNG,[2] QIAO YAN,[3] THOMAS R. INSEL,[2] AND ZUOXIN WANG[1]*

[1]Department of Psychology and Neuroscience Program, Florida State University, Tallahassee, Florida 32306

[2]Center for Behavioral Neuroscience, Emory University, Atlanta, Georgia 30322

[3]Department of Neuroscience, Amgen Inc., Thousand Oaks, California 91320

ABSTRACT

Brain-derived neurotrophic factor (BDNF) has been linked to the development, differentiation, and plasticity of the central nervous system. In the present study, we first used a highly specific affinity-purified antibody and a cRNA probe to generate a detailed mapping of BDNF immunoreactive (BDNF-ir) staining and mRNA labeling throughout the forebrain of female prairie voles. Our data revealed that (1) BDNF-ir cells were present essentially in the brain regions in which BDNF mRNA-labeled cells were found; (2) BDNF-ir fibers were distributed extensively throughout many forebrain regions; and (3) BDNF mRNA was also detected in some thalamic regions in which BDNF-ir fibers, but not immunostained cells, were present. With few exceptions, the distribution pattern of BDNF in the vole brain generally resembled the pattern found in rats. In a second experiment, we examined the effects of estrogen on BDNF expression. Ovariectomized prairie voles that were treated with estradiol benzoate had a higher level of BDNF mRNA labeling in the dentate gyrus and CA3 region of the hippocampus, as well as in the basolateral nucleus of the amygdala, than did ovariectomized voles that were treated with vehicle. In addition, estrogen treatment increased the density of BDNF-ir fibers in the lateral septum, dorsolateral area of the bed nucleus of the stria terminalis, and lateral habenular nucleus. These data suggest that estrogen may regulate BDNF at the level of gene and protein expression, and thus, BDNF may be in a position to mediate the effects of estrogen on the brain of the prairie vole. J. Comp. Neurol. 433:499–514, 2001.

Indexing terms: BDNF; neurotrophic factors; estrogen; vole

Neurotrophins are a group of proteins with important growth and trophic actions on development and maintenance of the central and peripheral nervous systems. Among neurotrophins, brain-derived neurotrophic factor (BDNF) was first purified from pig brains as a trophic factor for dorsal root ganglion cells in 1982 (Barde et al., 1982), and subsequently the gene was cloned in 1989 (Leibrock et al., 1989). An extensive body of literature has demonstrated that BDNF is broadly distributed throughout the central nervous system (CNS) in a variety of species, such as birds (Akutagawa and Konishi, 1998), rats (Castren et al., 1995; Conner et al., 1997b; Yan et al., 1997), mice (Hofer et al., 1990), pigs (Wetmore et al., 1990), nonhuman primates (Hayashi et al., 1997; Kawamoto et al., 1999), and humans (Phillips et al., 1991; Murer et al., 1999). BDNF plays an important role in promoting proliferation, survival, growth, and differentiation for a broad range of neuronal cell types, including basal forebrain cholinergic neurons (Alderson et al., 1990),

Grant sponsor: National Institutes of Health; Grant numbers: MHR29-54554, MHR01-58616.

*Correspondence to: Zuoxin Wang, Ph.D., Department of Psychology, Florida State University, Tallahassee, FL 32306-1270.
E-mail: zwang@psy.fsu.edu

Received 11 April 2000; Revised 16 January 2001; Accepted 7 February 2001

mesencephalic dopaminergic neurons (Hyman et al., 1991; Knusel et al., 1991), serotonergic neurons (Mamounas et al., 1995), cortical and hippocampal neurons (Ghohs et al., 1994; Nitta et al., 1999), striatal neurons (Ventimiglia et al., 1995), subependymal cells (Kirschenbaum and Goldman, 1995), cerebellar granule cells (Lindholm et al., 1993), and motoneurons (Yan et al., 1994). Moreover, BDNF influences neuronal plasticity (Lo, 1995; Thoenen, 1995; McCallister et al., 1997), and neurotransmitter and neurotrophic factor synthesis (Snider, 1994; Thoenen, 1995). In view of its importance and broad range of activities in neuronal populations, BDNF has gained attention as a potential therapeutic agent for neurological diseases (Lindsay et al., 1994).

Steroid hormones represent another class of ligands that have profound effects on the development of neuronal architecture and organization, as well as on the expression and maintenance of neuroendocrine and behavioral functions. Recent studies have shown that steroid hormones appear to be involved in the regulation of BDNF expression in the CNS. In songbirds, for example, testosterone treatment of female canaries or estrogen treatment of male juvenile zebra finches significantly increases their BDNF immunoreactivity or mRNA labeling in a forebrain song control nucleus (Dittrich et al., 1999; Rasika et al., 1999). In rats, acute or chronic stress alters BDNF mRNA and protein expression in the hippocampus as well as in several hypothalamic brain regions, and this effect is at least partially regulated through a corticosterone-mediated mechanism (Smith et al., 1995a,b; Schaaf et al., 1997; Schaaf et al., 1998). Estrogen is also an important regulator for BDNF expression in rats. The level of BDNF mRNA in the hippocampal formation fluctuates across the estrus cycle (Gibbs, 1998) and increases in response to acute estrogen replacement (Singh et al., 1995). Estrogen administration to ovariectomized rats results in an enhanced BDNF mRNA labeling in the cerebral cortex and olfactory bulb (Sohrabji et al., 1995). In addition, estrogen also alters neuronal sensitivity to BDNF by influencing BDNF receptor activities (Sohrabji et al., 1994a,b). Because both estrogen and BDNF have been implicated in aging-related neuronal disorders (Phillips et al., 1991; Conner et al., 1997a; Xu et al., 1998), the two may act in concert and/or reciprocally to stimulate the synthesis of proteins required for neuronal development, differentiation, and survival (Toran-Allerand, 1996).

The prairie vole (*Microtus ochrogaster*) is a highly social microtine rodent in which females are induced ovulators and form pair bonds with a male after mating (Richmond and Conaway, 1969; Williams et al., 1992). Exposure to a male or male sensory cues induces an increase in circulating estrogen associated with an induction of behavioral estrus in female prairie voles (Carter et al., 1987; Cohen-Parsons and Carter, 1987). Recently, such increased estrogen during male exposure or mating has been attributed to increased cell proliferation and survival, observed in selected brain areas in prairie voles (Fowler et al., 2000; Smith et al., 2001). Although these studies indicate that estrogen plays an important role in the regulation of cell proliferation, the underlying mechanism is yet unknown. Because estrogen influences BDNF and its receptors (Sohrabji et al., 1994b; Singh et al., 1995) and BDNF regulates cell proliferation and survival (Kirschenbaum and Goldman, 1995; Johnson et al., 1997; Zigova et al., 1998), we hypothesized that estrogen may act on BDNF, which, in turn, regulates cell proliferation and/or survival in female prairie voles. As an initial step in testing this hypothesis, we have used *in situ* hybridization and immunocytochemistry to generate a detailed mapping of the BDNF mRNA and protein in the forebrain of female prairie voles. In addition, we have examined the effects of estrogen on BDNF labeling by comparing ovariectomized female voles that were treated with either estradiol benzoate or vehicle, respectively. Elucidation of sites of BDNF synthesis and storage and of estrogen action on BDNF will provide important information guiding our further studies to examine BDNF functions.

MATERIALS AND METHODS

Subjects

Subjects were sexually naive female prairie voles (*M. ochrogaster*) that were the F3 generation of a laboratory breeding colony started from field-captured animals. Subjects were housed in plastic cages (20 × 50 × 40 cm) that contained cedar chips as bedding. Water and food were provided *ad libitum*. The cages were maintained on a

Abbreviations

3V	third ventricle
AC	anterior commissure
AD	anterodorsal thalamic nucleus
BLA	basolateral nucleus of the amygdala
BMA	basomedial nucleus of the amygdala
BSTd	bed nucleus of the stria terminalis, anterodorsal part
BSTv	bed nucleus of the stria terminalis, anteroventral part
CA1	CA1 region of the hippocampus
CA2	CA2 region of the hippocampus
CA3	CA3 region of the hippocampus
CeA	central nucleus of the amygdala
DG	dentate gyrus
DM	dorsomedial thalamic nucleus
EB	estradiol benzoate
Gr	granule cell layer of the dentate gyrus
LHb	lateral habenular nucleus
LS	lateral septum
LSd	lateral septum, dorsal part
LSv	lateral septum, ventral part
LV	lateral ventricle
MeA	medial nucleus of the amygdala
MHb	medial habenular nucleus
Mo	molecular layer of the dentate gyrus
MS	medial septum
Or	stratum oriens of the CA3
OT	optic tract
OVX	ovariectomized
PLC	prelimbic cortex
Po	polymorhic layer of the dentate gyrus
PVT	paraventricular thalamic nucleus
PVN	paraventricular nucleus of the hypothalamus
Py	pyramidal layer of the hippocampus
Rad	stratum radiatum of the CA3
Sl	stratum lucidum of the CA3
VA	ventral anterior thalamic nucleus
VMH	ventromedial nucleus of the hypothalamus
VPM	ventral posteromedial thalamic nucleus
VT	ventral thalamic nucleus, anterior part

14:10-hour light:dark cycle with lights on at 0700. The temperature was approximately 20 ± 1°C.

Experimental procedures

Experiment 1 was designed to map the distribution pattern of the BDNF mRNA and protein in the forebrain of female prairie voles. Females (70–90 days old) were either decapitated (n = 7) or perfused (n = 4). After decapitation, brains were immediately removed, frozen on dry ice, and subsequently cut on a cryostat. Brains were cut into 20-μm coronal sections, thaw-mounted on Superfrost/Plus slides (Fisher), and stored at −80°C until they were processed for BDNF *in situ* hybridization. Perfusion was performed by deeply anesthetizing subjects with sodium pentobarbital (1 mg/10 g body weight) and perfusing through the ascending aorta with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PBS, pH 7.4). Brains were removed, 40 μm coronal sections were cut with a vibratome, and floating sections were processed for BDNF immunocytochemistry.

Experiment 2 was designed to examine the effects of steroid hormone estrogen on the BDNF gene and protein expression. Female prairie voles (70–90 days old) were ovariectomized 2 weeks before testing. Thereafter, they were implanted with either Silastic tubing (2.0 mm i.d. × 4.0 mm o.d., 7.0 mm long) filled with estradiol benzoate (EB, Sigma) or vehicle alone (sesame oil, Sigma) for 3 days. Such EB treatment induces female prairie voles into behavioral estrus (Gingrich et al., 2000). Three days after tubing implants, 10 EB-treated and 10 vehicle-treated subjects were decapitated, brains were harvested, cut on a cryostat, and 20-μm coronal sections were processed for BDNF *in situ* hybridization. In addition, four EB-treated and three vehicle-treated subjects were perfused, and their brain sections were processed for BDNF immunocytochemistry.

BDNF *in situ* hybridization

A 500-base-pair rat BDNF cDNA was supplied by Dr. Mark Smith (National Institutes of Health). The [^{35}S]CTP-labeled BDNF antisense riboprobe was transcribed using T7 RNA polymerase from KpnI-digested pGEM4Z-rBDNF, and a BDNF sense probe was transcribed using Sp6 RNA polymerase from the same plasmid digested with HindIII. The probes were purified using the phenol extraction method followed by ethanol precipitation, and were characterized previously (Smith et al., 1995a).

BDNF *in situ* hybridization was performed according to the procedure described previously (Smith et al., 1995a). Briefly, slide-mounted 20-μm coronal sections were fixed in cold 4% paraformaldehyde in PBS buffer (pH 7.4) for 20 minutes, washed in cold PBS twice, digested with 5 μg/ml proteinase K in TE buffer (50 mM Tris-HCl and 5 mM EDTA, pH 8.0) for 15 minutes, and followed by 0.1 M triethanolamine (pH 8.0) containing 0.25% acetic anhydride for 10 minutes. Slides were washed in 2 × SSC, dehydrated in 70%, 95%, and 100% ethanol 3 minutes each, immersed in chloroform for 6 minutes, transferred quickly to 100% and then 95% ethanol, and air dried. Prehybridization was performed by applying each slide with 150-μl hybridization buffer containing 2.5 mg/ml tRNA at 55°C for 2 hours. The hybridization buffer contained 62.5% formamide, 12.5% dextran sulfate, 37.5 mM NaCl, 10 mM Tris-EDTA (pH 8.0), 1.25 × Dehardt's solution, and 10 mM dithiothreitol (DTT). After prehybridization, slides were washed with 2 × SSC, dehydrated, and air-dried. Each slide was then applied with 150 μl hybridization solution containing 0.5 mg/ml tRNA and 5×10^6 cpm/ml labeled probe. Slides were covered with parafilm, and hybridization was performed at 55°C for 16 hours. Thereafter, slides were washed in 50% formamide and 0.1% β-mercaptoethanol in 2 × SSC for 2 × 15 minutes at 55°C; digested in 25 μg/ml RNaseA in RNase buffer (8 mM Tris-HCl, 400 mM NaCl, 0.8 mM EDTA, pH 8.0) for 30 minutes at 37°C; blocked in 1 mM DTT in the same buffer for 30 minutes at 37°C; washed again in 50% formamide and 0.1% β-mercaptoethanol in 2 × SSC for 2 × 15 minutes at 55°C, and then washed in 50% formamide and 1% β-mercaptoethanol in 2 × SSC for 2 × 30 minutes at 55°C. After dehydration, slides were air dried and put on a BioMax MR film (Kodak) along with a [^{14}C] autoradiographic standard (Amersham, Buckinghamshire, England) for 2 days to generate autoradiograms for signal visualization. Thereafter, slides were dipped into NBT-2 emulsion (Kodak) and exposed for 4 weeks at 4°C. After developing, slides were stained with 0.5% cresyl violet, dehydrated, immersed in xylene, mounted with coverslips, and examined under a microscope. Control sections were hybridized with ^{35}S-labeled single-stranded sense cRNA probe, which did not generate any specific labeling.

BDNF immunocytochemistry

BDNF immunoreactive (BDNF-ir) staining was performed on the 40-μm floating sections. Sections were rinsed in 0.05 M Tris-buffered saline (TBS, pH 7.6) and treated with 0.006% H_2O_2 in TBS for 10 minutes. After rinsing in TBS, sections were incubated in TBS with 0.3% Triton X-100 (Tris-Triton) containing 10% normal goat serum (NGS) and 2% bovine albumin (BSA) for 1 hour, and then incubated in 200 ng/ml rabbit-anti-BDNF (Amgen, CA) in Tris-Triton containing 1% NGS and 2% BSA at 4°C for 48 hours. This affinity-purified rabbit polyclonal antibody to BDNF had been extensively characterized in previous studies, and the antibody is specific for BDNF with less than 0.5% cross-reactivity with other neurotrophins (Conner et al., 1997a,b; Yan et al., 1997). After incubation, sections were rinsed in the same solution without the primary antibody, and then incubated in 6 μl/ml biotinylated goat-anti-rabbit in Tris-Triton with 1% NGS and 2% BSA for 2 hours at room temperature. After rinsing with TBS, sections were incubated in ABC complex (Vector) for 90 minutes and stained in 0.05% 3-3′-diaminobenzidine (DAB) with 0.009% H_2O_2 and 0.06% nickel chloride. Sections were then mounted on slides, air dried, and coverslipped. Controls for the specificity of the antiserum included staining sections with anti-BDNF that was pretreated with 50 μM BDNF or omission of the primary antibody. Specific staining was not found in either case.

Data analysis

Analysis of the sections was done by the experimenter who was blind to the identity of the specimens. The quantity of BDNF mRNA labeling in selected brain regions was measured from the autoradiograms using a computerized image program (NIH IMAGE 1.60), permitting the conversion of optical density to nanocuries per gram tissue equivalents. The optical density was converted to nanocuries per gram tissue equivalents using a standard regres-

sion curve that was obtained by correlating the optical density with nanocuries per gram tissue equivalents derived from the coexposed standard with 10 counts ranging from 0.13 to 129.8 nCi/g tissue equivalents. The optical density of the BDNF mRNA images on the autoradiograms was within the linear range of the standard curve. The density of BDNF-ir fiber plexus in selected brain regions was also measured using the same computer image program, as described previously (Wang et al., 1996). To standardize measurements, the light and camera settings were kept constant across the images on the autoradiograms or brain sections so that the density of the background remained the same for all images. Data were quantified bilaterally from three to four sections per area per animal, and the means were used for data analysis. Treatment effects on BDNF mRNA labeling or BDNF-ir staining were analyzed by a t-test. The significance level was set at $P < 0.05$.

RESULTS

BDNF immunocytochemistry and *in situ* hybridization revealed a specific distribution pattern of BDNF-producing cells and fibers in well-defined regions in the forebrain of female prairie voles. The distributions of BDNF-ir and mRNA labeling within the forebrain regions are summarized in Table 1. Although the two techniques were performed on brain tissues from separate sets of voles, the data were presented together for the comparison of BDNF protein with mRNA.

BDNF-ir stained or mRNA-labeled cells

In nearly all cortical regions, scattered cells stained for BDNF-ir or labeled for BDNF mRNA were distributed throughout the rostrocaudal levels of the brain of the prairie vole. As previously reported in rats (Conner et al., 1997b; Yan et al., 1997), BDNF cells were present in all cortical layers: layer IV contained only a few lightly labeled cells, whereas layer VI contained the most robust BDNF-ir or mRNA labeling. In general, the pattern of BDNF-ir staining matched the pattern of BDNF mRNA labeling, as shown for the prelimbic cortex in Figure 1A,B.

Intense BDNF labeling was seen in the hippocampal formation (Figs. 2 and 3). In the CA2 and CA3 regions, cells that were stained for BDNF-ir or labeled for BDNF mRNA were found in the pyramidal layer (Fig. 2C–E and Fig. 3C), whereas heavy stained BDNF-ir fibers were present in the stratum lucidum (Fig. 2C). In the CA1 region, few scattered cells were found to be labeled for BDNF mRNA (Fig. 2F) or stained for BDNF-ir (Fig. 3D). Scattered cells labeled for BDNF mRNA or stained for BDNF immunoreactivity were also found in the stratum lucidum (Fig. 2H), stratum oriens (Fig. 2I,J), and stratum radiatum (Fig. 3A) of the CA3 region of the hippocampus. In the dentate gyrus (DG), BDNF-ir-stained or mRNA labeled cells were found in moderate densities in the granule cell layer (Fig. 2G and Fig. 3A,B). Some scattered BDNF mRNA-labeled cells were found in the polymorphic layer extending to the deeper hilus (Fig. 2G). However, heavily stained BDNF-ir mossy fibers in this area prevented identification of BDNF-ir cells.

Another brain region that contained intense BDNF-ir or mRNA labeling was the amygdaloid complex. BDNF-ir- or BDNF-mRNA-labeled cells were detected in the basolateral (BLA; Fig. 1C,D), basomedial (BMA), and medial nuclei of the amygdala (MeA). The latter two regions also contained BDNF-ir fibers at low densities. In contrast, intense BDNF-ir fibers were found in the central nucleus of the amygdala (CeA), where no BDNF-ir- or BDNF-mRNA-labeled cells were visualized.

In addition to the abovementioned areas, BDNF-labeled cells were also found in several other forebrain regions.

TABLE 1. Distribution of Brain-Derived Neurotrophic Factor (BDNF) in the Forebrain of Female Prairie Voles[1]

Area	BDNF mRNA	BDNF-ir cells	BDNF-ir fibers
Cortex			
Layer I	+	+	−
Layer II	++	++	−
Layer III	++	++	−
Layer IV	+	+	−
Layer V	++	++	−
Layer VI	++	++	−
Claustrum	+++	+++	−
Endopiriform nucleus	+++	+++	−
Anterioventral periventricular nucleus	++	++	++
Median preoptic nucleus	+	+	++
Lateral septal nucleus	+	+	++
Medial septal nucleus	++	++	+
Diagonal band nucleus	+	+	++
Medial preoptic area	+	+	++
Lateral habenular nucleus	−	−	++
Medial habenular nucleus	−	−	−
Bed nucleus of the stria terminalis (BST)			
Dorsal nucleus of the BST	−	−	+++
Ventral nucleus of the BST	−	−	++
Hippocampal formation			
CA1, stratum oriens	−	−	+
CA1, stratum pyramidale	+	+	+
CA1, stratum radiatum	−	−	−
CA2, stratum oriens	−	−	+
CA2, stratum pyramidale	+++	+++	+
CA2, stratum radiatum	+	?	++
CA3, stratum oriens	+	−	++
CA3, stratum pyramidale	+++	+++	+
CA3, stratum radiatum	+	?	+++
Dentate gyrus, polymorph layer/hilus	+	?	++++
Dentate gyrus, granule cell layer	+++	+++	+
Dentate gyrus, molecular layer	+	+	+
Amygdaloid nucleus (AMYG)			
Basolateral nucleus of the AMYG	+++	+++	−
Central nucleus of the AMYG	−	−	+++
Medial nucleus of the AMYG	+	+	+
Cortical nucleus of the AMYG	++	++	+
Basomedial nucleus of the AMYG	+	+	+
Thalamus			
Paraventricular thalamic nucleus	++	−	++
Ventral anterior thalamic nucleus	++	−	+
Ventral posteromedial thalamic nucleus	+	−	+
Anterodosal thalamic nucleus	++	−	+
Dorsomedial thalamic nucleus	++	−	++
Central medial thalamic nucleus	++	−	+
Paratenial	+	−	+
Hypothalamus			
Paraventricular hypothalamic nucleus	++	++	+
Dorsomedial hypothalamic nucleus	+	+	+
Ventromedial hypothalamic nucleus	++	++	+
Anterior hypothalamic nucleus	+	+	+
Dorsolateral nucleus	+	+	+

[1]Immunoreactive-stained fibers: −, none; +, slight staining; ++, moderate staining; +++, heavy staining; ++++, extremely heavy staining. Immunoreactive-stained or mRNA-labeled cells: −, none; +, few scattered cells; ++, moderate number of cells; +++, densely packed cells; ?, could not be determined.

Fig. 1. Photomicrographs displaying cells stained for brain-derived neurotrophic factor (BDNF) immunoreactivity (brightfield) or labeled for BDNF mRNA (darkfield) in the prelimbic cortex (PLC) (**A,B**), basolateral nucleus of the amygdala (BLA) (**C,D**), or lateral part of the medial septum (MS) (**E,F**) of female prairie voles. Scale bar = 50 μm.

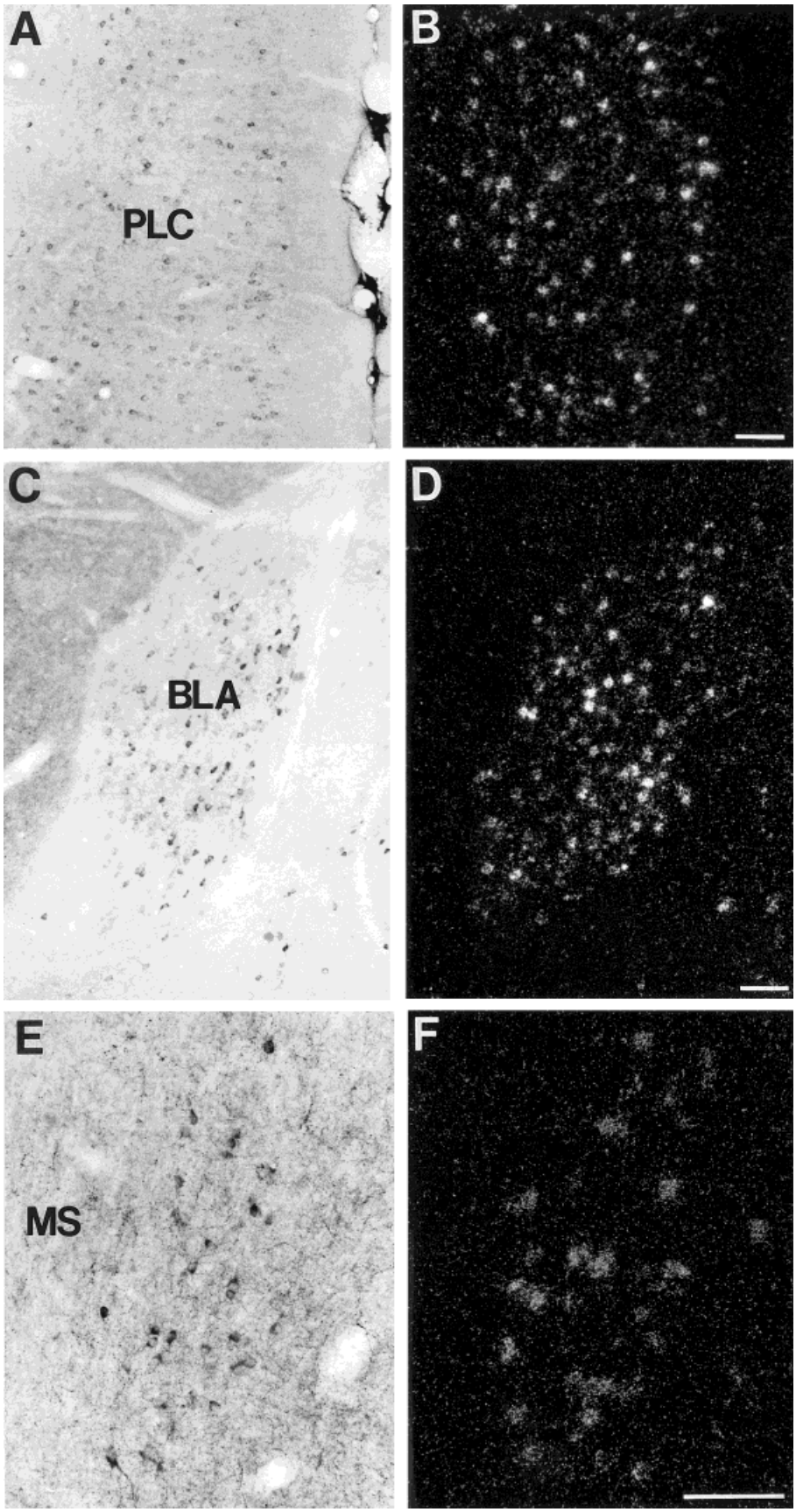

Figure 1

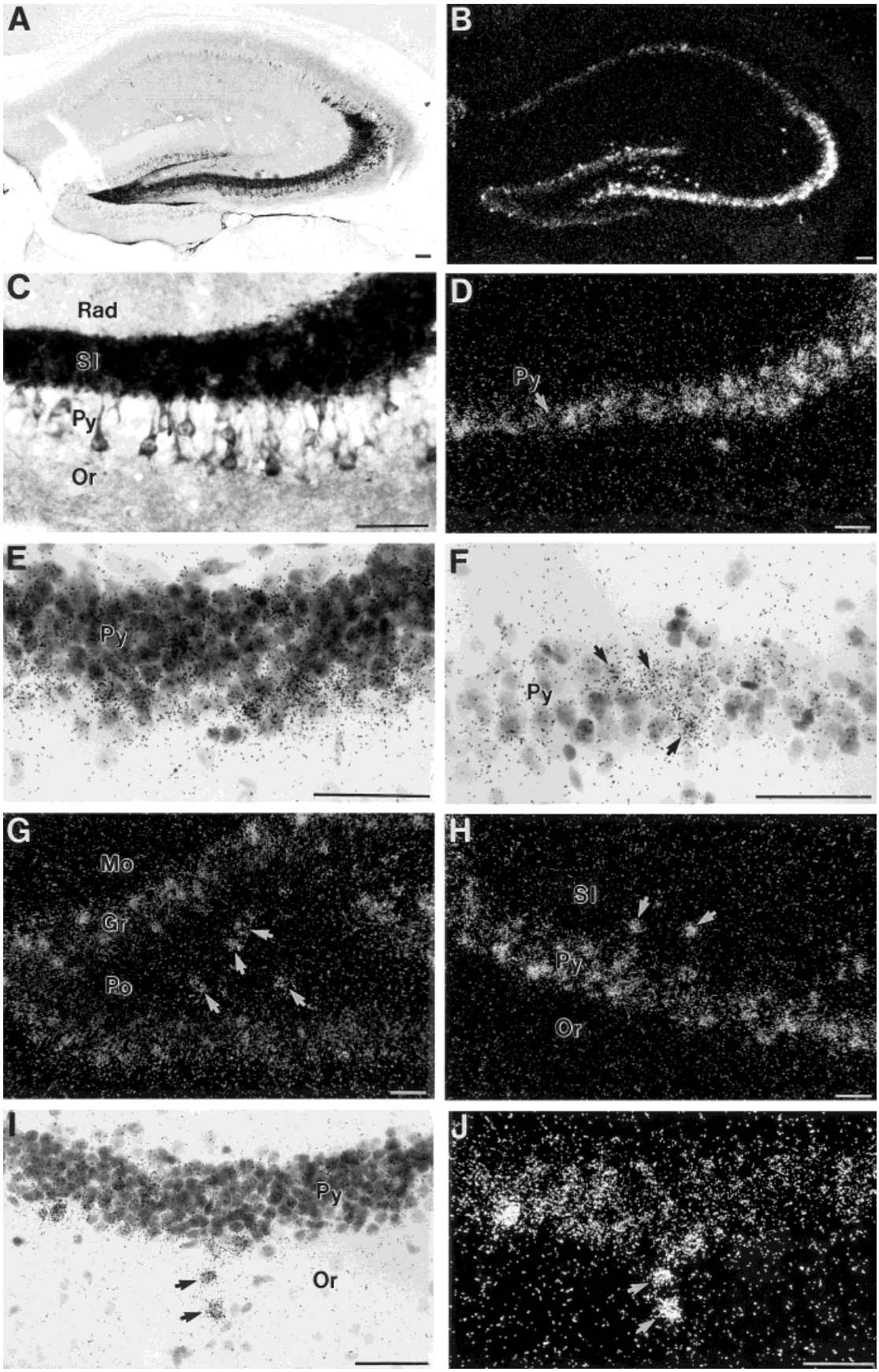

Figure 2

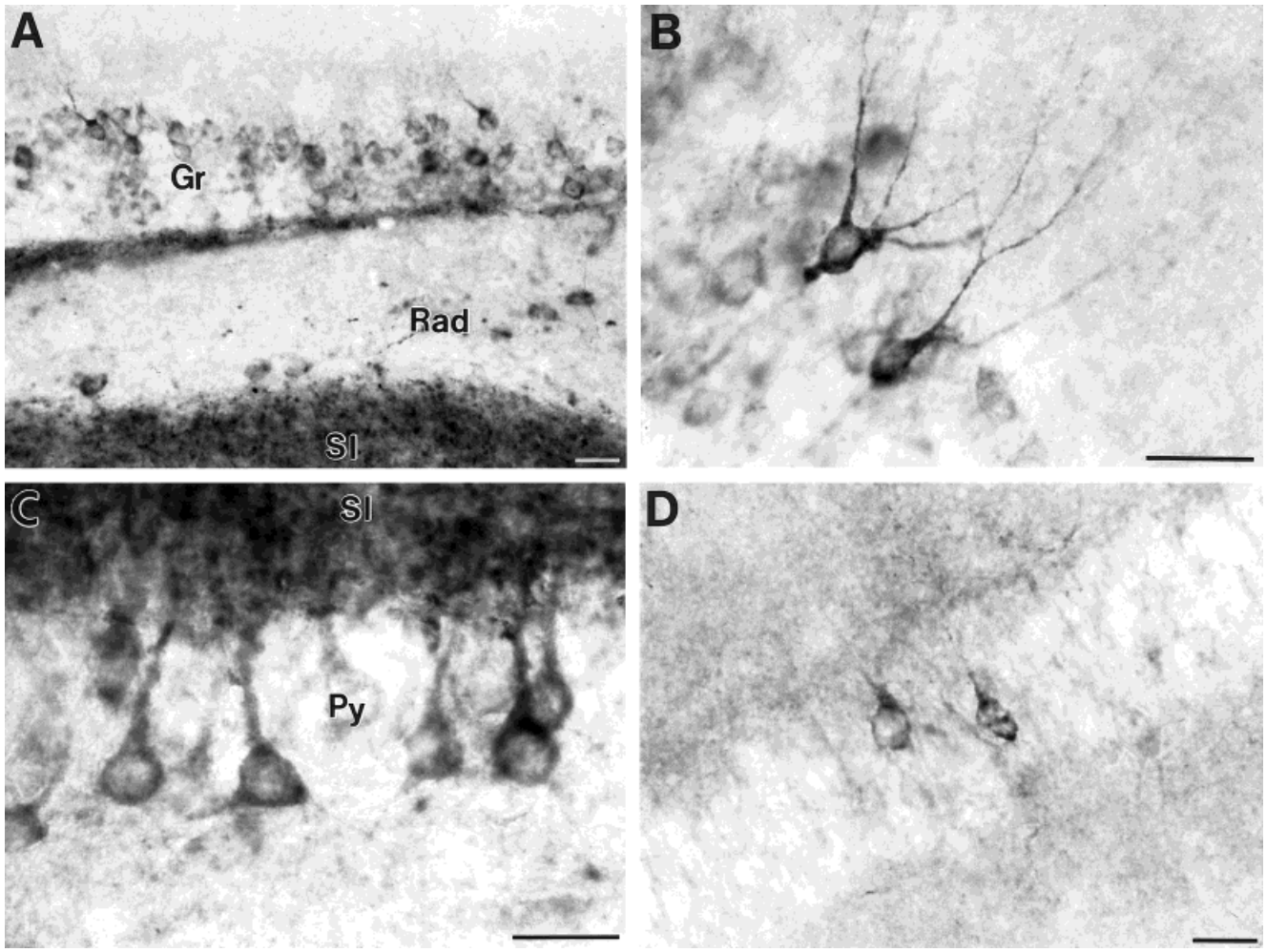

Fig. 3. Photomicrographs displaying brain-derived neurotrophic factor (BDNF) immunoreactive-stained cells in the granule cell layer (Gr) of the dentate gyrus (**A,B**), as well as in the pyramidal layer (Py) of the CA3 (**C**) and CA1 regions (**D**) of the hippocampus in female prairie voles. Scattered cells stained for BDNF immunoreactivity were also found in the stratum radiatum (Rad) of the CA3 region (A). SI, stratum lucidum. Scale bar = 20 μm.

The number of the cells containing BDNF-ir or BDNF-mRNA labeling was detected in moderate to intense densities throughout the rostrocaudal extent of the claustrum and dorsal endopiriform nucleus, as well as in the paraventricular (PVN; Fig. 4A,B), dorsomedial, and ventromedial (VMH; Fig. 4C,D) nuclei of the hypothalamus. Some scattered but well immunostained or mRNA-labeled BDNF cells were found in the lateral part of the medial septum (Fig. 1E,F), vertical limb of the diagonal band, median preoptic nucleus dorsal to the anterior commissure, anterioventral periventricular nucleus, medial preoptic nucleus, and anterior and lateral nuclei of the hypothalamus. In almost all of those regions, the distribution pattern of BDNF-ir cells resembled the pattern of BDNF-mRNA-labeled cells.

However, a mismatch between cells stained for BDNF-ir or labeled for BDNF mRNA was found in some areas of the prairie vole brain. This mismatch was restricted to the thalamic regions. Light or moderate BDNF mRNA labeling was found in the paraventricular (PVT; Fig. 5D), ventral anterior (Fig. 5E), anterodorsal (Fig 5F), dorsomedial (DM), and central medial thalamic nuclei. No BDNF-ir cells were found in those brain regions. Instead, BDNF-ir fibers in light or moderate density were present in some thalamic areas (see below).

Fig. 2. Photomicrographs displaying brain-derived neurotrophic factor (BDNF) immunoreactive staining (**A**) or BDNF mRNA labeling (**B**) in the hippocampus of female prairie voles. In the CA3 region, cells stained for BDNF immunoreactivity (**C**) or labeled for BDNF mRNA (**D**) were found in the pyramidal layer (Py), whereas BDNF immunoreactive mossy fibers were present in the stratum lucidum (Sl) (C). Many cells were labeled for BDNF mRNA in the pyramidal layer of the CA3 region (**E**), whereas only few labeled cells (arrows) were found in the pyramidal layer of the CA1 region (**F**). BDNF mRNA-labeled cells (arrows) were also found in the polymorphic layer (Po), especially in the deep hilus, of the dentate gyrus (**G**), in the stratum lucidum (Sl) (**H**) and the stratum oriens (Or) (**I,J**) of the CA3 region of the hippocampus. (I) and (J) were from the exact same field of the tissue. Gr, granule cell layer of the dentate gyrus; Mo, molecular cell layer of the dentate gyrus; Rad, stratum radiatum. Scale bar = 50 μm.

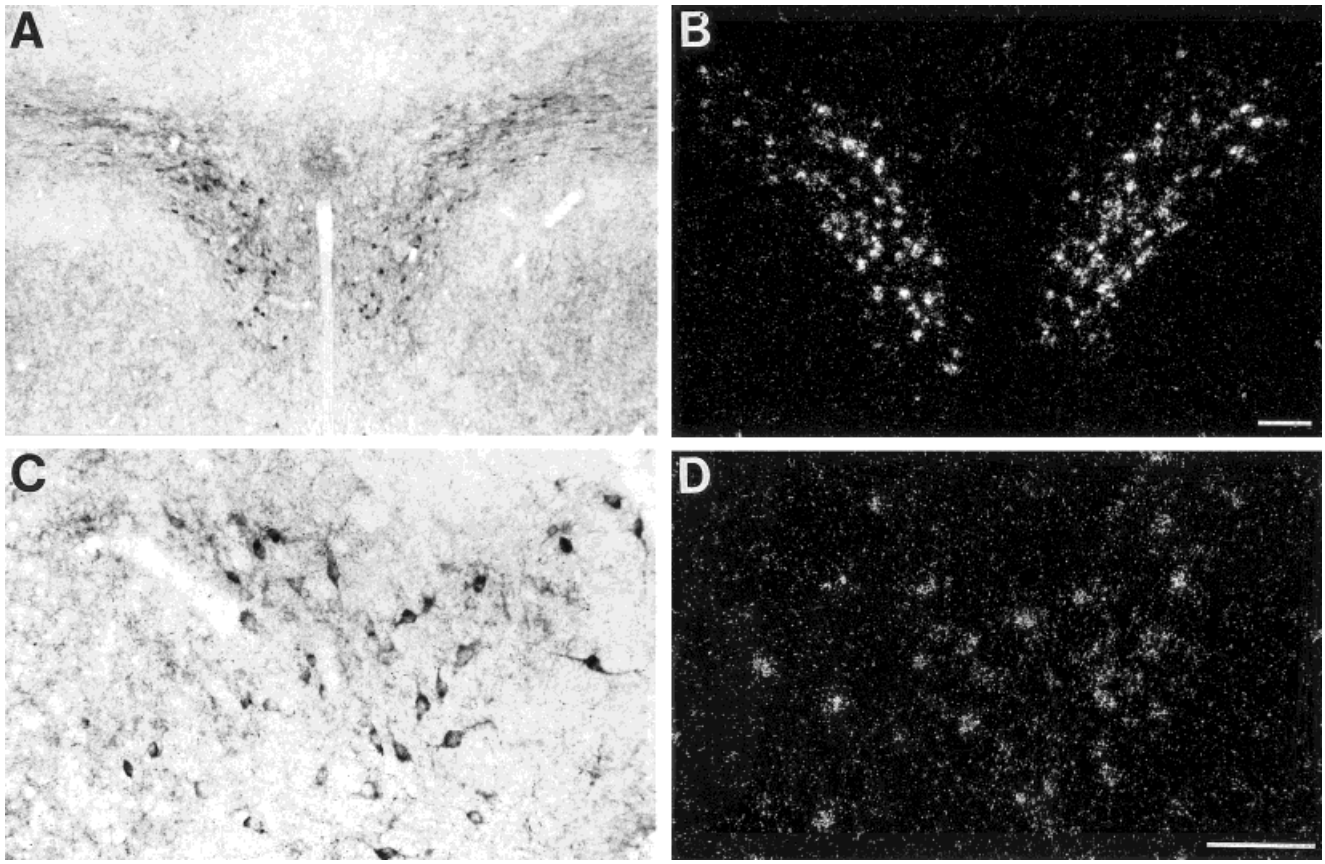

Fig. 4. Photomicrographs displaying cells stained for brain-derived neurotrophic factor (BDNF) immunoreactivity (brightfield) or labeled for BDNF mRNA (darkfield) in the paraventricular nucleus (**A,B**) or ventromedial nucleus of the hypothalamus (**C,D**) of female prairie voles. Scale bar = 50 μm.

BDNF-ir fibers

BDNF immunocytochemistry resulted in an extensive staining of BDNF-ir fibers in the prairie vole brain. The fibers were present either in the form of finely distinct individual fibers, fiber plexuses, or in the form of heavy punctate or noded fibers that form pericellular baskets (Conner et al., 1997).

Finely distinct fibers were the primary source of BDNF-ir terminals found in many forebrain regions. For example, in the lateral septum (Fig. 6F), lateral habenular nucleus, and ventral part of the bed nucleus of the stria terminalis (Fig. 6D), BDNF-ir fibers formed plexuses of a moderate density with individually distinguishable fibers. In the hypothalamus, neocortex, and most aspects of the amygdaloid complex, finely distinct BDNF-ir fibers were widely spread in low to moderate densities. Finally, BDNF-ir fibers at low to moderate densities were also found in most thalamic regions, including the PVT (Fig. 5A), paratenial, DM (Fig. 5B), ventral (Fig. 5B), and ventral posteromedial (Fig. 5C) thalamic nuclei.

Heavily stained BDNF-ir fibers were found in several brain regions. In the dorsal part of the bed nucleus of the stria terminalis (Fig. 6A) and central nucleus of the amygdala (Fig. 6E), BDNF-ir fibers were present in the form of pericellular baskets with individual fibers not distinguishable. In the deep hilus of the DG and the stratum lucidum of the CA3 (Fig. 2C and Fig. 6B), BDNF-ir appeared in the form of heavily stained mossy fibers. An intense staining of BDNF-ir fibers was also found in the median preoptic nucleus dorsal to the anterior commissure.

Estrogen effects on BDNF-ir and mRNA labeling

The effect of estrogen on BDNF expression was assessed by comparing regional BDNF mRNA or immunoreactive staining in ovariectomized female prairie voles that were treated with EB or vehicle. Treatment effects on BDNF mRNA were measured in the granule cell layer of the DG, the pyramidal layer of the CA1 and CA3 regions of the hippocampus, BLA, BMA, and MeA regions of the amygdaloid complex, prelimbic cortex, and PVN and VMH of the hypothalamus. EB treatment significantly increased BDNF mRNA labeling in a regional-specific manner. The EB-treated females had almost twice the amount of BDNF mRNA labeling in the granule cell layer of the DG ($t = 2.43$, $P < 0.05$) or the pyramidal layer of the CA3 region ($t = 2.45$, $P < 0.05$) of the hippocampus than that of vehicle-treated females (Fig. 7A). EB treatment also enhanced BDNF mRNA labeling in the BLA of the amygdala ($t = 5.44$, $P < 0.01$; Fig. 7B and Fig. 8E,F). No treatment effects were detected on BDNF mRNA labeling in any other measured brain areas.

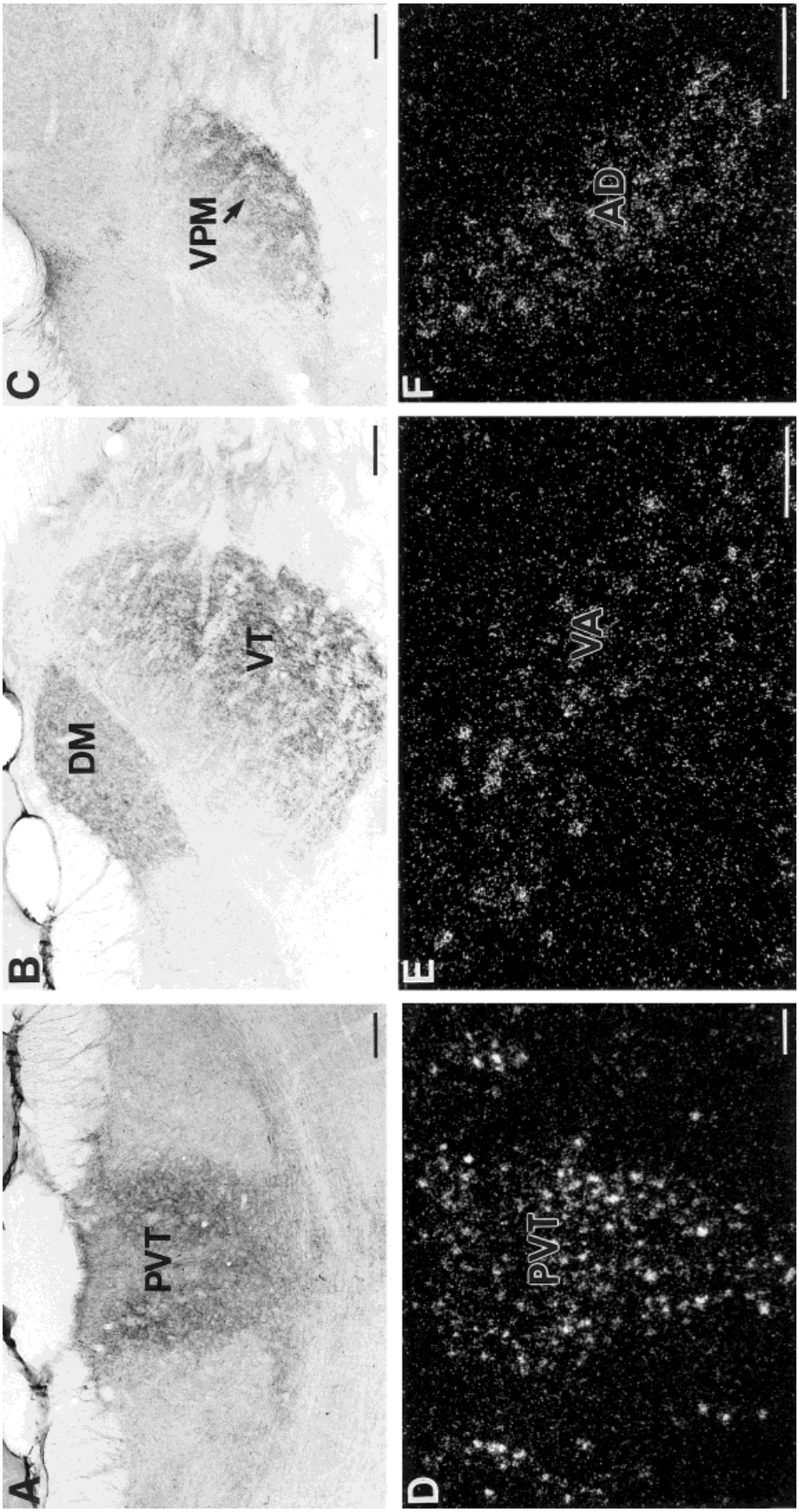

Fig. 5. Photomicrographs displaying brain-derived neurotrophic factor (BDNF) immunoreactive stained fibers (brightfield) in the paraventricular thalamic (PVT) (**A**), dorsomedial thalamic (DM) (**B**), anterior part of ventral thalamic (VT) (B), and ventral posteromedial thalamic (VPM) (**C**) nuclei of female prairie voles. The photomicrographs also display BDNF mRNA-labeled cells (darkfield) in the PVT (**D**), ventral anterior thalamic (VA) (**E**), and anterodorsal thalamic (AD) (F) nuclei of female prairie voles. Scale bar = 50 μm.

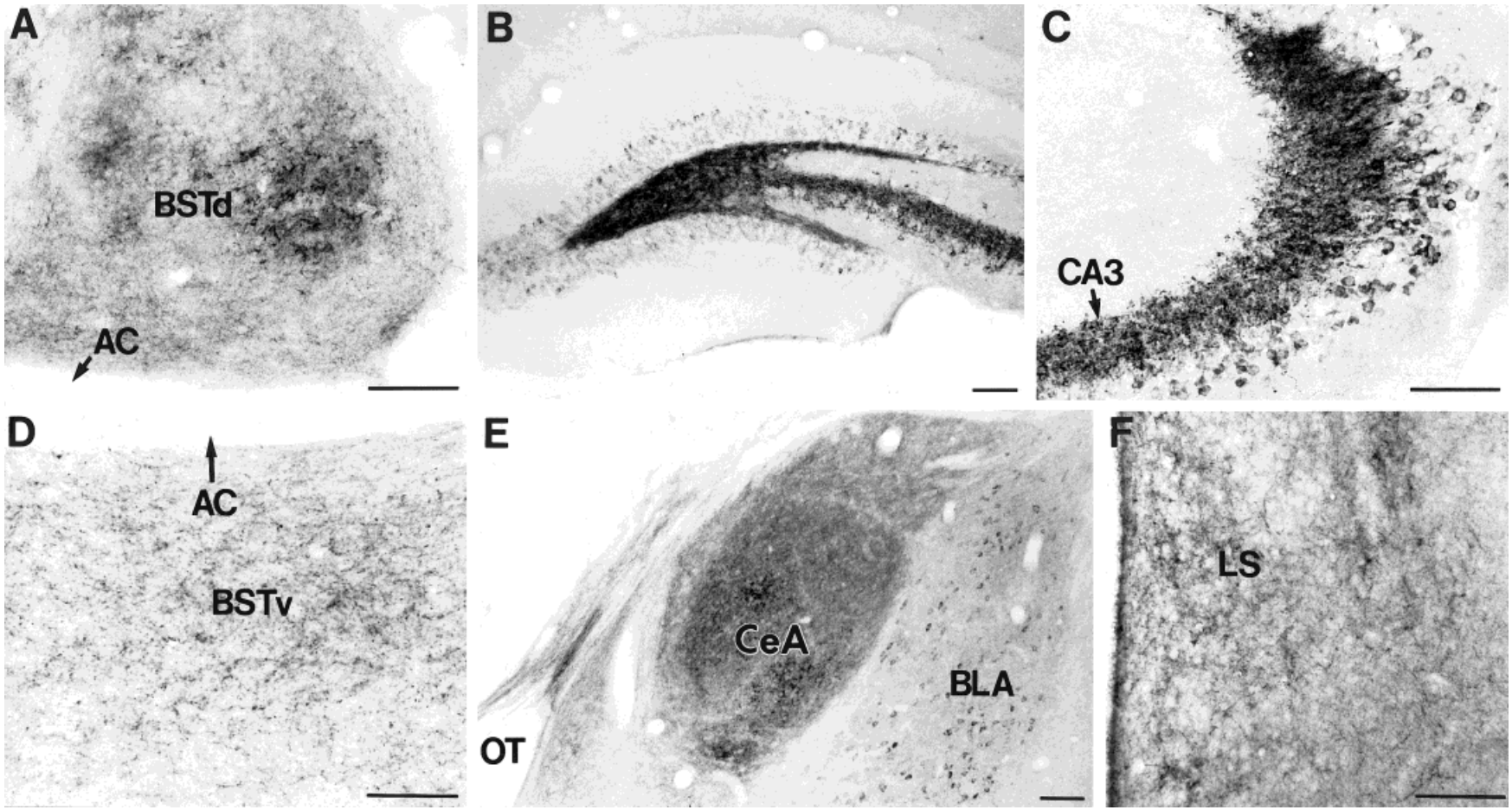

Fig. 6. Photomicrographs displaying brain-derived neurotrophic factor (BDNF) immunoreactive stained fibers in the dorsal area (BSTd) (**A**) or ventral area (BSTv) (**D**) of the bed nucleus of the stria terminalis, the dentate gyrus (**B**), and the CA3 region (**C**) of the hippocampus, central nucleus of the amygdala (CeA) (**E**), and lateral septum (LS) (**F**) of female prairie voles. AC, anterior commissure; BLA, basolateral nucleus of the amygdala; OT, optic tract. Scale bar = 50 μm.

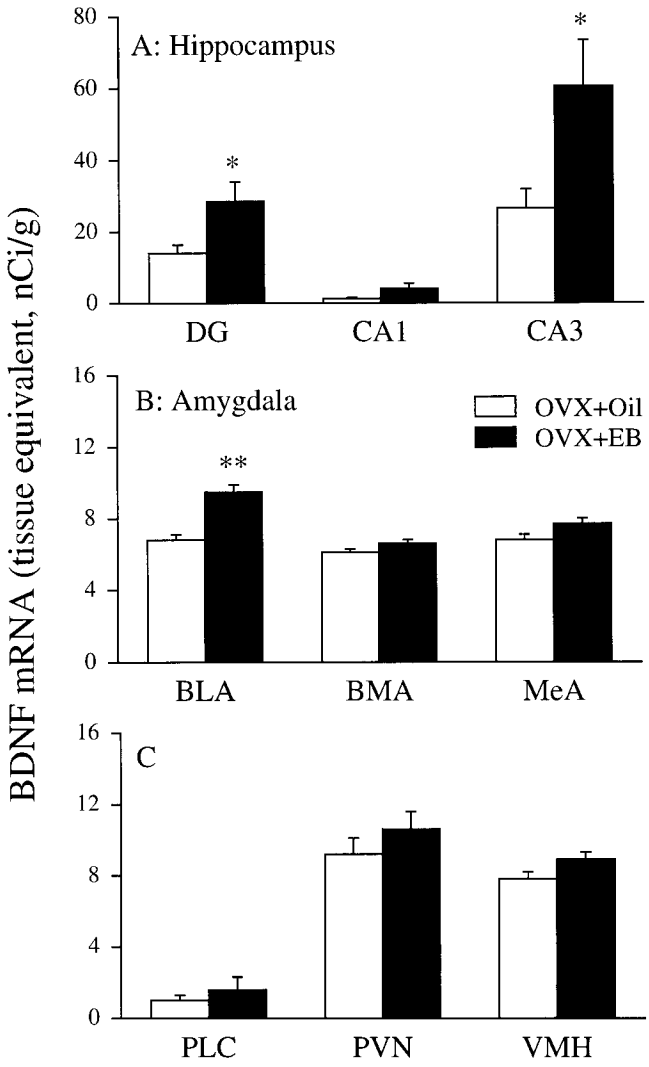

Fig. 7. The levels of brain-derived neurotrophic factor (BDNF) mRNA labeling in the hippocampus (**A**), amygdaloid complex (**B**), prelimbic cortex (PLC), paraventricular nucleus (PVN), and ventromedial nucleus (VMH) of the hypothalamus (**C**) of female prairie voles that were ovariectomized (OVX) and treated with either oil vehicle (Oil) or estradiol benzoate (EB). EB-treated females had a higher level of BDNF mRNA labeling in the granule cell layer of the dentate gyrus (DG) and the pyramidal layer of the CA3 region (CA3) of the hippocampus, as well as in the basolateral nucleus of the amygdala (BLA) in comparison to the oil-treated females. No significant differences were found in the pyramidal layer of the CA1 region (CA1) of the hippocampus, the basomedial (BMA) and mediadorsal nuclei (MeA) of the amygdala, PLC, PVN, and VMH. $*P < 0.05$, $**P < 0.01$.

The density of BDNF-ir fibers was also measured in selected brain regions using a computerized image program. Such measurement has been reliably used in our previous studies examining the density of vasopressin-ir fibers (Wang et al., 1993, 1996). EB-treated females had a higher density of BDNF-ir fibers than vehicle-treated females in the ventral part of the lateral septum ($t = 3.20$, $P < 0.05$), dorsal part of the BST ($t = 3.59$, $P < 0.05$) and lateral habenular nucleus ($t = 5.26$, $P < 0.01$; Fig. 8A-D, Fig. 9). No group differences were found in other measured brain regions including the dorsal part of the lateral septum, ventral part of the bed nucleus of the stria terminalis, paraventricular thalamic nucleus, and central nucleus of the amygdala. Because the polymorphic layer of the DG and the stratum lucidum of the CA3 of the hippocampus were highly saturated with the BDNF-ir fibers, the density of BDNF-ir fibers was not measured in those areas.

DISCUSSION

In the present study, we have used a highly specific antibody and a riboprobe to determine the distribution pattern of the BDNF protein and mRNA in the forebrain of female prairie voles. Our data have revealed a detailed mapping of BDNF-producing cells and their projections. In addition, we have found that the ovarian steroid hormone estrogen enhanced regional BDNF mRNA expression and immunostaining, suggesting that estrogen may influence BDNF gene expression and protein availability in the prairie vole brain.

Distribution pattern of BDNF-producing cells and fibers

The BDNF mRNA labeling in the brain of female prairie voles was primarily found in cell bodies but not in their projections, indicating that active BDNF synthesis was occurring in the cell body. On the other hand, BDNF protein was generally localized in two compartments, in the cell bodies and in their fibers/projections. Immunostaining for BDNF in the cell bodies resulted in diffuse cytoplasmic labeling in well-defined brain regions, such as the neocortex, hippocampal formation, amygdaloid complex, and hypothalamic areas. In general, the distribution pattern of BDNF-producing cells observed in voles is consistent, for the most part, with the pattern found in rats (Phillips et al., 1990; Wetmore et al., 1990; Wetmore et al., 1991; Gall et al., 1992; Castren et al., 1995; Dugich-Djordjevic et al., 1995; Kawamoto et al., 1996; Conner et al., 1997; Yan et al., 1997). Although BDNF-ir cells sometimes were not found in brain regions in which BDNF mRNA was detected (see below), almost all brain areas that contained BDNF-ir cells possessed BDNF mRNA, suggesting that BDNF protein in these cell bodies represents protein retained by cells producing BDNF (Conner et al., 1997).

A mismatch between BDNF mRNA-labeled and immunostained cells was found in several thalamic regions in the prairie vole brain. In these thalamic areas, *in situ* hybridization detected light to moderate levels of BDNF mRNA labeling, but immunocytochemistry failed to reveal BDNF-ir cells. The explanation for this discrepancy is not obvious, and several possibilities may exist. First, BDNF producing cells in these areas may synthesize and accumulate BDNF protein at a level that was too low to be detected by immunocytochemistry. Alternatively, BDNF protein in these areas might be in a prepro or unfolded state, which prevented recognition by the antibody (Yan et al., 1997). Another likely explanation is that BDNF might have been rapidly transported or secreted after synthesis, resulting in a depletion or lack of sufficient quantity of BDNF protein within a cell body to permit immunoreactive detection. This scenario is supported by the finding that cells synthesizing nerve growth factor in certain brain regions did not accumulate enough antigen to per-

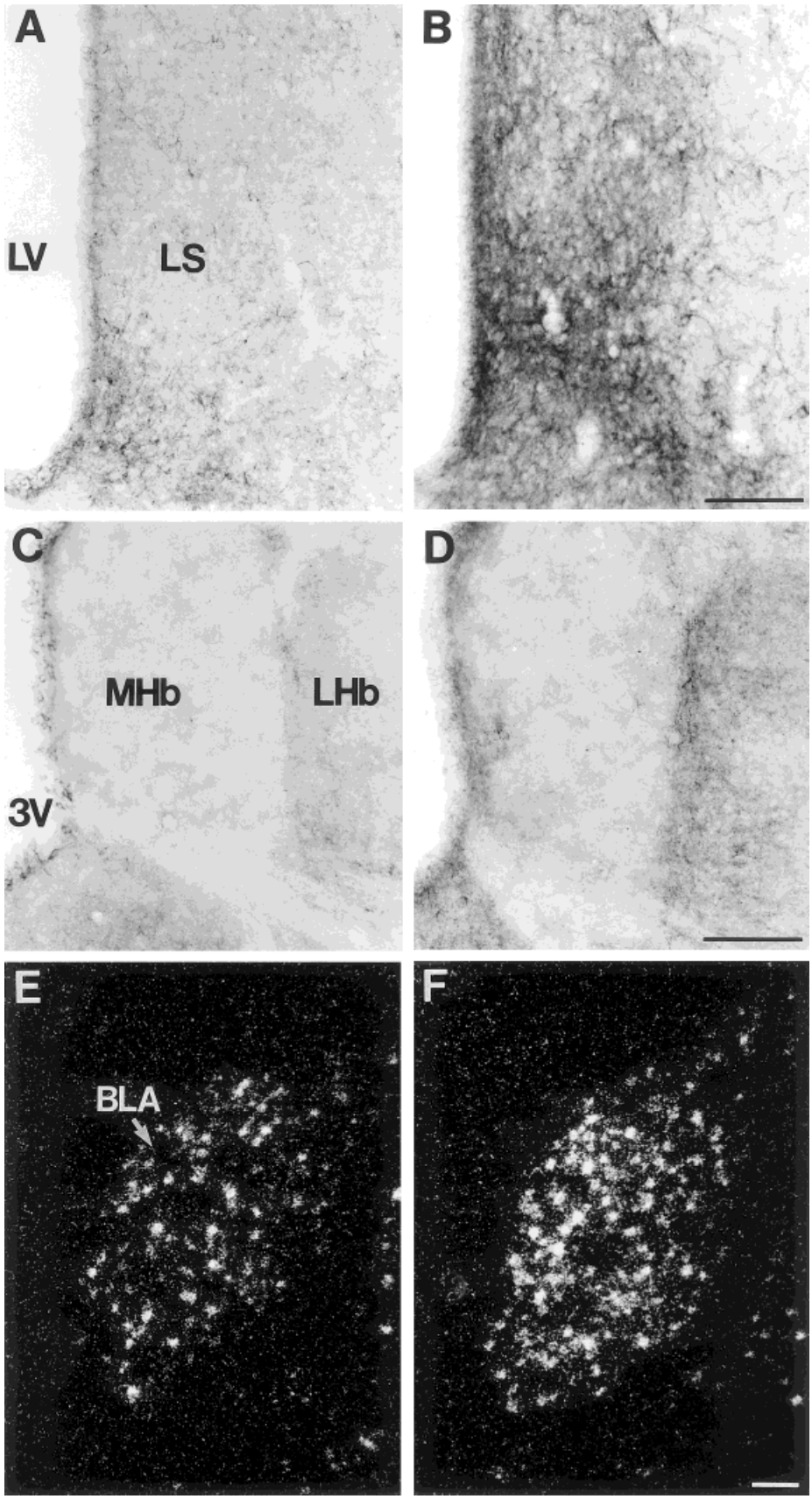

Figure 8

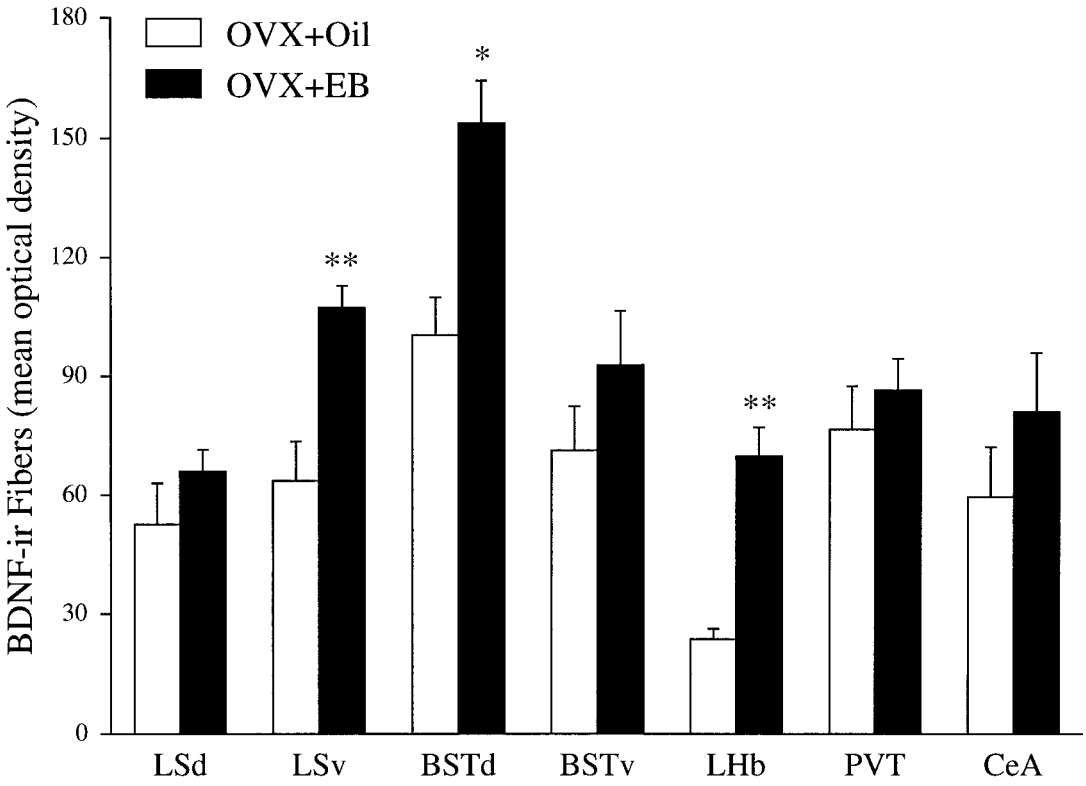

Fig. 9. The density of BDNF immunoreactive (BDNF-ir) stained fibers in selected forebrain regions of female prairie voles. Ovariectomized females that were treated with estradiol benzoate (OVX+EB) had a higher density of BDNF-ir fibers in the ventral part of the lateral septum (LSv), dorsal part of the bed nucleus of the stria terminalis (BSTd), and lateral habenular nucleus (LHb) in comparison to ovariectomized females that were treated with oil (OVX+Oil). No differences were found in the dorsal part of the lateral septum (LSd), ventral part of the BST (BSTv), paraventricular thalamic nucleus (PVT), and central nucleus of the amygdala (CeA). *$P < 0.05$; **$P < 0.01$.

mit immunostaining unless animals were treated with colchicine to block axonal transport (Conner et al., 1992; Conner and Varon, 1992). Finally, studies with vasopressin and oxytocin have demonstrated that messenger RNAs may also be transported (Jirikowski et al., 1990, 1992; Trembleau et al., 1994). Could it be possible that BDNF mRNA was anterogradely transported into the area and thus BDNF synthesis occurred in the fibers? Although our data could not rule in or rule out this possibility, it is unlikely that this would contribute to the observed discrepancy between the BDNF-ir staining and mRNA labeling, because silver grains formed clusters and were located within Nissl-stained cell bodies in the thalamus. Whatever the mechanism is, this discrepancy between BDNF-ir and mRNA labeling was not found in other brain areas, indicating its specificity in the thalamic region of the vole brain.

Extensive, but anatomically discrete BDNF-ir fiber staining was found throughout the forebrain areas in prairie voles, as previously demonstrated in rats (Conner et al., 1997). In agreement with the finding in rats, BDNF-ir fibers were present either in the form of distinguishable individual fibers or fiber plexuses, or in the form of mossy fibers or pericellular baskets (Conner et al., 1997; Yan et al., 1997). In general, the distribution of BDNF-ir fibers can be classified into three patterns in regard to the presence or absence of BDNF-ir or mRNA-labeled cells. In some brain regions, such as the ventral area of the bed nucleus of the stria terminalis, lateral septum, lateral habenular nucleus, and central nucleus of the amygdala, fibers were the sole structures containing BDNF. In these areas, neither BDNF-ir-stained nor BDNF mRNA-labeled cells were detected. In other regions, such as the hypothalamus, neocortex, and medial and basomedial nuclei of the amygdala, BDNF-ir fibers existed along with BDNF-ir or mRNA-labeled cells. In several thalamic regions, moreover, BDNF-ir fibers were present with BDNF mRNA-labeled but not immunostained cells. This widely spread distribution pattern of BDNF-ir fibers in voles suggests that BDNF protein storage is most prominent in the proximity of the neuronal terminals and that BDNF can be

Fig. 8. Photomicrographs displaying brain-derived neurotrophic factor immunoreactive (BDNF-ir) stained fibers (brightfield) in the lateral septum (LS) (**A,B**) and lateral habenular nucleus (LHb) (**C,D**), as well as BDNF mRNA-labeled cells (darkfield) in the basolateral nucleus of the amygdala (BLA) (**E,F**) of ovariectomized female prairie voles that were treated with either oil vehicle (left) or estradiol benzoate (EB; right). Females treated with EB had a higher density of BDNF-ir fibers or more BDNF mRNA-labeled cells than females treated with oil vehicle. 3V, third ventricle, LV, lateral ventricle; MHb, medial habenular nucleus. Scale bar = 50 μm.

released into many brain regions during physiological processes.

In comparison to rats, however, prairie voles showed differences in several aspects of regional BDNF mRNA and protein expression. For example, BDNF-ir fiber staining in the habenular nucleus was detected in both the medial and lateral nuclei in rats (Conner et al., 1997; Yan et al., 1997), but was exclusively localized in the lateral nucleus in prairie voles. Most thalamic regions did not contain BDNF protein or mRNA in rats (Conner et al., 1997; Yan et al., 1997) but were enriched with BDNF-ir fibers and/or mRNA-labeled cells in prairie voles. It is worthwhile to note that the same antibody used in our present study was used to map the distribution pattern of BDNF-ir staining in the rat brain (Conner et al., 1997; Yan et al., 1997). Therefore, the differences in the BDNF expression between rats and voles may likely reflect species-specific involvement of BDNF in the regulation of physiological functions.

Effects of estrogen on BDNF expression

In the present study, we found that estrogen treatment of ovariectomized prairie voles induced an increase not only in the level of BDNF mRNA labeling but also in the level of BDNF-ir staining in selected brain regions. These data suggest that estrogen influences BDNF by regulating its gene expression and protein availability in the prairie vole brain. It should be pointed out that the regional density of BDNF-ir fibers was measured by using a computerized image program (NIH IMAGE 1.60). Such computerized image analysis has provided a useful tool in estimating the magnitude of experimental effects in immunostaining (Bamshad et al., 1993; Mize, 1994; Wang et al., 1996). However, neither the avidin–biotin enhancement of signals nor diaminobenzidine reactions are linear, and many factors during the process, such as the antibody concentration, incubation time, and labeling intensity may influence the density of immunolabeling. Therefore, densitometric measurement of regional immunostaining should not be considered as quantification of the absolute antigen concentration (Mize, 1994). Instead, it serves as a semiquantitative measurement of the effect of an experimental variable on antibody labeling, especially for the immunostaining contained in dendrites and axon terminals that otherwise cannot be counted reliably.

Estrogen has been proven to be a potent regulator of BDNF in rats. BDNF mRNA in the hippocampal formation fluctuated across the estrous cycle (Gibbs, 1998). Ovariectomy decreased, whereas estrogen administration or estrogen plus progesterone treatment of ovariectomized rats increased BDNF mRNA expression in the subregions of the hippocampus (Singh et al., 1995; Gibbs, 1998). Estrogen was also found to enhance BDNF mRNA expression in the cerebral cortex and olfactory bulb of ovariectomized rats (Sohrabji et al., 1995). Finally, estrogen may alter neuronal sensitivity to BDNF by influencing BDNF receptor activity (Sohrabji et al., 1994a,b).

Although the fact that estrogen regulates BDNF has been described, the underlying mechanisms are still unknown. Nevertheless, several lines of evidence have indicated potential cellular and anatomical substrates for estrogen's regulation of BDNF. First, an estrogen receptor response element has been identified in the BDNF gene in rats, indicating that BDNF may respond directly to estrogen's actions (Sohrabji et al., 1995). Second, BDNF actions are mediated by two different membrane-bound receptors: the tyrosine kinase B receptors and the "low affinity" $p75^{NGFR}$ receptors, and this two-receptor system may have a significant biological role in the mediation of the functions of BDNF (Hempstead et al., 1991; Chao and Hempstead, 1995). It has been demonstrated that the estrogen receptor binding sites colocalize with the $p75^{NGFR}$ mRNA and immunoreactive protein not only in the developing but also in the adult rodent brain (Toran-Allerand et al., 1992). These data indicate that estrogen may directly regulate BDNF and its mediated cell functioning, by influencing BDNF or its receptors at the level of signal transduction or gene transcription (Sohrabji et al., 1995; Toran-Allerand, 1996). Certainly, we cannot exclude the possibility that estrogen may influence BDNF indirectly via protein–protein interactions with other transcription factors or through the activation of neurons that regulate BDNF and estrogen-responsive afferents to the target region.

What is the physiological significance of the regulation of BDNF by estrogen? A functional link between estrogen and BDNF has been demonstrated in rats. Estrogen induces an increase in dendritic spine density on hippocampal neurons both in vivo and in vitro (Woolley and McEwen, 1992; Murphy and Segal, 1996). Estrogen also stimulates a transient increase in the number of neurons in the DG of female rats (Tanapat et al., 1999). It has been shown that estrogen may act on BDNF, which, in turn, leads to a reduction in GABA and a subsequent increase in electrical activity and the formation of new dendritic spines (McAllister et al., 1996; Murphy et al., 1998). The physiological relevance of the BDNF upregulation by estrogen is still unknown in voles. In female prairie voles, an estrogen surge during estrus induction is associated with an increased number of bromodeoxyuridine-labeled cells from the subventricular zone (Smith et al., 2001), and mating for 2 days significantly increased the number of proliferating cells in the DG (Fowler et al., 2000). Because BDNF regulates cell proliferation and protects cells from apoptotic death in rats (Kirschenbaum and Goldman, 1995; Courtney et al., 1997; Zigova et al., 1998), it could be speculated that increased estrogen during estrus induction and mating acts on BDNF, which, in turn, enhances cell proliferation and survival in the prairie vole brain. This hypothesis can be tested in further experiments by administering BDNF or blocking its receptors and then examining markers for cell proliferation and survival. The fact that BDNF modulates the effects of steroid hormones on neuronal proliferation and survival is further supported by the fact that the stress hormone corticosterone simultaneously caused neuronal damage and a reduction in BDNF mRNA/protein, whereas exogenous BDNF treatment attenuated the corticosterone-induced neuronal death in rats (Smith et al., 1995a; Nitta et al., 1999).

CONCLUSION

In conclusion, we have systematically mapped the BDNF mRNA and protein in the forebrain of female prairie voles. Our data indicated that, with few exceptions, the distribution pattern of BDNF immunoreactivity and mRNA expression in voles generally resembles that found in rats. In comparison to rats, however, prairie voles showed some differences in the regional BDNF expression, suggesting possible species-specific involvement of

BDNF in physiological functions. We also found that estrogen treatment of ovariectomized female voles significantly increased BDNF mRNA and BDNF-ir labeling in several brain regions, suggesting that estrogen may regulate BDNF at the level of gene and protein availability. The functional significance of species-specific BDNF distribution and BDNF upregulation by estrogen in voles needs to be further studied.

ACKNOWLEDGMENTS

This research was supported by the FYAP Award of Florida State University to Z.W. C.D.F. was a recipient of the Art and Science Fellow Award from Florida State University. The authors thank Drs. Fred Stephen, Thomas Curtis, and Karen Berkley for a critical reading of the manuscript. We also thank Drs. Charles Ouimet and Thomas Houpt for their helpful discussion and suggestion throughout the course of this study.

LITERATURE CITED

Akutagawa E, Konishi M. 1998. Transient expression and transport of brain-derived neurotrophic factor in the male zebra finch's song system during vocal development. Proc Natl Acad Sci USA 95:11429–11434.

Alderson RF, Alterman AL, Barde YA, Lindsay RM. 1990. Brain-derived neurotrophic factor increases survival and differentiated functions of rat septal cholinergic neurons in culture. Neuron 5:297–306.

Bamshad M, Novak MA, De Vries GJ. 1993. Sex and species differences in the vasopressin innervation of sexually naive and parental prairie voles, *Microtus ochrogaster* and meadow voles, *Microtus pennsylvanicus*. J Neuroendocrinol 5:247–255.

Barde YA, Edgar D, Thoenen H. 1982. Purification of a new neurotrophic factor from mammalian brain. Eur Molec Biol Org J 1:549–553.

Carter CS, Getz LL, Gavish L, McDermott JL, Arnold P. 1987. Male-related pheromones and the activation of female reproduction in the prairie vole (Microtus ochrogaster). Biol Reprod 23:1038–1045.

Castren E, Thoenen H, Lindholm D. 1995. Brain-derived neurotrophic factor messenger RNA is expressed in the septum, hypothalamus and in adrenergic brain stem nuclei of adult rat brain and is increased by osmotic stimulation in the paraventricular nucleus. Neuroscience 64: 71–80.

Chao MV, Hempstead BL. 1995. p75 and Trk: a two-receptor system. Trends Neurosci 18:321–326.

Cohen-Parsons M, Carter CS. 1987. Males increase serum estrogen and estrogen receptor binding in brain of female voles. Physiol Behav 39:309–314.

Conner JM, Varon S. 1992. Distribution of nerve growth factor-like immunoreactive neurons in the adult rat brain following colchicine treatment. J Comp Neurol 326:347–362.

Conner JM, Muir D, Varon S, Hagg T, Manthorpe M. 1992. The localization of nerve growth factor-like immunoreactivity in the adult rat basal forebrain and hippocampal formation. J Comp Neurol 345:409–418.

Conner B, Young D, Yan Q, Faull RLM, Synek B, Dragunow M. 1997a. Brain-derived neurotrophic factor is reduced in Alzheimer's disease. Brain Res Mol Brain Res 49:71–81.

Conner JM, Lauterborn JC, Yan Q, Gall CM, Varon S. 1997b. Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat: evidence of anterograde axonal transport. J Neurosci 17:2295–2313.

Courtney MJ, Akerman KEO, Coffey ET. 1997. Neurotrophins protect cultured cerebellar granule neurons against the early phase of cell death by a two-component mechanism. J Neurosci 17:4201–4211.

Dittrich F, Feng Y, Metzdorf R, Gahr M. 1999. Estrogen-inducible, sex specific expression of brain-derived neurotrophic factor mRNA in a forebrain song control nucleus of the juvenile zebra finch. Proc Natl Acad Sci USA 96:8241–8246.

Dugich-Djordjevic MM, Peterson C, Isono F, Ohsawa F, Widmer HR, Denton TL, Bennett GL, Hefti F. 1995. Immunohistochemical visualization of brain-derived neurotrophic factor in the rat brain. Eur J Neurosci 7:1831–1839.

Fowler CD, Liu Y, Jia XX, Wang ZX. 2000. Social experience alters neurogenesis in the brain of adult female prairie voles. Soc Neurosci Abs 26:54.

Gall CM, Gold SJ, Isackson PJ, Seroogy KB. 1992. Brain-derived neurotrophic factor and neurotrophin-3 mRNAs are expressed in ventral midbrain regions containing dopaminergic neurons. Mol Cell Neurosci 3:56–63.

Ghohs A, Carnahan J, Greenberg ME. 1994. Requirement for BDNF in activity-dependent survival of cortical neurons. Science 263:1618–1623.

Gibbs RB. 1998. Levels of trkA and BDNF mRNA, but not NGF mRNA, fluctuate across the estrous cycle and increase in response to acute hormone replacement. Brain Res 787:259–268.

Gingrich B, Liu Y, Cascio C, Wang ZX, Insel TR. 2000. Dopamine D2 receptors in the nucleus accumbens are important for social attachment in female prairie voles. Behav Neurosci 114:173–183.

Hayashi M, Yamashita A, Shimizu K. 1997. Somatostatin and brain-derived neurotrophic factor mRNA expression in the primate brain: decreased levels of mRNAs during aging. Brain Res 749:283–289.

Hempstead BL, Martin-Zanca D, Kaplan DR, Parada LF, Chao MV. 1991. High-affinity NGF binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor. Nature 350:678–683.

Hofer M, Pagliusi SR, Hohn A, Leibrock J, Barde YA. 1990. Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. EMBO J 9:2459–2464.

Hyman C, Hofer M, Barde YA, Juhasz M, Yancopoulos G, Squinto SP, Lindsay RM. 1991. BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. Nature 350:230–232.

Jirikowski GF, Sanna PP, Bloom FE. 1990. mRNA coding for oxytocin is present in axons of the hypothalamo-neurohypophysial tract. Proc Natl Acad Sci USA 87:7400–7404.

Jirikowski GF, Sanna PP, Maciejewski-Lenoir D, Bloom FE. 1992. Reversal of diabetes insipidus in Brattleboro rats: intrahypothalamic injection of vasopressin mRNA. Science 255:996–998.

Johnson F, Hohmann SE, DiStefano PS, Bottjer SW. 1997. Neurotrophins suppress apoptosis induced by deafferentation of an avian motor-cortical region. J Neurosci 17:2101–2111.

Kawamoto Y, Nakamura S, Nakano S, Oka N, Akiguchi I, Kimura J. 1996. Immunohistochemical localization of brain-derived neurotrophic factor in adult rat brain. Neuroscience 74:1209–1226.

Kawamoto Y, Nakamura S, Kawamata T, Akiguchi I, Kimura J. 1999. Cellular localization of brain-derived neurotrophic factor-like immunoreactivity in adult monkey brain. Brain Res 821:341–349.

Kirschenbaum K, Goldman SA. 1995. Brain-derived neurotrophic factor promotes the survival of neurons arising from the adult rat forebrain subependymal zone. Proc Natl Acad Sci USA 92:210–214.

Knusel B, Winslow JW, Rosenthal A, Burton LE, Seid DP, Nikolics K, Fefti F. 1991. Promotion of central cholinergic and dopaminergic neuron differentiation by brain-derived neurotrophic factor but not neurotrophin-3. Proc Natl Acad Sci USA 88:961–965.

Leibrock J, Lottspeich F, Hohn A, Hofer M, Hengerer B, Masiakowski P, Thoenen H, Barde YA. 1989. Molecular cloning and expression of brain-derived neurotrophic factor. Nature 341:149–152.

Lindholm D, Dechant G, Heisenberg CP, Thoenen H. 1993. Brain-derived neurotrophic factor is a survival factor for cultured rat cerebellar granule neurons and protects them against glutamate-induced toxicity. Eur J Neurosci 5:1455–1464.

Lindsay RM, Wiegand SJ, Altar CA, DiStefano PS. 1994. Neurotrophic factors: from molecule to man. Trends Neurosci 17:182–190.

Lo DC. 1995. Neurotrophic factors and synaptic plasticity. Neuron 15:979–981.

Mamounas LA, Blue ME, Siuciak JA, Altar A. 1995. Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. J Neurosci 15:7929–7939.

McAllister AK, Katz LC, Lo DC. 1996. Neurotrophin regulation of cortical dendritic growth required activity. Neuron 17:1057–1064.

McCallister AK, Katz LC, Lo DC. 1997. Opposing roles for endogenous BDNF and NT-3 in regulating cortical dendritic growth. Neuron 18: 767–778.

Mize RR. 1994. Quantitative image analysis for immunocytochemistry and in situ hybridization. J Neurosci Methods 54:219–237.

Murer MG, Boissiere F, Yan Q, Hunot S,Villares J, Faucheux B, Agid Y, Hirsch E, Raisman-Vozari R. 1999. An immunohistochemical study of the distribution of brain-derived neurotrophic factor in the adult hu-

man brain, with particular reference to Alzheimer's disease. Neuroscience 88:1015–1032.

Murphy DD, Segal M. 1996. Regulation of dendritic spine density in cultured rat hippocampal neurons by steroid hormones. J Neurosci 16:4059–4068.

Murphy DD, Cole NB, Segal M. 1998. Brain-derived neurotrophic factor mediates estradiol-induced dendritic spine formation in hippocampal neurons. Proc Natl Acad Sci USA 95:11412–11417.

Nitta A, Ohmiya M, Sometani A, Itoh M, Nomoto H, Furukawa Y, Furukawa S. 1999. Brain-derived neurotrophic factor prevents neuronal cell death induced by corticosterone. J Neurosci Res 57:227–235.

Phillips HS, Hains JM, Laramee GR, Rosenthal V, Winslow JW. 1990. Widespread expression of BDNF but not NT3 by target areas of basal forebrain cholinergic neurons. Science 250:290–294.

Phillips HS, Hains JM, Laramee GR, Hohnson SA, Winslow JW. 1991. BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease. Neuron 7:695–702.

Rasika S, Alvarez-Buylla A, Nottebohm F. 1999. Neuron 22:53–82.

Richmond, ME, Conaway CH. 1969. Induced ovulation and oestrus in Microtus ochrogaster. J Reprod Fertil Suppl 6:357–376.

Schaaf M, de Jong J, de Kloet E, Vreugdenhil E. 1998. Downregulation of BDNF mRNA and protein in the rat hippocampus by corticosterone. Brain Res 813:112–120.

Schaaf MJM, Hoetelmans RWM, de Kloet ER, Vreugdenhil E. 1997. Corticosterone regulates expression of BDNF and trkB but not NT-3 and trkC mRNA in the rat hippocampus. J Neurosci Res 48:334–341.

Singh M, Meyer EM, Simpkins JW. 1995. The effect of ovariectomy and estradiol replacement on brain-derived neurotrophic factor messenger ribonucleic acid expression in cortical and hippocampal brain regions of female Sprague-Dawley rats. Endocrinology 136:2320–2324.

Smith M, Makino S, Kvetnansky R, Post R. 1995a. Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. J Neurosci 15:1768–1777.

Smith MA, Makino S, Kim SY, Kvetnansky R. 1995b. Stress increases brain-derived neurotropic factor messenger ribonucleic acid in the hypothalamus and pituitary. Endocrinology 136:3743–3750.

Smith MT, Pencea V, Wang ZX, Luskin MB, Insel TR. 2001. Increased cell proliferation in the rostral migratory stream of the estrous prairie voles. Horm Behav 39:11–21.

Snider WD. 1994. Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. Cell 77:627–638.

Sohrabji F, Greene LA, Miranda RC, Toran-Allerand CD. 1994a. Reciprocal regulation of estrogen and NGF receptors by their ligands in PC12 cells. J Neurobiol 25:974–988.

Sohrabji F, Miranda RC, Toran-Allerand CD. 1994b. Estrogen differentially regulates estrogen and nerve growth factor receptor mRNAs in adult sensory neurons. J Neurosci 14:459–471.

Sohrabji F, Miranda RCG, Toran-Allerand D. 1995. Identification of a putative estrogen response element in the gene encoding brain-derived neurotrophic factor. Proc Natl Acad Sci USA 92:11110–11114.

Tanapat P, Hastings NB, Reeves AJ, Gould E. 1999. Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. J Neurosci 15:5792–5801.

Thoenen H. 1995. Neurotrophins and neuronal plasticity. Science 270:593–598.

Toran-Allerand C. 1996. Mechanisms of estrogen action during neural development: mediation by interactions with the neurotrophins and their receptors. J Steroid Biochem Mol Biol 56:169–178.

Toran-Allerand C, Miranda R, Bentham W, Sohrabji F, Brown T, Hochberg R, MacLusky N. 1992. Estrogen receptors colocalize with low-affinity nerve growth factor receptors in cholinergic neurons of the basal forebrain. Proc Natl Acad Sci USA 89:4668–4672.

Trembleau A, Morales M, Bloom FE. 1994. Aggregation of vasopressin mRNA in a subset of axonal swellings of the median eminence and posterior pituitary: light and electron microscopic evidence. J Neurosci 14:39–53.

Ventimiglia R, Mather PE, Jones BE, Lindsay RM. 1995. The neurotrophins BDNF, NT-3 and NT-4/5 promote survival and morphological and biochemical differentiation of striatal neurons in vitro. Eur J Neurosci 7:213–222.

Wang ZX, Bullock NA, De Vries GJ. 1993. Sexual differentiation of vasopressin projections of the bed nucleus of the stria terminals and medial amygdaloid nucleus in rats. Endocrinology 132:2299–2306.

Wang ZX, Zhou L, Hulihan TJ, Insel TR. 1996. Immunoreactivity of central vasopressin and oxytocin pathways in microtine rodents: a quantitative comparative study. J Comp Neurol 366:726–737.

Wetmore C, Ernfors P, Persson H, Olson L. 1990. Localization of brain-derived neurotrophic factor mRNA to neurons in the brain by in situ hybridization. Exp Neurol 109:141–152.

Wetmore C, Cao Y, Pettersson RF, Olson L. 1991. Brain-derived neurotrophic factor: subcellular compartmentalization and interneuronal transfer as visualized with anti-peptide antibodies. Proc Natl Acad Sci USA 88:9843–9847.

Williams JR, Catania KC, Carter CS. 1992. Development of partner preferences in female prairie voles (Microtus ochrogaster): the role of social and sexual experience. Horm Behav 26:339–349.

Woolley C, McEwen B. 1992. Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. J Neurosci 12:2549–2554.

Xu H, Gouras GK, Greenfield JP, Vincent B, Naslund J, Mazzarelli L, Fried G, Jovanovic JN, Seeger M, Relkin NR, Liao F, Checler F, Buxbaum JD, Chait BT, Thinakaran G, Sisodia SS, Wang R, Greengard P, Gandy S. 1998. Estrogen reduces neuronal generation of Alzheimer β-amyloid peptides. Nature Med 4:447–451.

Yan Q, Matheson C, Lopex OT, Miller JA. 1994. The biological responses of axotomized adult motoneurons to brain-derived neurotrophic factor. J Neurosci 4:5191–9281.

Yan Q, Rosenfeld RD, Matheson CR, Hawkins N, Lopez OT, Bennett L, Welcher AA. 1997. Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system. Neuroscience 78:431–448.

Zigova T, Pencea V, Wiegand SJ, Luskin MB. 1998. Intraventricular administration of BDNF increases the number of newly generated neurons in the adult bulb. Mol Cell Neurosci 4:234–245.