Sex- and Estrous Cycle-Related Differences in the Effects of Acute Antipsychotic Drug Administration on Neurotensin-Containing Neurons in the Rat Brain

BECKY KINKEAD, STEVEN M. LORCH, MICHAEL J. OWENS, and CHARLES B. NEMEROFF

Laboratory of Neuropsychopharmacology, Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, Georgia

Accepted for publication June 26, 2000

ABSTRACT

The effects of a single injection of haloperidol (2.0 mg/kg), a typical antipsychotic drug, on neurotensin (NT) concentrations and NT/neuromedin N (NT/NN) mRNA expression in adult female and male rats were examined. There were significant estrous cycle stage-related differences in both NT concentrations and NT/NN mRNA expression in female control rats. Although acute administration of haloperidol increased NT concentrations and NT/NN mRNA expression in the caudate/putamen and nucleus accumbens of both male and female rats, haloperidol did not increase NT/NN mRNA expression during diestrus 2 or NT concentrations during proestrus in the nucleus accumbens of female rats. These results indicate the presence of both sex- and estrous cycle-related differences in the regulation of NT-containing neurons and in the effects of antipsychotic drug administration on the NT system of the rat brain.

A burgeoning literature supports the view that sex differences in the course and treatment response of patients with schizophrenia exist. These include the following: 1) a delayed age of onset in women, possibly attributable to the number of women becoming schizophrenia during their 40s and 50s; 2) better treatment response in women; 3) less severe course of illness; and 4) better outcome in women (Castle et al., 1995). It is unclear, however, whether the sex differences observed in schizophrenia are due to actual sex differences in the manifestation of the disorder, or whether women are more susceptible to distinct subtypes of schizophrenia. Because of the increased incidence of psychotic episodes at times of low estrogen (menopause and the puerperal period), however, estrogen has been hypothesized to have a protective, possibly antidopaminergic, effect in schizophrenia.

The neurotensin (NT) system is one neurotransmitter system regulated by estrogen. NT is a tridecapeptide that was first structurally characterized from extracts of bovine hypothalamus by Carraway and Leeman (1973). Estrogen has been repeatedly demonstrated to exert effects on NT-containing neurons in the rat brain (Alexander et al., 1989a; Herbisson and Theodosis, 1991; Watters and Dorsa, 1998). Moreover, numerous studies have demonstrated interactions between dopamine (DA) neurons and estrogen (Becker, 1999), and the former has long been known to be intimately related to NT circuits.

NT was first hypothesized to be an endogenous antipsychotic drug due to the numerous similarities between the effects of centrally administered NT and peripherally administered antipsychotic drugs (Nemeroff, 1980; Bissette and Nemeroff, 1995). Clinical studies of drug-free schizophrenic patients have repeatedly demonstrated that there is a subset of such patients with decreased cerebrospinal fluid (CSF) concentrations of NT. After antipsychotic drug treatment, these CSF NT concentrations are increased toward control concentrations (Lindström et al., 1988; Garver et al., 1991; Sharma et al., 1997). There also appears to be a correlation between NT concentrations in the CSF and the magnitude of psychopathology, including negative symptoms (Garver et al., 1991; Breslin et al., 1994). Schizophrenic patients with low CSF concentrations of NT are lithium nonresponders, and have a greater degree of thought disorder, negative symptoms, delusions-hallucinations, behavioral disorganization, and impaired functioning.

One of the most consistent findings implicating the NT circuits to the mechanism of action of antipsychotic drugs is the effect of antipsychotic drug administration on regional NT concentrations in the rat brain. Govoni et al. (1980) first reported that the clinically efficacious antipsychotic drugs haloperidol, chlorpromazine, trifluoperazine, and pimozide...
specifically increased NT concentrations in the nucleus accumbens and caudate/putamen of rats, whereas clinically ineffective phenothiazines (e.g., promazine and promethazine), as well as other classes of psychotherapeutic agents such as tricyclic antidepressants, anxiolytics, and antihistamines failed to alter NT concentrations in any brain region examined (Govoni et al., 1980; Myers et al., 1992). In contrast to the effects of typical antipsychotic drugs, antipsychotic drugs classified as “atypical” due to their lack of extrapyramidal side effects and superior efficacy in treatment-resistant schizophrenia (e.g., clozapine) increase NT concentrations only in the nucleus accumbens (Kilts et al., 1988).

Administration of the typical antipsychotic drug haloperidol also increases the number of NT/neuromedin N (NT/NN) mRNA-expressing neurons in the neostriatum of the rat brain. Merchant et al. (1992), using in situ hybridization histochemistry, demonstrated that a single dose of haloperidol leads to a nearly 3-fold increase in the number of cells expressing NT/NN mRNA in the dorsolateral striatum, whereas the atypical antipsychotic drug clozapine had no effect in this brain region. Both clozapine and haloperidol were found to increase NT/NN mRNA levels in the shell section of the nucleus accumbens.

To date, all of the studies examining the effects of antipsychotic drugs on the NT system have been conducted in adult male rats. For all of the above-cited reasons it was of interest to scrutinize the effects of antipsychotic drug administration in female as well as male rats. These studies examined basal levels of NT/NN mRNA expression and NT concentrations in both male and female rats, as well as the effects of an acute injection of the typical antipsychotic drug haloperidol on NT/NN mRNA expression, as well as NT tissue concentrations.

**Materials and Methods**

**Animals and Housing.** Sexually mature male and female Sprague-Dawley rats (150–200 g; Harlan Sprague-Dawley, Inc., Indianapolis, IN) were housed in same sex groups of four, under 24-h light/dark cycle (lights on 7:00 AM; lights off 7:00 PM) in an environmentally controlled animal facility. Food and water were available ad libitum. All rats were handled daily for 1 week before treatment. The Emory Institutional Animal Care and Use Committee approved all animal protocols.

**Treatment and Tissue Preparation.** Stage of estrus was assessed in female rats by vaginal lavage. Based on the number and type of cells present in the daily swab, females were divided into four groups: diestrus 1 (D1), diestrus 2 (D2), proestrus (P), and estrus (E). Only those rats showing at least two regular 4-day cycles were considered in this study. Male (n = 5–10) and female rats (n = 5–10/group) received a single s.c. injection of either haloperidol (2.0 mg/kg) or vehicle (1.0 ml/kg, 0.3% tartaric acid).

For measurement of NT concentrations by radioimmunoassay (RIA), vaginal lavages were obtained from all female rats 1 h before treatment (4:00 PM) and 1 h before they were sacrificed (10:00 AM, 18 h after the single injection). Based on the final swab and the results of the previous day’s swabs, the females were categorized as being either in D1, D2, P, or E at the time of death. For measurement of NT/NN mRNA expression by RNase protection assay (RPA) and in situ hybridization, vaginal lavages were obtained 1 h before the rats were injected (9:00 AM) and at the time of kill (4:00 PM, 7 h after the single injection).

Rats used for examination of NT concentrations or NT/NN mRNA expression by RPA were sacrificed by decapitation, and the brains were rapidly removed and frozen. The brains were dissected free-hand on ice based on the method of Glowinski and Iversen (1966). The brain regions dissected included the prefrontal cortex, nucleus accumbens, anterior and posterior caudate/putamen, hippocampus, substantia nigra (SN), and ventral tegmental area (VTA). Individual brain regions were stored at −70°C in polypropylene microcentrifuge tubes until assay.

Animals used for in situ hybridization analysis of NT/NN mRNA expression were anesthetized using Euthanasia-5 solution (Henry Schein, Inc., Port Washington, NY) and transcardially perfused via the ascending aorta with cold 0.9% NaCl (200 ml) followed by cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.6) for 8 min (250 ml). The brains were then removed and postfixed in 4% paraformaldehyde for 24 h at 4°C and then transferred to 20% sucrose for 48 h. After postfixing, the brains were rinsed in double-distilled H₂O, dried, and stored at −70°C until use. Tissue sections (30 μm) were cut on a microtome and collected in 24-well series. The level of the appearance of the anterior commissure was marked for future reference. Tissue sections were stored in cryoprotectant solution (30% ethylene glycol and 20% glycerol in 25 mM phosphate buffer, pH 7.4) at −20°C. Before slide mounting the tissue, the tissue slices were rinsed in 50 mM phosphate buffer (pH 7.6) to remove cryoprotectant. The slides were then allowed to dry at room temperature and stored at −20°C until use.

**RPA.** Total RNA was extracted from individual brain regions using a phenol/guanidinium isothiocyanate/chloroform method. The tissue was homogenized in TRI Reagent (phenol and guanidine isothiocyanate in a monophase solution; Molecular Research Center, Inc., Cincinnati, OH), chloroform was added, and the sample was centrifuged at 12,000 g for 15 min at 4°C. The homogenate then separates into three phases, with the top aqueous phase containing the RNA. The RNA was then precipitated with isopropanol, washed with 75% ethanol, and resuspended in distilled DEPC-treated water. Total RNA content was determined by optical densitometry at 260 nm.

Riboprobes were prepared as described above for the in situ hybridization with the exception that [³²P]UTP was incorporated as the radiolabel. The probe was then gel purified and used in conjunction with the Ambion RPA II RNase protection kit. Nonlabeled sense strand RNA (for determination of a specific signal) was generated using SP6 RNA polymerase and XbaI linearized plasmid using a similar method.

The RNase protection assay was performed using the RPA II ribonuclease protection assay kit (Ambion, Austin, TX). Briefly, extracted RNA was combined with [³²P]-labeled probe and allowed to hybridize overnight at 45°C. [³²P]-Labeled antisense probe to rat actin RNA was also added to serve as an internal RNA-loading control for each sample. An RNase cocktail (RNase A/RNase T1) was added, and the samples were incubated for 30 min. The RNases were then deactivated using an ethanol mixture (solution Dx, RPA II kit; Ambion), the protected fragments ethanol precipitated, and resuspended in gel-loading buffer (solution E, RPA II kit; Ambion). The protected fragments were separated on a denaturing polyacrylamide gel. To determine the amount of nonspecific probe binding, nonlabeled sense strand RNA (a different length than the native sense strand RNA) was added to separate samples. The gel was then exposed to an autoradiographic film, and the resulting bands were quantified by densitometry on a Nikon microphot with a charge-coupled device video system using an Apple Quadra 950 computer with NIH IMAGE software. Hybridization times and exact buffer concentrations were adjusted to maximize sensitivity and specificity.

**In Situ Hybridization.** Template plasmid consisting of a 336-base pair EcoRv/BglII fragment (nucleotides 626–961) of the rat NT/NN gene (Kishlauksia et al., 1988) in a BamH1/SmaI-digested pGEM-4 (Promega, Madison, WI) was generously provided by P. Dobner (University of Massachusetts Medical Center, Worcester, MA). [³²P]-Labeled antisense riboprobes were generated using EcolI linearized plasmid, nucleotides, [³²P]-UTP, and T7 RNA polymerase (protocol adapted from the T7/T3 MAXImiscript kit; Ambion).
Unincorporated nucleotides were removed from the reactions using Quick Spin Columns (Boehringer Mannheim, Indianapolis, IN). The \(^{35}\)S-labeled riboprobes were then diluted to 1 \(\times\) 10\(^7\) cpm/\(\mu\)l in hybridization buffer [62.5% formalde, 12.5% dextran sulfate, 0.375 M NaCl, 2.5% Denhardt’s solution, 12.5 mM Tris (pH 8.0), 1.25 mM EDTA, pH 8.0] and stored at \(-20^\circ\)C until use.

The protocol for in situ hybridization was adapted from the methods of Simmons et al. (1989). Briefly, the slide-mounted tissue was digested with proteinase K followed by acetylation in acetic anhydride. The slides were then rinsed in 2\% standard saline citrate buffer (NaCl/sodium citrate) and then quickly dehydrated in ascending concentrations of fresh ethanol. After dehydration, slides were dried at room temperature, and then 100 \(\mu\)l (1 \(\times\) 10\(^6\) cpm) of riboprobe mixture (riboprobe in hybridization solution with RNA and dithiothreitol) was added to each slide. The slides were then covered with parafilm, and stored overnight at 60°C in covered trays.

The paraffin was removed after incubation for 24 h, and the slides were rinsed in 4\% standard saline citrate before RNase digestion (1500 dilution of 10.0 mg/ml RNase A). The slides were then rinsed, gradually desalted, and incubated for 30 min at 60°C. Slides were then quickly dehydrated in ethanol (containing salt and dithiothreitol), drained well, allowed to dry, and exposed to Kodak Biomax MR film. After exposure to film, the slides were emulsion coated and counterstained.

Film autoradiograms were digitized and quantified using NIH IMAGE. A \(^{14}\)C standard curve (Amersham, Piscataway, NJ) was included in each film cassette for use in standardizing quantification between films. NT/NN mRNA expression was measured in the core and shell subdivisions of the nucleus accumbens at 2.4 mm anterior to bregma according to the atlas of Palkovits and Brownstein (1988). RIA. NT concentrations were determined using a highly specific and sensitive NT RIA. Brain regions were extracted in ice-cold 1.0 M HCl by ultrasonic dismembration, and the homogenates centrifuged at 10,000 \(\times\) g for 15 min at 4°C. The supernatant was then transferred to a fresh microcentrifuge tube, vortexed, and duplicate 100-\(\mu\)l aliquots were transferred to borosilicate glass tubes and stored at \(-70^\circ\)C. On the day of the assay the frozen aliquots were lyophilized, reconstituted in assay buffer, and then assayed by a single equilibrium RIA according to methods previously described (Bissette et al., 1984). The assay buffer consisted of 10 mM NaH\(_2\)PO\(_4\), 0.15 M NaCl, 0.01% NaN\(_3\), 0.1% gelatin, 2.5 mM EDTA, and 0.05% Triton X-100 adjusted to pH 7.6 with NaOH. The antiserum used (Peninsula Laboratories, Inc., Belmont, CA) is directed against NT. The NT/NN mRNA expression was below detectable limits (ND) in the prefrontal cortex.

### Results

#### Sex- and Estrous Cycle-Related Differences in Basal NT/NN mRNA Expression and Response to Acute Antipsychotic Drug Administration

Comparison of basal NT/NN mRNA expression (determined by RPA) in female versus male rats showed that female rats had significantly higher levels of basal NT/NN mRNA expression in the nucleus accumbens (\(P < .05\)) and the SN (\(P < .05\)). Furthermore, basal NT/NN mRNA expression in the anterior caudate/putamen was significantly lower in female rats compared with male rats (\(P < .05\)).

There was a significant effect of estrous cycle stage in the nucleus accumbens, anterior caudate/putamen, and VTA. Analysis by one-way ANOVA (Fig. 1a) demonstrated that these estrous cycle-related differences were due to differences in basal NT/NN mRNA expression in all three brain regions; NT/NN mRNA expression was highest in female rats during D2.

Two-way ANOVA of NT/NN mRNA expression (sex \(\times\) treatment) and (cycle \(\times\) treatment) indicated a significant treatment effect in the nucleus accumbens (Fig. 2), anterior caudate/putamen (Fig. 3), and the posterior caudate/putamen (Fig. 4). Administration of haloperidol significantly increased NT/NN mRNA expression in the anterior and posterior caudate/putamen in male and female rats in all stages of the estrous cycle. In the nucleus accumbens, haloperidol did not increase NT/NN mRNA expression in female rats during D2. There were no significant (sex \(\times\) treatment) or (cycle \(\times\) treatment) interactions, sex \(\times\) treatment interactions, or significant differences by one-way ANOVA, Student-Newman-Keuls multiple comparisons test was applied to identify significantly differing groups.

#### Table 1

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>NT/NN mRNA Expression (\times) 10(^{-3}) (Relative to Actin)</th>
<th>NT-Like Immunoreactivity</th>
<th>pg/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Prefrontal cortex</td>
<td>ND</td>
<td>ND</td>
<td>48.3 (\pm) 9.0 (10)</td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td>58.1 (\pm) 9.1 (5)</td>
<td>91.5 (\pm) 5.2 (35)*</td>
<td>199.1 (\pm) 12.9 (9)</td>
</tr>
<tr>
<td>Anterior caudate</td>
<td>14.5 (\pm) 3.1 (9)</td>
<td>17.0 (\pm) 1.9 (31)*</td>
<td>58.8 (\pm) 4.0 (9)</td>
</tr>
<tr>
<td>Posterior caudate</td>
<td>35.8 (\pm) 1.1 (5)</td>
<td>31.5 (\pm) 3.2 (31)</td>
<td>103.8 (\pm) 5.8 (10)</td>
</tr>
<tr>
<td>SN</td>
<td>27.6 (\pm) 2.4 (5)</td>
<td>44.3 (\pm) 2.4 (30)*</td>
<td>425.1 (\pm) 33.2 (9)</td>
</tr>
<tr>
<td>VTA</td>
<td>28.1 (\pm) 2.7 (5)</td>
<td>32.3 (\pm) 1.2 (33)</td>
<td>625.3 (\pm) 16.8 (9)</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>6.7 (\pm) 2.3 (5)</td>
<td>12.9 (\pm) 1.0 (33)</td>
<td>195.1 (\pm) 12.6 (8)</td>
</tr>
</tbody>
</table>

*\(P < .05\) compared with NT/NN mRNA expression or NT-like immunoreactivity in the same brain region of male rats. NT/NN mRNA expression was below detectable limits (ND) in the prefrontal cortex.
Sex- and Estrous Cycle-Related Differences in Basal NT Concentrations and Response to Acute Antipsychotic Drug Administration. There was a significant effect of sex in the prefrontal cortex, posterior caudate/putamen, SN, and VTA. Further comparison of basal NT tissue concentrations in female versus male rats demonstrated that female rats had significantly higher levels of basal NT-like immunoreactivity in the prefrontal cortex (P < .05) and SN (P < .05) (Table 1).

Fig. 2. Estrous cycle-related differences in the effects of acute haloperidol administration on NT/NN mRNA expression and NT concentrations in females and males rats in the nucleus accumbens. Male (n = 10) and female rats (n = 5–10/each estrous cycle stage) received a single s.c. injection of either haloperidol (2.0 mg/kg) or vehicle. NT/NN mRNA expression was measured by RNase protection assay. NT-like immunoreactivity was determined by RIA. Two-way ANOVA (cycle × treatment) indicated a significant treatment effect (F1,66 = 27.6, P < .001) and (F1,66 = 84.8, P < .001) on NT/NN mRNA expression and NT-like immunoreactivity, respectively. There was a significant (cycle stage × treatment) interaction in the nucleus accumbens (F3,66 = 4.6, P < .001). *P < .05 compared with corresponding control. Cross-hatched bar is percentage of control of vehicle-treated animals ± S.E.M. S.E.M. shown is the largest S.E.M. from a control group.

Fig. 3. Estrous cycle-related differences in the effects of acute haloperidol administration on NT/NN mRNA expression and NT concentrations in female and males rats in the anterior caudate/putamen. Male (n = 10) and female rats (n = 5–10/each estrous cycle stage) received a single s.c. injection of either haloperidol (2.0 mg/kg) or vehicle. NT/NN mRNA expression was measured by RNase protection assay. NT-like immunoreactivity was determined by RIA. Two-way ANOVA (cycle × treatment) indicated a significant treatment effect (F1,61 = 31.1, P < .001) and (F1,61 = 154.6, P < .001) on NT/NN mRNA expression and NT-like immunoreactivity, respectively. *P < .05 and **P < .001 compared with corresponding control. Cross-hatched bar is percentage of control of vehicle-treated animals ± S.E.M. S.E.M. shown is the largest S.E.M. from a control group.
reactivity was determined by RIA. Two-way ANOVA (cycle expression was measured by RNase protection assay. NT-like immunoreactivity in male and female rats (n = 5–10/each estrous cycle stage) received a single s.c. injection of either haloperidol (2.0 mg/kg) or vehicle. NT/NN mRNA expression was measured by RIA. Two-way ANOVA (cycle × treatment) indicated a significant treatment effect (F^1.24_4 = 51.9, P < .001) and (F^1.87_7 = 212, P < .001) on NT/NN mRNA expression and NT-like immunoreactivity, respectively. *P < .05 and **P < .01 compared with corresponding control. Cross-hatched bar is percentage of control of vehicle-treated animals ± S.E.M. S.E.M. shown is the largest S.E.M. from a control group.

There was a significant effect of estrous cycle stage on NT-like immunoreactivity in the prefrontal cortex, posterior caudate/putamen, SN, and VTA (Fig. 1b). Analysis by one-way ANOVA demonstrated that these estrous cycle-related differences were due to differences in basal NT-like immunoreactivity in the VTA only. In the VTA, NT-like immunoreactivity was significantly higher during D1 (P < .05) compared with NT-like immunoreactivity in all other estrous cycle stages.

Two-way ANOVA of NT-like immunoreactivity (sex × treatment) and (cycle × treatment) indicated a significant treatment effect in the nucleus accumbens (Fig. 2), anterior caudate/putamen (Fig. 3), and the posterior caudate/putamen (Fig. 4). Haloperidol significantly increased NT-like immunoreactivity in male and female rats in the anterior and posterior caudate/putamen. There were no significant (sex × treatment) interactions in any other brain region examined. In the nucleus accumbens, there was a significant (cycle stage × treatment) interaction. In this brain region, haloperidol increased NT-like immunoreactivity in males and in females in all stages of the estrous cycle except for P.

**Discussion**

In the same year that NT was hypothesized to be an endogenous neuroleptic (Nemeroff, 1980), the first report appeared concerning the effects of antipsychotic drug administration on NT-like immunoreactivity was published (Giovoni et al., 1980). To date, all clinically effective antipsychotic drugs examined have specific effects on the NT system of the rat brain (for review, see Kinkead et al., 1999). Previously, acute administration of haloperidol has been shown to increase NT/NN mRNA expression (Merchant et al., 1992) and NT-like immunoreactivity (Frey et al., 1986; Eggerman and Zahm, 1988; Zahm, 1992; Zahm et al., 1996) in both the dorsal and ventral striatum of adult male rats. Within the ventral striatum, the effects of haloperidol have been reported to be limited to the shell subdivision (Merchant et al., 1992; Zahm, 1992; Zahm et al., 1996).

The results of these current studies demonstrate the necessity of considering both sex and the estrous cycle stage of female rats when examining the regulation of NT-containing neurons. Significant differences in basal NT/NN mRNA expression and NT concentrations between male and female rats were found in the prefrontal cortex, nucleus accumbens, hippocampus, and SN. In addition to differences between the sexes, both NT/NN mRNA expression and NT concentrations varied significantly across the estrous cycle of female rats in the VTA, nucleus accumbens, and anterior caudate/putamen. Analysis of NT/NN mRNA expression by in situ hybridization demonstrated that the estrous cycle-related differences in basal NT/NN mRNA expression were limited to the shell subdivision of the nucleus accumbens, and not to the core.
The highest levels of NT/NN mRNA expression are seen in D2. It is possible from the time course of the increase in NT/NN mRNA expression during D2, to increases in NT concentrations during P that the increases in peptide concentrations may be due to the increase in gene expression. NT neurons in the VTA project to the nucleus accumbens (specifically the shell subdivision of the nucleus accumbens) and NT neurons within the nucleus accumbens have many local axon collaterals (Kalivas and Miller, 1984). It is unclear whether the estrous cycle-related increases in NT peptide are associated with changes in peptide release. Microdialysis studies are needed to specifically answer this question.

After examination of the effects of acute administration of the typical antipsychotic drug haloperidol, striking results were found in the nucleus accumbens. Haloperidol administration increased NT/NN mRNA expression and NT concentrations in both male and female rats. In contrast, haloperidol administration did not increase NT/NN mRNA expression in female rats in D2, or NT concentrations in female rats in P. In both cases, it appears that haloperidol did not have a significant effect due to the significantly higher basal NT/NN mRNA expression and NT concentrations during D2 and P, respectively. In situ hybridization analysis of NT/NN mRNA expression in the nucleus accumbens demonstrated that the lack of effect of haloperidol administration during D2 was confined to the shell subdivision of the nucleus accumbens.

The unique estrous cycle regulation and effects of haloperidol administration on NT/NN mRNA expression in the shell subdivision of the nucleus accumbens are interesting in light of the role of this particular brain region in the antipsychotic properties of antipsychotic drugs. The induction of the immediate early gene c-fos in the nucleus accumbens was shown to be an excellent predictor of antipsychotic drug potential (Robertson et al., 1994). In addition, both typical and atypical antipsychotic drugs increase NT/NN mRNA expression in the shell subdivision of the nucleus accumbens, whereas only typical antipsychotic drugs increase NT/NN mRNA expression in the caudate/putamen (Merchant et al., 1992). Estrous cycle-related regulation of NT/NN mRNA expression and NT tissue concentrations (and subsequently NT release?) might be the mechanism by which ovarian hormones exert antipsychotic-like properties.

Although it is unclear which hormone (or hormones) is responsible for the estrous cycle regulation of the NT system, previous studies have demonstrated that estrogen can regulate NT/NN gene expression in hypothalamic nuclei of the rat (Alexander et al., 1989a,b; Alexander, 1993; Alexander and Leeman, 1994). Alexander et al. (1989) demonstrated that estrogen-induced increases in NT/NN mRNA expression in the preoptic nuclei are essential for the preovulatory surge of luteinizing hormone. It was further demonstrated that in ovariectomized rats, estrogen differentially regulates NT/NN mRNA expression in subdivisions of the arcuate nucleus and the median eminence and that there are estrous cycle-related differences in NT/NN mRNA expression in the dorsomedial division of the arcuate nucleus (Alexander, 1993). These experiments extend the findings of ovarian hormone regulation of NT-containing neurons to nonhypothalamic nuclei. Recently, Watters and Dorsa (1998) described a mechanism by which estrogen may regulate NT/NN mRNA expression (the NT gene lacks an estrogen response element) via interactions with the cAMP cascade.

Although NT/NN mRNA expression is regulated by estrogen, it is possible that other ovarian hormones influence NT-containing neurons in these brain regions or that the effects of estrogen on NT-containing neurons are secondary to the effects of estrogen on other neurotransmitter systems. For example, extracellular DA levels, as well as DA-mediated behaviors are both regulated by ovarian hormone levels (Castner et al., 1993; Morissette and Di Paolo, 1993; Diaz-Veliz et al., 1994; Xiao and Becker, 1994; Becker, 1999). The effects of estrogen on the DA system are complex, nevertheless, it has been hypothesized that estrogen has an antidiopaminergic (possibly antipsychotic drug-like) effect. Additionally, the fact that haloperidol administration did not further increase the elevated NT/NN mRNA expression and NT concentrations seen during D2 and P, respectively, indicates possible regulation of NT-containing neurons via the same mechanisms. These findings, in combination with the close association between NT and DA in both the mesolimbic and nigrostriatal pathways, indicate the possibility that ovarian hormone effects on NT-containing neurons may be secondary to modulation of the DA system.

These results are of interest in light of the evidence that NT may function as an endogenous antipsychotic agent (Nemeroff, 1980) and the evidence of sex differences in schizophrenia (Castle et al., 1995; Canuso et al., 1998). Women have a better treatment response, less severe symptomatology, and a better outcome compared with men, leading to the hypothesis that estrogen may have a protective effect in schizophrenia (Seeman, 1996). Women also have a higher incidence of neuroleptic-induced Parkinsonism, akathisia, and tardive dyskinesia. A recent study examining the neuroleptic-like properties of progesterone demonstrated that progesterone also shares many of the same behavioral effects as atypical antipsychotic drugs (Ruppel et al., 1999). Further studies are needed to determine the mechanism behind the regulation of the NT-containing neurons in these brain regions, as well as to determine whether NT release is altered in concert with the mRNA and peptide concentration changes.

Acknowledgment

We thank David Knight for superb technical assistance.

References


**Send reprint requests to:** Charles B. Nemeroff, M.D., Ph.D., Laboratory of Neuropsychopharmacology, Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Suite 4000 WMRB, 1639 Pierce Dr., Atlanta, GA 30322. E-mail: cnemero@emory.edu  

---

**Missing author information:**
