Cellular and Molecular Neurobiology, Vol. 16, No. 3, 1996

Estrogen Control of Central Neurotransmission: Effect on Mood, Mental State, and Memory

George Fink,[1,2] Barbara E. H. Sumner,[1] Roberta Rosie,[1] Oliver Grace,[1] and John P. Quinn[1]

Received April 3, 1995; accepted May 30, 1995

KEY WORDS: estrogen as psychoprotectant; estradiol-17ß; testosterone; aromatase; serotonin; 5-hydroxytryptamine; 5-hydroxytryptamine$_2$ receptors; cerebral cortex; nucleus accumbens; arginine vasopressin; bed nucleus of the stria terminalis; social memory; psychosis; depression; premenstrual syndrome; postnatal depression; schizophrenia; mania; Tourette's syndrome.

SUMMARY

1. Estrogen exerts profound effects on mood, mental state and memory by acting on both "classical" monoamine and neuropeptide transmitter mechanisms in brain. Here we review an example of each type of action.

2. With respect to the effect of estrogen on central monoamine neurotransmission, low levels of estrogen in women are associated with the premenstrual syndrome, postnatal depression and post-menopausal depression. Sex differences in schizophrenia have also been attributed to estrogen. Previous studies have shown that estrogen stimulates a significant increase in dopamine$_2$ (D_2) receptors in the striatum. Here we show for the first time that estrogen also stimulates a significant increase in the density of 5-hydroxytryptamine$_{2A}$ (5-HT_{2A}) binding sites in anterior frontal, cingulate and primary olfactory cortex and in the nucleus accumbens, areas of the brain concerned with the control of mood, mental state, cognition, emotion and behavior. These findings explain, for example, the efficacy of estrogen therapy or 5-HT uptake blockers such as fluoxetine in treating the depressive symptoms of the premenstrual syndrome,

[1] MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ, U.K.

[2] To whom correspondence should be addressed at University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ, U.K.

0272-4340/96/0600-0325$09.50/0

and suggest that the sex differences in schizophrenia may also be due to an action of estrogen mediated by way of 5-HT_{2A} receptors.

3. With respect to the effect of estrogen on central neuropeptide transmission, estrogen stimulates the expression of the arginine vasopressin (AVP) gene in the bed nucleus of the stria terminalis (BNST) in rodents. This results in a 100-fold increase in AVP mRNA in the BNST and a massive increase in AVP peptide in the BNST and its projections to the lateral septum and lateral habenula. The BNST-AVP system enhances and/or maintains "social" or "olfactory" memory, and thus provides a powerful model for correlating transcriptional control of neuropeptide gene expression with behavior. Whether similar mechanisms operate in the human remain to be determined.

4. These two examples of the action of estrogen on central neurotransmission are discussed in terms of their immediate clinical importance for the treatment of depressive symptoms, their use as powerful models for investigations on the steroid control of central neurotransmitter mechanisms, and the role of estrogen as "Nature's" psychoprotectant.

INTRODUCTION

Estrogen has long been known to exert powerful effects on brain function. Thus, for example, in spontaneously ovulating mammals such as man, monkey, sheep and rat the ovulatory surge of luteinizing hormone (LH) is triggered by a spontaneous surge of estradiol-17ß secreted by the ovary. This estradiol surge acts on the brain and the anterior pituitary gland to initiate a positive feedback cascade which involves the release of the decapeptide, LH-releasing hormone (LHRH), and an increase in pituitary responsiveness to LHRH (Everett, 1988; Fink, 1979, 1988, 1994). Pituitary responsiveness to LHRH is further increased by a unique property of LHRH which is to increase pituitary responsiveness to itself, the "self-priming" or "self-sensitizing" effect of LHRH. The latter coordinates the increase release of LHRH into hypophysial portal blood with the increase in pituitary responsiveness so that both reach a peak simultaneously and thereby ensure a massive ovulatory surge of LH (Fink, 1979, 1988, 1995). The preovulatory surge of estrogen also plays a pivotal role in inducing lordosis in the female (Pfaff, 1980). The action of estradiol in triggering the surge of LHRH is mediated by several neurotransmitter systems and receptors, in particular 5-HT_{2A} (Dow, *et al.* 1994; Sumner and Fink, 1995a, b), α_1 adrenoreceptors (Rosie *et al.* 1993; Sarkar and Fink, 1981), and two pharmacologically distinct dopamine receptors one of which inhibits while the other stimulates LHRH release (Sarkar and Fink, 1981).

Clinical observations suggest that in addition to its role in neuroendocrine control, estrogen and its male counterpart, testosterone, exert powerful effects on mood, mental state, behavior and memory. Thus, the early studies of Dalton (1959) showed that of 276 women admitted to psychiatric hospitals, 46% were admitted at or immediately before menstruation. The incidence of suicide was also much greater during the luteal compared with the follicular phase of the

cycle (MacKinnon and MacKinnon, 1956) or immediately before or during menstruation (Dalton, 1959). The premenstrual syndrome and the high incidence of depression in women after the menopause are, respectively, associated with the precipitous fall in plasma estrogen concentrations towards the end of the menstrual cycle and the more gradual fall in estrogen levels towards undetectable levels at the time of the menopause (O'Brien, 1993; Reid and Yen, 1981; Studd and Zamblera, 1994). Estrogen has been used as an effective therapy in women with persistent depression which was resistant to conventional therapies (Klaiber, *et al.* 1979). Similarly, in the majority of patients with puerperal illness, which presents as severe depression (postnatal depression), mania, schizoaffective manic disorder or other types of psychosis, the first symptoms occur in the first week after delivery when there is a precipitous fall in plasma estradiol concentrations (Dean and Kendell, 1981). Puerperal psychosis has been attributed to an estrogen-dependent disorder of D_2 receptors (Cookson, 1985; Wieck, *et al.* 1991).

Estrogen has also been implicated in schizophrenia in that, the average age of onset of schizophrenia is significantly later in women than in men, there is a qualitative difference in the symptoms of schizophrenia in women compared with men, and there is a second peak of schizophrenia onset in women, but not men, after the age of 40 (Angermeyer, *et al.* 1990; Bleuler, 1950; Di Paolo, 1994; Häfner, *et al.* 1993; Kraepelin, 1971; Lewis, 1992; Loranger, 1984; Riecher-Rössler and Häfner, 1993; Seeman and Lang, 1990).

With respect to testosterone, substantial clinical evidence suggests that the Gilles de la Tourette's Syndrome, and obsessive compulsive disorder and chronic tics in families with Tourette's Syndrome, are all inherited as a highly penetrant autosomal dominant trait the expression of which is exacerbated significantly by androgens (Friedhoff, 1986; Leckman and Scahill, 1990; Pauls and Leckman, 1986). Furthermore, affective and psychotic symptoms may be associated with the use of anabolic steroids (Pope and Katz, 1988). Testosterone also plays a critical role in "social" or "olfactory" memory in rodents (Bluthé, *et al.* 1993a, b; Dantzer and Bluthé, 1992; Fink, 1994). This action of testosterone would appear to be mediated by way of its enzymatic (aromatase) conversion to estrogen.

Although the clinical and experimental observations, reviewed above, suggest that estrogen may be "Nature's psychoprotectant", and may also play a crucial role in at least one type of memory, the precise mechanisms by which estrogen exerts its effects have not been established. In the case of schizophrenia, experimental studies suggest that the protective effect of estrogen may be due to its neuroleptic-like effect, even though estrogen also increases the density of D_2 receptors in striatum (Di Paolo, 1994; Häfner, *et al.* 1993). The dopamine hypothesis has also been invoked for postnatal affective psychosis (Cookson, 1985; Wieck, *et al.* 1991). That is, the precipitous, postnatal fall in estrogen levels is thought to expose supersensitive D_2 receptors in forebrain. Much less, however, is known about how estrogen could protect against the depressive symptoms of the premenstrual syndrome, post-menopausal depression and, indeed, postnatal depression compared with other forms of puerperal psychosis.

Here we review two of our studies, the first of which suggests that the anti-depressant/psychotic action of estrogen may be mediated by a serotonergic

mechanism involving 5-HT_{2A} receptors, while the second shows that the action of estrogen and testosterone (by way of estrogen) in inducing or maintaining "social" or "olfactory" memory involves a significant increase in the amount of arginine vasopressin (AVP) in the bed nucleus of the stria terminalis (BNST) and its projections to the lateral habenula and lateral septum. This is associated with a more than 100-fold increase in the amount of AVP mRNA in the BNST probably as a consequence of increased AVP gene transcription brought about by a complex interaction between the activated estrogen receptor and other transcription factors which control AVP gene expression.

STUDY I: ESTROGEN INCREASES THE DENSITY OF 5-HT_{2A} RECEPTORS IN CEREBRAL CORTEX AND NUCLEUS ACCUMBENS IN THE FEMALE RAT

The profound effects of estradiol-17ß on mood and mental state in the human are likely to be mediated by monoamine mechanisms. The functional psychoses are broadly divided into the affective disorders and schizophrenia. Affective disorders have long been assumed to involve defective monoamine function in brain (e.g. Ashcroft, *et al.* 1972). The efficacy of 5-HT uptake inhibitors in the treatment of depression has suggested that major depression is due mainly to a disorder of central 5-HT transmission (Kaplan and Sadock, 1985). Schizophrenia, on the other hand, has generally been throught to be due to a disorder of central dopaminergic transmission. However, recent psychopharmacological data suggest that as well as playing a pivotal role in major depression (Kaplan and Sadock, 1985) serotonergic (5-HT) mechanisms may also be responsible, at least in part, for schizophrenia. Thus, for example, powerful "atypical" antipsychotics such as clozapine and risperidone, which are highly effective in the treatment of schizophrenia, bind with much greater affinity to 5-HT_{2A} than dopamine receptors (Edwards, 1994; Janssen, *et al.* 1988; Leysen, *et al.* 1992; Meltzer, *et al.* 1989; Seeman, 1992; Strange, 1994), and ritanserin, a 5-HT_{2A} receptor antagonist, is an effective adjunctive treatment for the negative symptoms of schizophrenia (Duinkerke, *et al.* 1993). This hypothesis receives further support from the finding that the density of 5-HT_{2A} receptors, assessed by ^{3}H-ketanserin binding in post mortem brain tissue, was significantly lower in the prefrontal, but not the occipital cortex of patients with chronic schizophrenia who died of natural causes compared with psychotics who died of suicide, control subjects, and non-psychotic suicide victims (Laruelle, *et al.* 1993). The experimental evidence for the dopamine hypothesis of schizophrenia depends to a large extent on the use of spiperone as a ligand (Di Paolo, 1994); but, spiperone also has a high affinity for 5-HT_{2A} receptors (Hoyer, *et al.* 1994).

Bearing these facts in mind, and prompted by our finding that estradiol, in its positive feedback mode for LHRH/LH release, stimulates a massive increase in the expression of 5-HT_{2A} receptor mRNA in the dorsal raphe nucleus (Sumner and Fink, 1993), we investigated the possible effects of estradiol on the density of 5-HT_{2A} receptors in brain (Sumner and Fink, 1995a, b).

Materials and Methods

As more fully described previously (Sumner and Fink, 1995a, b) twelve adult female COB Wistar rats were bilaterally ovariectomized under halothane anesthesia on the morning of diestrus, and immediately injected s.c. with 30 μg estradiol benzoate (EB) in 0.2 ml arachis oil, or with 0.2 ml arachis oil alone (six rats per group). Next day at the expected time of the ovulatory surge of LH secretion, the animals were decapitated under sodium pentobarbitone anesthesia, and their brains removed and frozen for in vitro receptor autoradiography. Trunk blood was measured for LH by radioimmunoassay (mean ± SEM: 3.7 ± 0.3 ng NIDDK rat LH/RP/2/ml in EB-treated rats, and 0.8 ± 0.1 ng/ml in oil-treated rats). Student's t-test and the Mann-Whitney U-test confirmed that, as expected, the EB had significantly stimulated LH release ($P = 0.0001$ and $P < 0.01$ respectively).

Brains from EB- and oil-treated animals were processed in pairs. Serial 15 μm coronal sections were lightly fixed for 5 min in 1% paraformaldehyde in phosphate-buffered saline (pH 7.4), and then (after washes) incubated in buffered 2 nM ^{3}H-ketanserin hydrochloride (NEN: sp.act. 2223 GBq/mmol) as described by Pazos *et al.* (1985), except that our method included unlabelled 10^{-4}M prazosin to prevent ketanserin binding to α_1-adrenergic receptors. After the post-incubation washes the sections were dried, apposed to Hyperfilm-^{3}H together with a tritium standard (Amersham, U.K.) and exposed at 0–4°C for 5 weeks. Control sections (blanks) were incubated with both labelled and unlabelled ketanserin, and processed similarly.

Silver grain densities were measured on the developed autoradiographic images with an Optomax image-analyser (Synoptics, Cambridge, U.K.). After subtraction of blanks, the grain densities were converted to binding values (fmol/mg tissue) by reference to the calibrated standard scale and the specific activity of the ligand.

For each neuroanatomical region, a mean ± SEM binding value was calculated from data collected over ten sections per animal. The means were analyzed for statistical significance by paired tests (paired t-tests, and Wilcoxon signed rank test) because the brains had been processed in pairs throughout.

Results

The highest density of 5-HT_{2A} receptors was found in the anterior cingulate, frontal, and piriform cortex, the claustrum and the nucleus accumbens (Table 1; Fig. 1). This distribution is similar to that found in previous autoradiographic studies which used either [^{3}H] ketanserin or the hallucinogens [^{125}I]-LSD (lysergic acid diethylamide) or [^{125}I]-DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane) as 5-HT_{2A} ligands (Appel, *et al.* 1990; Hoyer, *et al.* 1994; Palacios, *et al.* 1990; Pazos, *et al.* 1985). As also shown previously, the density of 5-HT_{2A}

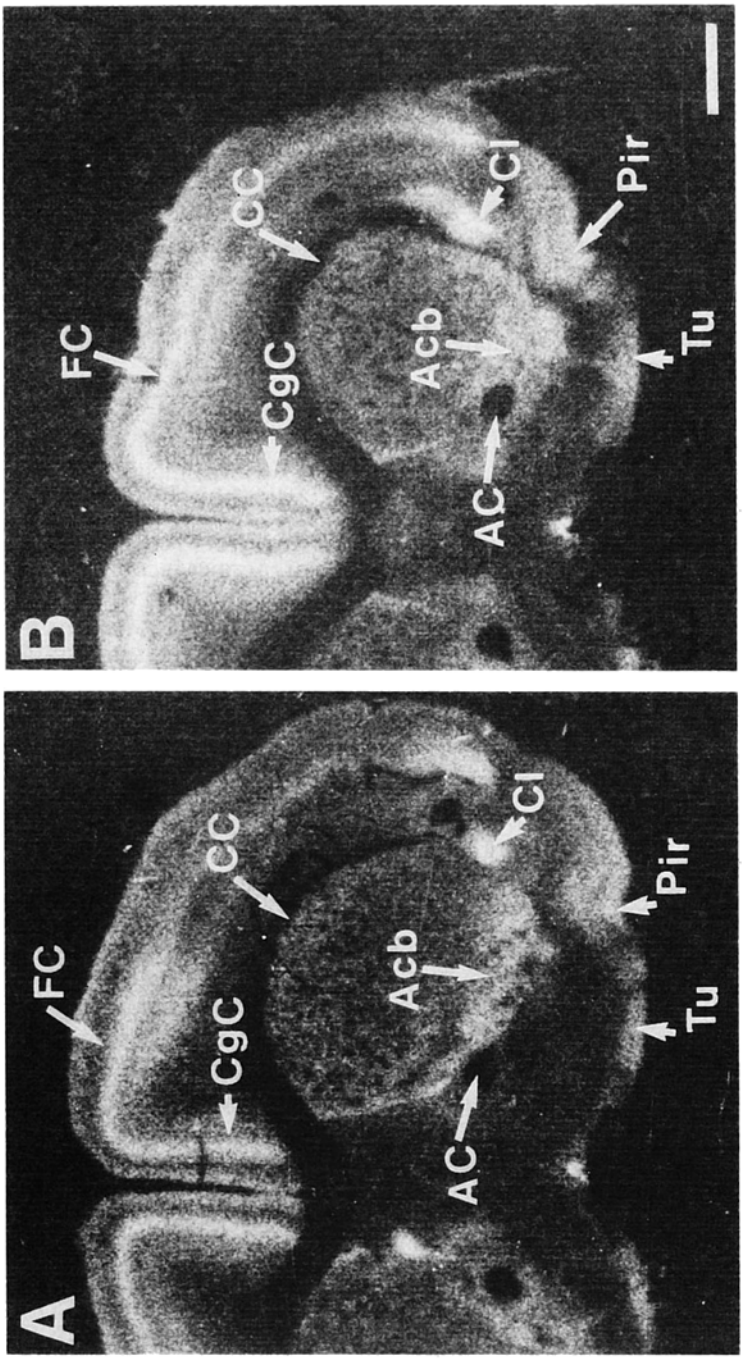

Fig. 1. Dark-field film autoradiographs showing the distribution of 5-HT_{2A} receptors in anterior forebrain (A, B) and cerebral cortex (C, D). The density of 5-HT_{2A} receptors, determined by the binding of ^{3}H-ketanserin in the presence of prazosin, in frontal (FC), cingulate (CgC) and piriform (Pir) cortex, and in olfactory tubercle (Tu) and nucleus accumbens (Acb), was significantly greater in animals treated with estrogen (B and D) compared with oil-treated control animals (A and C). AC, anterior commissure; CC, corpus callosum; Cl, claustrum. Scale, 1 mm. From Sumner and Fink (1995a) with permission.

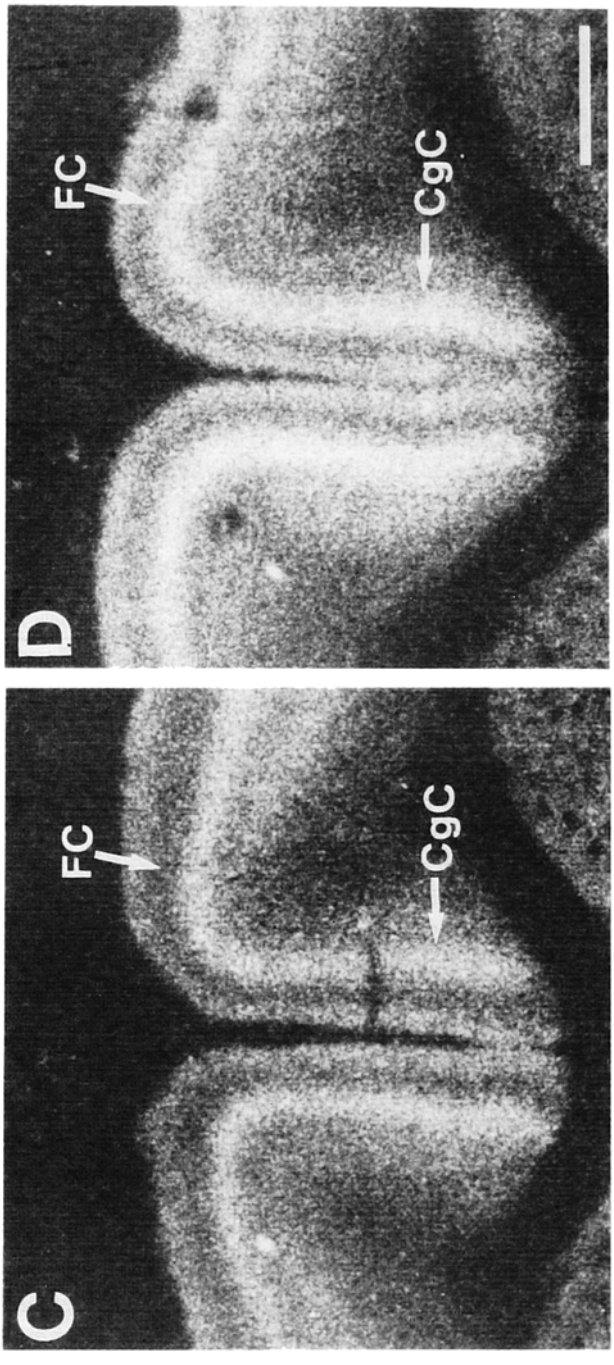

Fig. 1. *(Continued.)*

Table I. Effects of Acute Estradiol Benzoate (EB) on Mean ± SEM Binding (fmol/mg Tissue) of ^{3}H-Ketanserin (in the Presence of Prazosin) in Different Regions of Female Rat Brain

				Statistical significance	
Brain region	Oil-treated	EB-treated	Percentage change	Paired t-test	Wilcoxon signed rank
Anterior cingulate cortex	72.2 ± 6.7	89.2 ± 7.8	24% increase	P = 0.0010	P = 0.0312
Anterior frontal cortex (areas 1 & 2)	67.9 ± 4.9	96.1 ± 6.6	41% increase	P = 0.0004	P = 0.0312
Piriform cortex	62.2 ± 6.7	72.4 ± 5.5	16% increase	P = 0.0153	P = 0.0312
Olfactory tubercle	46.5 ± 5.0	53.7 ± 4.2	16% increase	P = 0.0357	P = 0.0312
Nucleus accumbens	50.2 ± 3.2	56.4 ± 2.7	12% increase	P = 0.0059	P = 0.0312
Claustrum	60.3 ± 3.3	63.0 ± 5.5	4% increase	Not significant	Not significant
Antero-ventral part of the periventricular nucleus	37.1 ± 1.7	42.8 ± 4.5	15% increase	Not significant	Not significant
Medial preoptic area	18.0 ± 2.1	17.1 ± 1.5	5% decrease	Not significant	Not significant
Hippocampus (dentate gyrus)	21.5 ± 1.9	19.5 ± 4.4	9% decrease	Not significant	Not significant
Hippocampus (pyramidal cell fields)	18.9 ± 4.6	19.9 ± 2.4	5% increase	Not significant	Not significant
Dorsal raphe nucleus (ventromedial part)	31.1 ± 3.4	34.4 ± 2.3	11% increase	Not significant	Not significant
Dorsal raphe nucelus (dorsomedial part)	28.3 ± 1.9	31.6 ± 1.5	11% increase	Not significant	Not significant
Dorsal raphe nucleus (lateral part)	24.5 ± 1.4	35.6 ± 2.1	45% increase	P = 0.0040	P = 0.0312
Locus coeruleus	21.9 ± 4.8	20.9 ± 3.2	4% decrease	Not significant	Not significant

binding sites in frontal and cingulate cortex was greatest in laminae I, Va and IV (Appel, *et al.* 1990; Blue *et al.* 1988; Hoyer, *et al.* 1994) (Fig. 1). The density of the 5-HT_{2A} binding sites (Fig. 1; Table I) corresponds closely with the concentrations of 5-HT_{2A} receptor mRNA in brain (Mengod, *et al.* 1990; Pompeiano, *et al.* 1994; Sumner and Fink, 1993; Sumner, *et al.* 1992).

Estradiol treatment significantly increased the density of 5-HT_{2A} binding sites in the anterior frontal, anterior cingulate and piriform cortex, the olfactory tubercle, the nucleus accumbens and the lateral dorsal raphe nucleus (Table I; Fig. 1). Preliminary studies had shown that a dosage of 10 μg EB (as used in Sumner and Fink, 1993) significantly increased the density of 5-HT_{2A} receptors in four out of six animals in frontal cortex and in three out of six animals in cingulate cortex. In the definitive, study, using the 30 μg dose of EB (Sumner and Fink, 1995a, b), all six out of six animals treated with EB showed a significant increase in 5-HT_{2A} receptor density in frontal and cingulate cortex compared with that in oil-treated control animals.

Discussion

These findings provide the first experimental evidence for the fact that estradiol in its positive feedback mode for LHRH/LH release increases the density of 5-HT_{2A} receptors in cerebral cortex and nucleus accumbens. The data provide a rational explanation for the fact that the administration of estrogen or 5-HT uptake blockers, such as fluoxetine ("Prozac"), are effective in the treatment

of the depressive symptoms of the premenstrual syndrome (O'Brien, 1993; Rapkin, 1992; Studd and Zamblera, 1994; Wood, *et al.* 1992). The data may also explain why estrogen therapy was effective in reducing significantly the severity of symptoms in women with persistent major depressive disorder (Klaiber, *et al.* 1979), and why 5-HT_2 receptors have been implicated in suicide (Zifa and Fillion, 1992).

Our findings are also relevant to puerperal psychosis which presents as major depression, mania, schizoaffective or other psychosis (Dean and Kendell, 1981). The majority of patients with puerperal psychosis are admitted to hospital during the second postnatal week, but symptoms most frequently begin during the first week after delivery of the infant (Dean and Kendell, 1981) during which the plasma concentrations of estrogen drop precipitously. Some authors attribute puerperal psychosis to a disorder of central dopaminergic mechanisms (Cookson, 1985; Wieck, *et al.* 1991), but our data raise the possibility that some of the symptoms of puerperal psychosis involve 5-HT_{2A} receptors. This is especially likely for postnatal depression which accounted for 69% (46% major depressive disorder; 23% minor depressive disorder) of the 71 patients with puerperal psychosis studied by Dean and Kendell (1981).

For reasons outlined in the introduction, estrogen is also thought to play a major role in the sex difference in schizophrenia: that is, the later onset of schizophrenia in women than in men, a second peak of onset of schizophrenia in women, but not in men after the age of 40 years, and the qualitative sex-difference in symptoms (Cookson, 1985; Di Paolo, 1994; Lewis, 1992; Seeman and Lang, 1990). These clinical observations suggest that estrogen "protects" against schizophrenia. The mechanism of this apparent antipsychotic effect of estrogen is not known, but, in line with the dopamine hypothesis of schizophrenia, has been postulated to be due to estrogen inhibition of dopamine activity (Cookson, 1985; Di Paolo, 1994; Wieck, *et al.* 1991). However, recent pharmacological data, reviewed in the Introduction to this Study, suggest that as well as playing a pivotal role in affective psychosis, a 5-HT_{2A} mechanism may also be involved in schizophrenia.

There is no conflict between the dopamine hypothesis of schizophrenia and puerperal psychosis (Cookson, 1985; Di Paolo, 1994; Wieck, *et al.* 1991) and our hypothesis that estradiol could also affect mood and mental state by way of 5-HT_{2A} receptors. Dopamine and 5-HT receptors may both be involved either in series or in parallel in the same disorder. Thus, for example, it is conceivable that abnormal dopaminergic activity might be responsible for florid symptoms, such as hallucinations, associated with the acute onset of schizophrenia while abnormal 5-HT function might be responsible for the more refractory, negative symptoms of schizophrenia. The latter respond to clozapine and risperidone which have a high affinity for 5-HT_{2A} receptors (Edwards, 1994; Janssen, *et al.* 1988; Leysen, *et al.* 1992; Meltzer, *et al.* 1989), or adjunctive treatment with the 5-HT_{2A} antagonist, ritanserin (Duinkerke, *et al.* 1993). Alternatively, since amphetamine, which stimulates dopamine release, and lysergic acid diethylamide (LSD), which is a 5-HT_{2A} receptor agonist (Appel, *et al.* 1990; Hoyer, *et al.* 1994; Palacios, *et al.* 1990; Pazos, *et al.* 1985; Zifa and Fillion, 1992), both cause hallucinations it is

conceivable that abnormal serotonergic and dopaminergic mechanisms could act in concert to cause hallucinations in schizophrenia and other psychoses.

The significant stimulation of 5-HT_{2A} receptors by estrogen in the nucleus accumbens deserves emphasis because this nucleus receives major inputs from the amygdala, anterior cingulate and the piriform cortex, and projects, by relay in striatum and thalamus, to the primary motor cortex (Zahm and Brog, 1992). The role of 5-HT in the nucleus accumbens is not known but estradiol significantly decreases 5-HT metabolism in this nucleus (Shimizu and Bray, 1993). The nucleus accumbens also projects to the hypothalamus as do the frontal, cingulate and piriform cortex, and so the estradiol-stimulated increase in the density of 5-HT_{2A} receptors in these brain regions (Fig. 1; Table I) may also be related to the control of pituitary hormone secretion and mating and motor behavior (Dow, *et al.* 1994; Hunter, *et al.* 1985; James, *et al.* 1989; Levy, *et al.* 1994; Mendelson and Gorzalka, 1986; Nauta, 1963). The 5-HT_{2A} receptor subtype plays a key role in food intake and sleep (Levy, *et al.* 1994; Zifa and Fillion, 1992), and also mediates the actions of hallucinogens such as LSD and DOI (Zifa and Fillion, 1992) and some of the actions of cocaine (Levy, *et al.* 1994).

The mechanism by which estradiol increases the density of 5-HT_{2A} receptors is not yet established. There is good evidence that the cortical 5-HT_{2A} receptors are post-synaptic (Hoyer, *et al.* 1994; Leysen, *et al.* 1983; Zifa and Fillion, 1992), but acute treatment with 10 μg estradiol benzoate did not increase the concentration of 5-HT_{2A} receptor mRNA in brain with the exception of the dorsal raphe nucleus (Sumner and Fink, 1993). A similar lack of concordance between the density of receptors and their mRNA was found for D_2 receptors in striatum (Di Paolo, 1994; Lévesque, *et al.* 1992). There are several feasible explanations for this of which two are the subject of our further studies. First, the effect of estrogen on monoamine receptors in forebrain may involve post-translational events which affect receptor binding activity rather than an increase in actual receptor protein. The potent stimulatory effect of glucocorticoids on monoamine-synthesising enzymes is a precedent for this explanation (Weiner and Ganong, 1978). Secondly, the effect of estradiol on monoamine receptors in forebrain could involve classical genomic mechanisms, but because of relative mRNA instability (e.g. due to tissue-specific short poly-A mRNA tail) the message degrades too fast to allow detection of an increase in 5-HT_{2A} receptor mRNA in cortex. However, before embarking on investigation of these two possibilities, we (B Sumner and G Fink in progress) are determining first whether the higher dose of 30 μg of estradiol benzoate, used in the definitive studies on 5-HT_{2A} receptors (Sumner and Fink, 1995a, b; Fig. 1 and Table I), affects 5-HT_{2A} receptor mRNA in cerebral cortex and nucleus accumbens.

The mechanism by which estradiol-17ß could exert an antipsychotic effect by increasing the density of 5-HT_{2A} binding sites is also not clear in that, since the effective antipsychotic, clozapine, is a 5-HT_{2A} antagonist, one might predict that the antipsychotic action of E_2 would depend upon a decrease rather than an increase in 5-HT_{2A} binding sites in brain. However, the same line of reasoning must also apply to the conventional neuroleptics which are mainly D_2 receptor antagonists. Thus, estrogen appears to have a neuroleptic-like effect on D_2

receptors, but, as in the case of 5-HT_{2A} binding sites, D_2 binding sites are also increased significantly by estradiol (Cookson, 1985; Di Paolo, 1994). So these data point towards the fact that an increase in 5-HT_{2A} and D_2 binding sites results in increased efficacy of conventional neuroleptics as well as atypical antipsychotics, even though in the case of D_2 receptors estrogen appears to act as an antagonist. The human post-morten brain studies which showed that schizophrenia was associated with a decreased density of 5-HT_{2A} binding sites in prefrontal, but not occipital cortex (Laruelle, *et al.* 1993), further support this hypothesis. Now that this principle has been defined, experiments can be designed to determine the precise mechanism involved in the interaction between estradiol, 5-HT_{2A} receptors and atypical and possibly conventional antipsychotics.

Relevant to this is the precise nature and connections of the 5-HT_{2A} receptor expressing neurons in cerebral cortex and nucleus accumbens. Available information is sparse, but the morphological and electrophysiological characteristics of 5-HT_{2A} receptor expressing neurons in cerebral cortex suggest that a subpopulation may be GABAergic (Morilak, *et al.* 1993; Zifa and Fillion, 1992), and that in prefrontal cortex 5-HT_{2A} receptor activation counteracts 5-HT_1 receptor mediated inhibition of cell firing (Aghajanian, *et al.* 1987; Sheldon and Aghajanian, 1990). The 5-HT_{2A} receptors involved in the control of cortical pyramidal neurons appear to be located on the pyramidal neurons themselves as well as on a proportion of interneurons, thought to be GABAergic (23% of the NMDA excitable interneurons in the piriform cortex), and which evoke inhibitory post synaptic potentials in cortical pyramidal neurons (Sheldon and Aghajanian, 1990). Since the net effect of 5-HT_{2A} activation in cerebral cortex would appear to facilitate neuronal firing (Aghajanian, *et al.* 1987), our data (Sumner and Fink, 1995a, b) suggest that estradiol, by significantly increasing 5-HT_{2A} receptor density in cerebral cortex, would enhance significantly the propensity of pyramidal cells to fire in response to a 5-HT stimulus. This proposition can be tested electrophysiologically. Exposure to high levels of estradiol for 2-5 days results in increased spontaneous firing of dopamine-containing or "dopamine-dependent" neurons of the nigro-striatal system (Chiodo and Caggiula, 1983; Tansey, *et al.* 1983). Whether a similar mechanism also obtains for cortical cells that express 5-HT_{2A} receptor must now be determined. The action of estradiol on D_2 neurons in striatum also challenges conventional wisdom in that there are no estradiol receptors in striatum and so the action of estradiol might be mediated by membrane rather than "classical" cytoplasmic receptors (Di Paolo, 1994; Tansey, *et al.* 1983).

Any hypothesis which suggests that the occurrence of depression and schizophrenia might be prevented by the action of estrogen on 5-HT_{2A} and D_2 receptors in brain must also explain why the overall incidence of schizophrenia in men is similar to that in women and why the incidence of depression is less in men than in women. The most obvious cause is that the major genetic differences between the two sexes may result in major differences in central neurotransmitter mechanisms. In addition, however, the plasma concentrations of testosterone in men is nearly 1000 times greater than the highest plasma concentrations of estradiol in women (Wilson and Foster, 1992). Data from several species

including man show that androgens are converted to estrogens by the brain (Naftolin, *et al.* 1975). Animal studies provide compelling evidence for the fact that the action of testosterone on the brain is mediated to a large extent by its conversion to estradiol-17ß by way of the aromatase enzyme (e.g. see Fink, 1988). Thus, even with a relatively small (0.1 to 1.0%) androgen-to-estrogen conversion rate, neurons in male brain will be exposed and respond to concentrations of estrogen which are likely to be as high as those reached in the female brain.

Finally, since estrogen induces brain progesterone receptors (McEwen, *et al.* 1981), the possibility cannot be excluded that the action of estrogen could be mediated, at least in part, by progesterone. Progesterone and estrogen treatment affect 5-HT uptake and turnover in several areas of brain (Cone, *et al.* 1981; Hackmann, *et al.* 1973; Ladisich, 1977). This too is the subject of further study.

Conclusions

In summary we show for the first time that estrogen induces a significant increase in 5-HT_{2A} receptors in regions of the cerebral cortex and nucleus accumbens concerned with cognition, emotion and neuroendocrine and motor control. These findings, together with the known effects of estradiol on striatal D_2 receptors, provide a rational neurobiological basis for the profound effect of estrogen on mood, mental state and motor activity, and a robust model for behavioral and microphysiological studies aimed at understanding the precise mechanism of estrogen action on the brain.

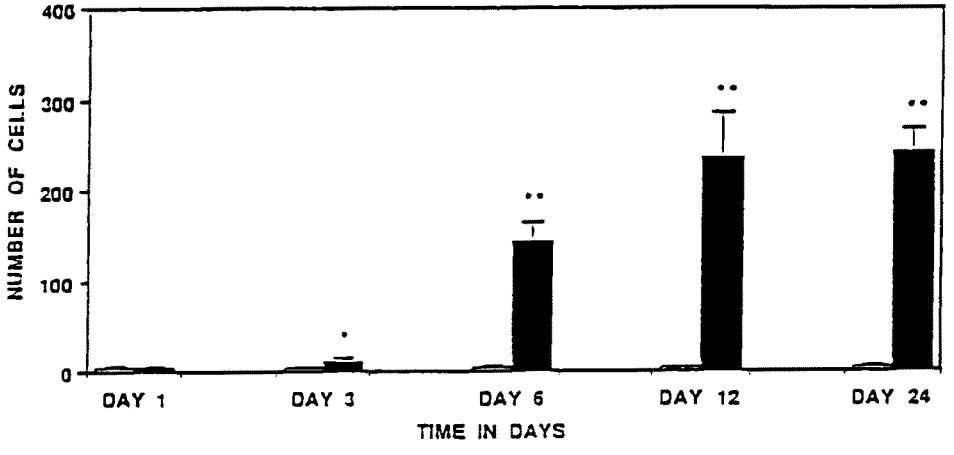

Fig. 2. The mean (±SEM) number of cells expressing AVP mRNA in the BNST of hypogonadal (*hpg*) mice at different times after implanting either empty (open bars) or testosterone-propionate filled (closed bars) silicone elastomer capsules. Note the exponential increase in the number of cells expressing AVP mRNA between days 3 and 6 of implanting the testosterone-propionate filled capsules. Significance of differences (Mann-Whitney *U* test): *$P < 0.05$; **$P < 0.01$. From Rosie *et al.* (1993) with permission.

STUDY II: STEROID EFFECTS ON AVP NEURONS OF THE BED NUCLEUS OF THE STRIA TERMINALIS

Effects of Gonadal Steroids on AVP Peptide Concentrations

De Vries *et al.* (1986) first demonstrated that there is a marked sex difference in the vasopressinergic innervation of the rat brain outside the classical hypothalamic-neurohypophysial system. These sex steroid-dependent differences are most pronounced in the lateral habenula and lateral septum in which there is a dense plexus of AVP containing fibres which project from BNST perikarya. The density of the AVP neurons is significantly greater in intact male compared with intact female brains. The BNST-AVP system plays a crucial role in 'social' or 'olfactory' memory (Bluthé, *et al.* 1993a, b; Dantzer and Bluthé, 1992; Dantzer, *et al.* 1988). We confirmed the findings of De Vries *et al.* (1986) in the mouse and showed that no immunoreactive AVP fibres are present in the lateral habenula of the LHRH deficient mutant hypogonadal (*hpg*) mouse (Mayes, *et al.* 1988). AVP-containing fibres in the lateral habenula can be induced in *hpg* mice by the insertion of a hypothalamic graft from normal mice into the third ventricle. The graft innervates the hypophysial portal vessels with LHRH-containing terminals which stimulate gonadotropin and thereby testosterone secretion which in turn stimulates the male pattern of AVP-containing fibres in the habenula (Mayes, *et al.*, 1988). The administration of testosterone or estrogen to male *hpg* mice also results in the normal development of AVP fibres in the lateral habenula. However, administration of 5α-dihydrotestosterone, a potent androgen which unlike testosterone cannot be converted by aromatase to estrogen, did not stimulate the appearance of AVP in the BNST-habenula/septum system, showing that the stimulatory action of testosterone depends upon its conversion to estrogen (Mayes, *et al.*, 1988). The substantially higher concentrations of AVP fibres in normal male compared with normal female brain probably reflects the fact that the amount of estrogen in the male brain is higher than in the female presumably because, as in man (see above), the amount of testosterone available for conversion to estrogen by aromatase in the normal male brain in rodents exceeds significantly the amount of estrogen in the normal female.

Effects of Gonadal Steroids on AVP mRNA Levels

Our recent studies were designed to determine whether the action of the gonadal sex steroids was due to stimulation of AVP biosynthesis as assessed by the concentration of AVP mRNA in the BNST. The concentration of AVP mRNA was determined by *in situ* hybridization using a ^{35}S-labelled 49 mer oligonucleotide probe complementary to the 5′ end of the glycopeptide coding domain of AVP mRNA (Rosie, *et al.* 1993). By selecting the coding domain for the glycopeptide we avoided confusion with oxytocin the transcript of which is similar to that of AVP, but does not contain the coding domain for the c-terminal glycopeptide. Exposure to supraphysiological concentrations of testosterone for

Transcription factor interactions with the AVP proximal Promoter

-365

GCTAGTCCTT GGTGAATGAG ACCTGGGGAC CCCTCTAGTC TGTTGAGAGC

TGCTGAAATG CTCAACTATG ATTTCCAGGT GACCCTCAAG TCGGCTCACC

TCCCTGATTG CACAGCACCA ATCACTGTGG CGGTGGCTCC CGTCACACGG

TGGCCAGTGA CAGCCTGATG GCTGGCTCCC CTCCTCCACC ACCCTCTGCA

TTGACAGGCC CACGTGTGTC CCCAGATGCC **TGAATCA**CTG CTGACAGCTT

GGGACCTGTC AGCTGTGGGC TCCTGGGGAG CCACTGGGGA GGGGGTTAGC

AGCCACGCTG TCGCCTCCTA GCCAACACCT GCAGACATAA ATAGACAGCC

CAGCCCGCTC AGGC -1

Fig. 3. Transcription factor interactions with the AVP proximal promoter. The bHLH factor binding sites, CANNTG, are boxed, the Sp1 binding sites are underlined and the AP1 site is shown in bold.

6–12 days induced an exponential increase of more than 100-fold in the number of cells that express AVP mRNA in the BNST in *hpg* mice (Fig. 2). Similar results were obtained in the adult rat (Miller, *et al.* 1989). The action of testosterone is "all-or-none" in that the level of AVP mRNA in the AVP-positive cells was similar at all times after testosterone implantation and also similar or less than that in the few AVP-positive cells in animals not treated with testosterone (Rosie, *et al.* 1993). The relatively long time taken for testosterone to exert its effect on AVP mRNA in BNST neurons is consistent with its effect on AVP immunoreactivity in the lateral habenula and lateral septum and on behavior, and suggests that the action of testosterone may be mediated by indirect or slow intracellular transduction mechanisms and/or increased stability of AVP mRNA.

Regulation of AVP Gene Transcription

While an effect on mRNA stability cannot be excluded, the more than 100-fold increase in AVP mRNA levels induced by testosterone makes it unlikely, and we have, therefore, begun an investigation on the effect of E_2 on AVP gene transcription. AVP gene expression is regulated by the interaction of transcription factors with discrete DNA motifs within the promoter region. In many genes the promoter region immediately upstream of the transcriptional start site imparts significant control over expression. We have initiated studies to correlate differences in AVP gene expression with transcription factor interactions within

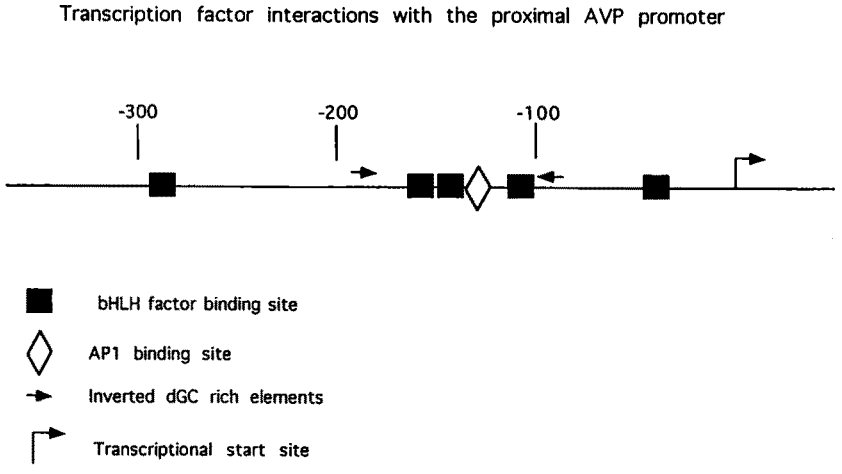

Fig. 4. Transcription factor interactions with the proximal AVP promoter. This schematic diagram shows the approximate location of potential cis acting elements within the AVP promoter.

the promoter. As a first step a detailed study was carried out on the potential transcription factor interactions within the proximal AVP promoter region spanning −365 to +1 (Mohr and Richter, 1990) using DNAase 1 footprint and electrophoretic mobility shift analysis (EMSA) (Grace *et al.* manuscript in preparation). These interactions are summarised in Figs 3 and 4. We have found a clustering of binding motifs in the proximal AVP promoter region and characterized the classes of transcription factor that interact with these motifs. Three classes of transcription factor have been demonstrated to bind to this region by EMSA: (i) AP1, the heterodimeric complex composed of proteins related to c-Jun and c-Fos, (ii) the Sp1 factor, and (iii) the basic helix-loop-helix (bHLH) protein family. The bHLH proteins are often involved in regulating tissue specific as well as stimulus inducible gene expression (Therrien and Drouin, 1993; Wright, 1992). It is striking that there is a clustering of five bHLH binding sites, also termed E box binding motifs, in this short region of the promoter. We have demonstrated by EMSA that each of these E box motifs has a different specificity and affinity for distinct bHLH complexes, raising the possibility that each distinct motif has a unique role to play in AVP gene expression. In particular the domain spanning −155 to −106 which contains three E box motifs flanking the AP1 motif is a potential complex regulatory region. In other promoters, the E box motif adjacent to AP1 elements has been demonstrated to act synergistically. Indeed, in the tyrosine hydroxylase gene promoter this arrangement of motifs acts as a tissue specific enhancer (Yoon and Chikaraishi, 1992). The definition of transcription factor interactions within the AVP promoter should allow the construction of testable models to correlate estradiol induced, tissue-specific AVP gene expression with specific regulatory domains in the promoter.

Conclusions

The general importance of these studies is that they demonstrate the value of the BNST-AVP system for studies on the way estrogen can exert effects on behavior by affecting neuropeptide gene expression in a tissue-specific manner. Whether the effects of estradiol on "social" or "olfactory" memory in rodents is also the case in man is the subject of further studies. These findings also emphasize the principle, which may prove to be generally applicable, that, in brain, the effects of testosterone would appear to be mediated by estrogen produced as a consequence of the aromatase converting enzyme. Whether this applies also to the effect of androgens in exacerbating the signs and symptoms of Tourette's Syndrome and genetically related disorders awaits to be determined.

CONCLUSION

Because of their potent actions on brain-pituitary functions, and because so much is already known about steroid receptors and their interactions with steroid response elements on genes in general, the study of steroid action on brain and pituitary offers a powerful method for establishing key principles about the molecular mechanisms that underlie central neurotransmission. This point is illustrated here by two separate studies.

The first demonstrates that estrogen, in its positive feedback mode for gonadotropin release, stimulates a significant increase in the density of 5-HT_{2A} binding sites in anterior frontal, cingulate and primary olfactory cortex and in the nucleus accumbens. These data provide the first experimental evidence for the fact that the psychoprotective effects of estrogen may be mediated by a 5-HT_{2A} receptor as well as a D_2 receptor mechanism. Our data explain the efficacy of estrogen therapy and the 5-HT uptake blocker, fluoxetine, for the treatment of the depressive symptoms of the premenstrual syndrome, and major depression in general. Our findings, together with the high affinity of atypical antipsychotics, like clozapine, for 5-HT_{2A} receptors, also suggest that schizophrenia may involve a serotonergic as well as dopaminergic component. Once the mechanism of action of estradiol on 5-HT_{2A} and D_2 receptors has been established, it may be possible to track back and determine the nature of the disorders that underlie the several functional psychoses.

Our second study illustrates the mechanism by which E_2, or testosterone by its conversion to E_2, can exert effects on memory and behavior by increasing significantly the expression of the neuropeptide AVP in the bed nucleus of the stria terminalis.

Both studies provide a firm basis for sophisticated molecular studies on how the same steroid, estrogen, modulates brain function by way of widely different molecular mechanisms. Whether these molecular models apply to man can now be tested with the aid of positron and single photon emission tomography (PET and SPET) coupled with modern human genetics. Our approach, therefore,

provides a fundamental bridge between neuroimaging on the one hand and human molecular genetics on the other.

ACKNOWLEDGMENTS

We thank Ann Fink for helpful discussions, John Bennie and Sheena Carroll for the LH radioimmunoassay, the National Hormone and Pituitary Program of NIDDK, Baltimore, Maryland, USA and the Scottish Antibody Production Unit, Carluke, Scotland, UK for radioimmunoassay materials and Lorraine Martin for the careful preparation of the manuscript. Some sections of this MS are similar to those of Sumner and Fink (1995a) and Rosie *et al.* (1993), and are reproduced with permission of the publishers of the *Journal of Steroid Biochemistry and Molecular Biology* and *Molecular and Cellular Neurosciences.*

REFERENCES

Aghajanian, G. K., Sprouse. J. S., and Rasmussen, K. (1987). Physiology of the midbrain serotonin system. In *Psychopharmacology: The Third Generation of Progress* (H. Y. Meltzer, Ed.), Raven Press, New York, pp. 141–149.

Angermeyer, M. C., Kühn, L., and Goldstein, J. M. (1990). Gender and the course of schizophrenia: Differences in treated outcomes. *Schizophr. Bull.* **16:**293–307.

Appel, N. M., Mitchell, W. M., Garlick, R. K., Glennon, R. A., Teitler, M., and De Souza, E. B. (1990). Autoradiographic characterization of (±)-1-(2.5-dimethoxy-4-[^{125}I]iodophenyl)-2-aminopropane ([^{125}I]DOI) binding to 5-HT_2 and 5-HT_{1C} receptors in rat brain. *J. Pharmacol. Exp. Ther.* **255:**843–857.

Ashcroft, G. W., Eccleston, D., Murray, L. G., Glen, A. I. M., Crawford, T. B. B., Pullar, I. A., Shields, P. J., Walter, D. S., Blackburn, I. M., Connechan, J., and Lonergan, M. (1972). Modified amine hypothesis for the aetiology of affective illness. *Lancet* **3:**573–577.

Bleuler, E. (1950). *Dementia Praecox or the Group of Schizophrenias*, International Universities Press, New York.

Blue, M. E., Yagaloff, K. A., Mamounas, L. A., Hartig, P. R., and Molliver, M. E. (1988). Correspondence between 5-HT_2 receptors and serotonergic axons in rat neocortex. *Brain Res.* **453:**315–328.

Bluthé, R.-M., Gheusi, G., and Dantzer, R. (1993a). Gonadal steroids influence the involvement of arginine vasopressin in social recognition in mice. *Psychoneuroendocrinology* **18:**323–335.

Bluthé, R. M., Suarez, S., Fink, G., Roques, B., and Dantzer, R. (1993b). Social recognition in mice is modulated by androgen-dependent vasopressinergic and cholecystokininergic neurotransmission. Proceedings of the Society for Neuroscience 23rd Annual Meeting, Washington, DC, p. 73.6.

Chiodo, L. A., and Caggiula, A. R. (1983). Substantia nigra dopamine neurons: Alterations in basal discharge rates and autoreceptor sensitivity induced by estrogen. *Neuropharmacology* **22:**593–599.

Cone, R. I., Davis, G. A., and Coy, R. W. (1981). Effects of ovarian steroids on serotonin metabolism within grossly dissected and microdissected brain regions of the ovariectomized rat. *Brain Res. Bull.* **7:**639–644.

Cookson, J. C. (1985). The neuroendocrinology of mania. *J. Affect. Disord.* **8:**233–241.

Dalton, K. (1959). Menstruation and acute psychiatric illnesses. *Br. Med. J.* **1:**148–149.

Dantzer, R., and Bluthé, R.-M. (1992). Vasopressin involvement in antipyresis, social communication and social recognition: A synthesis. *Crit. Rev. Neurobiol.* **6:**243–255.

Dantzer, R., Koob, G. F., Bluthé, R.-M., and Le Moal, M. (1988). Septal vasopressin modulates social memory in male rats. *Brain Res.* **457:**143–147.

Dean, C., and Kendell, R. E. (1981). The symptomatology of puerperal illnesses. *Br. J. Psychiatry* **139:**128–133.

De Vries, G. J., Duetz, W., Buijs, R. M., van Heerikhuize, J., and Vreeburg, J. T. M. (1986). Effects

of androgens and estrogens on the vasopressin and oxytocin innervation of the adult rat brain. *Brain Res.* **399:**296–302.

Di Paolo, T. (1994). Modulation of brain dopamine transmission by sex steroids. *Rev. Neurosci.* **5:**27–42.

Dow, R. C., Williams, B. C., Bennie, J., Carroll, S., and Fink, G. (1994). A central 5-HT_2 receptor mechanism plays a key role in the proestrous surge of luteinizing hormone but not prolactin in the rat. *Psychoneuroendocrinology* **19:**395–399.

Duinkerke, S. J., Botter, P. A., Jansen, A. A. I., van Dongen, P. A. M., van Haaften, A. J., Boom, A. J., van Laarhoven, J. H. M., and Busard, H. L. S. M. (1993). Ritanserin, a selective 5-HT(2/1C) antagonist, and negative symptoms in schizophrenia. A placebo-controlled double-blind trial. *Br. J. Psychiatry* **163:**451–455.

Edwards, J. G. (1994). Risperidone for schizophrenia. *Br. Med. J.* **308:**1311–1312.

Everett, J. W. (1988). Pituitary and hypothalamus: Perspectives and overviews. In *The Physiology of Reproduction* (E. Knobil and J. Neill, Eds.), Raven Press, New York, pp. 1143–1159.

Fink, G. (1979). Neuroendocrine control of gonadotrophin secretion. *Br. Med. Bull.* **35:**155–160.

Fink, G. (1988). The G. W. Harris Lecture. Steroid control of brain and pituitary function. *Q. J. Exp. Physiol.* **73:**257–293.

Fink, G. (1994). Molecular principles from neuroendocrine models: Steroid control of central neurotransmission. In *Progress in Brain Research—Neuroscience: From the Molecular to the Cognitive* (F. Bloom, Ed.), Elsevier, Amsterdam, pp. 139–147.

Fink, G. (1995). The self-priming effect of LHRH; A unique servomechanism and possible cellular model for memory. *Front. Neuroendocrinol.* **16:**1–8.

Friedhoff, A. J. (1986). Insights into the pathophysiology and pathogenesis of Gilles de la Tourette syndrome. *Rev. Neurol.* **142:**860–864.

Hackmann, E., Wirz-Justice, A., and Lichtsteiner, M. (1973). The uptake of dopamine and serotonin in rat brain during progesterone decline. *Psychopharmacologia* **32:**183–191.

Häfner, H., Riecher-Rössler, A., An Der Heiden, W., Maurer, K., Fätkenheuer, B., and Löffler, W. (1993). Generating and testing a causal explanation of the gender difference in age at first onset of schizophrenia. *Psychol. Med.* **23:**925–940.

Hoyer, D., Clarke, D. E., Fozard, J. R., Hartig, P. R., Martin, G. R., Mylecharane, E. J., Saxena, P. R., and Humphrey, P. P. A. (1994). VII. International Union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.* **46:**157–203.

Hunter, A. J., Hole, D. R., and Wilson, C. A. (1985). Studies into the dual effects of serotonergic pharmacological agents on female sexual behaviour in the rat: Preliminary evidence that endogenous 5-HT is stimulatory. *Pharmacol. Biochem. Behav.* **22:**5–13.

James, M. D., Lane, S. M., Hole, D. R., and Wilson, C. A. (1989). Hypothalamic sites of action of the dual effect of 5-HT on female sexual behaviour in the rat. In *Behavioral Pharmacology of 5-HT* (P. Bevan, A. R. Cools, and T. Archer, Eds.), Lawrence Erlbaum Associates, Hillsdale, NJ, pp. 73–77.

Janssen, P. A. J., Niemegeers, C. J. E., Awouters, F., Schellekens, K. H. L., Megens, A. A. H. P., and Meert, T. F. (1988). Pharmacology of risperidone (R 64 766), a new antipsychotic with serotonin-S2 and dopamine-D2 antagonistic properties. *J. Pharmacol. Exp. Ther.* **244:**685–693.

Kaplan, H. I., and Sadock, B. J. (1985). *Comprehensive Textbook of Psychiatry*, Williams and Wilkins, Baltimore, MD.

Klaiber, E. L., Broverman, D. M., Vogel, W., and Kobayashi, Y. (1979). Estrogen therapy for severe persistent depressions in women. *Arch. Gen. Psychiatry* **36:**550–554.

Kraepelin, E. (1971). *Dementia Praecox and Paraphrenia*, Krieger, Huntington, NY.

Ladisich, W. (1977). Influence of progesterone on serotonin metabolism: A possible causal factor for mood changes. *Psychoneuroendocrinology* **2:**257–266.

Laruelle, M., Abi-Dargham, A., Casanova, M. F., Toti, R., Weinberger, D. R., and Kleinman, J. E. (1993). Selective abnormalities of prefrontal serotonergic receptors in schizophrenia. *Arch. Gen. Psychiatry* **50:**810–818.

Leckman, J. F., and Scahill, L. (1990). Possible exacerbation of tics by androgenic steroids. *N. Engl. J. Med.* **322:**1674.

Lévesque, D., Gagné, B., Barden, N., and Di Paolo, T. (1992). Chronic estradiol treatment increases anterior pituitary but not striatall D_2 dopamine receptor mRNA levels in rats. *Neurosci. Lett.* **140:**5–8.

Levy, A. D., Baumann, M. H., and Van de Kar, L. D. (1994). Review of the influence of cocaine on the monoaminergic regulation of neuroendocrine function. *Front. Neuroendocrinol.* **15:**85–156.

Lewis, S. (1992). Sex and schizophrenia: Vive la différence. *Br. J. Psychiatry* **161:**445–450.

Leysen, J. E., Van Gompel, P., Verwimp, M., and Niemegeers, C. J. E. (1983). Role and localisation

of serotonin (S2)-receptor binding sites: Effects of neuronal lesions. In *CNS Receptors—From Molecular Pharmacology to Behaviour* (P. Mandel and F. V. de Feudis, Eds.), Raven Press, New York, pp. 373–383.

Leysen, J. E., Janssen, P. M. F., Gommeren, W., Wynants, J., Pauwels, P. J., and Janssen, P. A. J. (1992). *In vitro* and *in vivo* receptor binding and effects on monoamine turnover in rat brain regions of the novel antipsychotics risperidone and ocaperidone. *Mol. Pharmacol.* **41**:494–508.

Loranger, A. W. (1984). Sex difference in age at onset of schizophrenia. *Arch. Gen. Psychiatry* **41**:157–161.

MacKinnon, P. C. B., and MacKinnon, I. L. (1956). Hazards of the menstrual cycle. *Br. Med. J.* **1**:555.

Mayes, C. R., Watts, A. G., McQueen, J. K., Fink, G., and Charlton, H. M. (1988). Gonadal steroids influence neurophysin II distribution in the forebrain of normal and mutant mice. *Neuroscience* **25**:1013–1022.

McEwen, B. S., Biegon, A., Rainbow, T. C., Paden, C., Snyder, L., and DeGroff, V. (1981). The interaction of estrogens with intracellular receptors and with putative neurotransmitter receptors: Implications for the mechanisms of activation of regulation of sexual behavior and ovulation. In *Steroid Hormone Regulation of the Brain* (K. Fuxe, J. A. Gustafsson, and L. Wetterberg, Ed.), Pergamon, New York, pp. 15–29.

Meltzer, H. Y., Matsubara, S., and Lee, J.-C. (1989). Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin$_2$ pK_i values. *J. Phamacol. Exp. Ther.* **251**:238–246.

Mendelson, S. D., and Gorzalka, B. B. (1986). Serotonin type 2 antagonists inhibit lordosis behaviour in the female rat: Reversal with quipazine. *Life Sci.* **38**:33–39.

Mengod, G., Pompeiano, M., Martinez-Mir, M. I., and Palacios, J. M. (1990). Localization of the mRNA for the 5-HT_2 receptor by *in situ* hybridization histochemistry. Correlation with the distribution of receptor sites. *Brain Res.* **524**:139–143.

Miller, M. A., Urban, J. H., and Dorsa, D. M. (1989). Steroid dependency of vasopressin neurons in the bed nucleus of the stria terminalis by *in situ* hybridization. *Endocrinology* **125**:2335–2340.

Mohr, E., and Richter, D. (1990). Sequence analysis of the promoter region of the rat vasopressin gene. *FEBS Lett.* **260**:305–308.

Morilak, D. A., Garlow, S. J., and Ciaranello, R. D. (1993). Immunocytochemical localization and description of neurons expressing serotonin$_2$ receptors in the rat brain. *Neuroscience* **54**:701–717.

Naftolin, F., Ryan, K. J., Davies, I. J., Reddy, V. V., Flores, F., Petro, Z., Kuhn, M., White, R. J., Takoaka, Y., and Wolin, L. (1975). The formation of estrogens by central neuroendocrine tissues. *Recent Prog. Horm. Res.* **31**:295–315.

Nauta, W. J. H. (1963). Central nervous organization and the endocrine motor system. In *Advances in Neuroendocrinology* (A. V. Nalbandov, Ed.), University of Illinois Press, Urbana, pp. 5–12.

O'Brien, P. M. S. (1993). Helping women with premenstrual syndrome. *Br. Med. J.* **307**:1471–1475.

Palacios, J. M., Waeber, C., Hoyer, D., and Mengod, G. (1990). Distribution of serotonin receptors. *Ann. N.Y. Acad. Sci.* **600**:36–52.

Pauls, D. L., and Leckman, J. F. (1986). The inheritance of Gilles de la Tourette's syndrome and associated behaviors. *N. Engl. J. Med.* **315**:993–997.

Pazos, A., Cortés, R., and Palacios, J. M. (1985). Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. *Brain Res.* **346**:231–249.

Pfaff, D. W. (1980). *Estrogens and Brain Function,* Springer, New York.

Pompeiano, M., Palacios, J. M., and Mengod, G. (1994). Distribution of the serotonin 5-HT_2 receptor family mRNAs: Comparison between 5-HT_{2A} and 5-HT_{2C} receptors. *Mol. Brain Res.* **23**:163–178.

Pope, H. G. J., and Katz, D. L. (1988). Affective and psychotic symptoms associated with anabolic steroid use. *Am. J. Psychiatry* **145**:487–490.

Rapkin, A. J. (1992). The role of serotonin in premenstrual syndrome. *Clin. Obstet. Gynecol.* **35**:629–636.

Reid, R. L., and Yen, S. S. C. (1981). Premenstrual syndrome. *Am. J. Obstet. Gynecol.* **139**:85–104.

Riecher-Rössler, A., and Häfner, H. (1993). Schizophrenia and oestrogens—Is there an association? *Eur. Arch. Psychiatry Clin. Neurosci.* **242**:323–328.

Rosie, R., Wilson, H., and Fink, G. (1993). Testosterone induces an all-or-none, exponential increase in arginine vasopressin mRNA in the bed nucleus of stria terminalis of the *hypogonadal* mouse. *Mol. Cell. Neurosci.* **4**:121–126.

Sarkar, D. K., and Fink, G. (1981). Gonadotropin-releasing hormone surge: possible modulation through postsynaptic α-adrenoreceptors and two pharmacologically distinct dopamine receptors. *Endocrinology* **108**:862–867.

Seeman, M. V., and Lang, M. (1990). The role of estrogens in schizophrenia gender differences. *Schizophr. Bull.* **16**:185–195.

Seeman, P. (1992). Dopamine receptor sequences. Therapeutic levels of neuroleptics occupy D2 receptors, clozapine occupies D4. *Neuropsychopharmacology* **7**:261–284.

Sheldon, P. W., and Aghajanian, G. K. (1990). Serotonin (5-HT) induces IPSPs in pyramidal layer cells of rat piriform cortex: Evidence for the involvement of a 5-HT_2-activated interneuron. *Brain Res.* **506**:62–69.

Shimizu, H., and Bray, G. A. (1993). Effects of castration, estrogen replacement and estrus cycle on monoamine metabolism in the nucleus-accumbens, measured by microdialysis. *Brain Res.* **621**:200–206.

Strange, P. G. (1994). Dopamine D4 receptors: curioser and curioser. *Trends Phamacol. Sci.* **15**:317–319.

Studd, J., and Zamblera, D. (1994). Premenstrual depression. *Focus Depress.* **2**:6–9.

Sumner, B. E. H., and Fink, G. (1993). Effects of acute estradiol on 5-hydroxytryptamine and dopamine receptor subtype mRNA expression in female rat brain. *Mol. Cell. Neurosci.* **4**:83–92.

Sumner, B. E. H., and Fink, G. (1995a). Estrogen increases the density of 5-HT_{2A} receptors in cerebral cortex and nucleus accumbens in the female rat. *J. Steroid Biochem. Mol. Biol.* **54**:5–20.

Sumner, B. E. H., and Fink, G. (1995b). Oestradiol-17ß in its positive feedback mode significantly increases 5-HT_{2A} receptor density in the frontal, cingulate and piriform cortex of the female rat. *J. Physiol.* **483.P**:52P.

Sumner, B. E. H., Rosie, R., and Fink, G. (1992). Relative density of 5-hydroxytryptamine receptor subtype mRNAs in female rat neuroendocrine brain determined by *in situ* hybridization histochemistry. *Mol. Cell. Neurosci.* **3**:215–223.

Tansey, E. M., Arbuthnott, G. W., Fink, G., and Whale, D. (1983). Oestradiol-17ß increases the firing rate of antidromically identified neurones of the rat neostriatum. *Neuroendocrinology* **37**:106–110.

Therrien, M., and Drouin, J. (1993). Cell-specific helix-loop-helix factor required for pituitary expression of the pro-opiomelanocortin gene. *Mol. Cell. Biol.* **13**:2342–2353.

Weiner, R. I., and Ganong, W. F. (1978). Role of brain monoamines and histamine in regulation of anterior pituitary secretion. *Physiol. Rev.* **58**:905–976.

Wieck, A., Kumar, R., Hirst, A. D., Marks, M. N., Campbell, I. C., and Checkley, S. A. (1991). Increased sensitivity of dopamine receptors and recurrence of affective psychosis after childbirth. *Br. Med. J.* **303**:613–616.

Wilson, J. D., and Foster, D. W. (Eds.) (1992). *Williams Textbook of Endocrinology*, 8th ed., W B Saunders, Philadelphia, 1712.

Wood, S. H., Mortola, J. F., Chan, Y. F., Moossazadeh, F., and Yen, S. S. C. (1992). Treatment of premenstrual syndrome with fluoxetine: A double-blind, placebo-controlled, crossover study. *Obstet. Gynecol.* **80**:339–344.

Wright, W. E. (1992). Muscle basic helix-loop-helix proteins and the regulation of myogenesis. *Curr. Opin. Gen. Dev.* **2**:243–248.

Yoon, S. O., and Chikaraishi, D. M. (1992). Tissue-specific transcription of the rat tyrosine hydroxylase gene requires synergy between an AP-1 motif and an overlapping E box-containing dyad. *Neuron* **9**:55–67.

Zahm, D. S., and Brog, J. S. (1992). On the significance of subterritories in the "accumbens" part of the rat ventral striatum. *Neuroscience* **50**:751–767.

Zifa, E., and Fillion, G. (1992). 5-hydroxytryptamine receptors. *Pharmacol. Rev.* **44**:401–458.