Pergamon

0960-0760(95)00075-5

J. Steroid Biochem. Molec. Biol. Vol. 54, No. 1/2, pp. 15–20, 1995

0960-0760/95 $9.50 + 0.00

Estrogen Increases the Density of 5-Hydroxytryptamine$_{2A}$ Receptors in Cerebral Cortex and Nucleus Accumbens in the Female Rat

Barbara E. H. Sumner and George Fink*

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

Estrogen exerts a profound effect on mood and mental function in man. Based on our finding that estradiol selectively stimulates the expression of 5-hydroxytryptamine$_{2A}$ (5-HT$_{2A}$) receptor mRNA in the dorsal raphe nucleus of the female rat, we investigated the effects of estradiol on the density of 5-HT$_{2A}$ receptors in brain. The distribution and density of 5-HT$_{2A}$ receptors were determined by *in vitro* binding of [^{3}H]ketanserin in the presence of prazosin to exclude binding to α_1-adrenoreceptors. Brains were collected, processed and analysed in pairs from six estradiol- and six vehicle-treated animals. Our results show that a single pulse of estradiol induces a significant increase in the density of 5-HT$_{2A}$ receptors in female rat forebrain, particularly the anterior frontal, anterior cingulate and primary olfactory cortex and the nucleus accumbens. Since these brain regions play a pivotal role in cognition and emotion, as well as neuroendocrine and motor control, our findings provide the first experimental evidence for the fact that estrogen could alter mood and mental state by increasing the density of 5-HT$_{2A}$ receptors in cerebral cortex and nucleus accumbens.

J. Steroid Biochem. Molec. Biol., Vol. 54, No. 1/2, pp. 15–20, 1995

INTRODUCTION

The female sex steroid, estradiol-17β, exerts a profound effect on mood and mental state in the human. Thus, the sharp drop in plasma estradiol concentrations is thought to play a key role in precipitating the psychological symptoms of the premenstrual syndrome [1–3] and affective psychosis after childbirth [4]. The action of estradiol is likely to be mediated by monoamine, and in particular, 5-hydroxytryptamine (5-HT) mechanisms which play a major role in cognition, mood and behavior [5, 6]. Estrogen has also been implicated as a 'psychoprotectant' with respect to schizophrenia because the average age of onset of schizophrenia is significantly later in women than in men, and a second peak of onset of schizophrenia in women occurs after the menopause when there is a marked fall in plasma estradiol concentrations [7, 8]. Prompted by our finding that estradiol, in its positive feedback mode for luteinizing hormone (LH) release, stimulates a massive increase in the expression of 5-HT$_{2A}$ receptor mRNA in the dorsal raphe nucleus [9], we have investigated the possible effects of estradiol on the density of 5-HT$_{2A}$ receptors in brain. A preliminary report of this work has been presented [10].

*Correspondence to G. Fink.
Received 22 Dec. 1994; accepted 9 Feb. 1995

MATERIALS AND METHODS

Animal experimentation

Experiments were carried out on adult female COB Wistar rats, 200–250 g body weight, bred in the Department of Pharmacology and maintained under conditions of controlled lighting (lights on 0500–1900 h) and temperature (22°C), with free access to diet 41B and tap water. The experimental model was similar to that used before for studies on the positive feedback effect of estradiol-17β on the biosynthesis and release of luteinizing hormone releasing hormone (LHRH) [9]. Briefly, 12 rats were bilaterally ovariectomized under halothane anesthesia on the morning of diestrus between 0900 h and 1200 h, and immediately injected s.c. either with 30 μg estradiol benzoate (EB) (Paines & Byrne Limited, West Byfleet, Surrey,

U.K.) in 0.2 ml arachis oil or 0.2 ml arachis oil alone (six rats per group). At 1630–1730 h of the next day (presumptive proestrus), around the expected time of the spontaneous surge of LH, the animals were decapitated under sodium pentobarbitone anesthesia, and the brains rapidly removed and frozen at −45°C in isopentane, followed by storage at −70°C until microtomy. The EB treatment was effective since all the uteri in the EB-treated rats were markedly distended with fluid, and the plasma LH concentrations, determined in trunk blood by radioimmunoassay [11], were significantly elevated in the EB (3.7 ± 0.3 ng NIDDK rat LH/RP/2/ml) compared with the oil-treated rats (0.8 ± 0.1 ng/ml) ($P = 0.0001$, t-test, 2-tailed; $P < 0.01$, Mann–Whitney U-test, 2-tailed).

Determination of 5-HT$_{2A}$ receptor distribution in brain

The ligand–binding procedure was based on that of Pazos *et al.* [12] in which [^{3}H]ketanserin was used as a ligand. Ketanserin still remains the most selective ligand for 5-HT$_{2A}$ receptors [13, 14]. However, to prevent binding to α_1-adrenoreceptors, the density of binding sites was determined in the presence of the specific α_1-adrenoreceptor antagonist, prazosin.

The frozen brains were serially sectioned at 15 μm in a cryostat at −16 C. Coronal sections were thaw-mounted on to slides cleaned with chromic acid, and subbed with gelatine and poly-L-lysine, and stored at −70°C. Slides from each pair of animals (one experimental and one control) were co-processed. The slides were brought to room temperature, lightly fixed in freshly prepared 1% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4, Sigma) for 5 min, and then washed twice in PBS (5 min each).

Pre-incubation in 0.17 M Tris–HCl buffer, pH 7.7, for 15 min preceded incubation for 2 h at room temperature in the same buffer containing 2 nM [^{3}H]ketanserin hydrochloride (NEN, specific activity: 2223 GBq/mmol) and 10^{-4} M unlabelled prazosin (Research Biochemicals Incorporated) to prevent binding of [^{3}H]ketanserin to α_1-adrenoreceptors. For incubation the slides were placed horizontally in moist chambers and 50 μl of buffered ligand applied to each section. Control sections (blanks) were incubated in buffer containing 2 nM [^{3}H]ketanserin displaced by 10^{-4} M unlabelled ketanserin (gift to Dr B C Williams from Janssen Pharmaceuticals Ltd). After incubation, the sections were washed twice in ice-cold buffer without ligands for 10 min each, and then quickly dipped in ice-cold distilled water to remove salts. After several hours of vacuum desiccation, and overnight dry storage at room temperature, the slides were apposed to Hyperfilm-^{3}H (Amersham International plc, Amersham Place, Little Chalfont, Bucks, U.K.), and trapped between glass plates, wrapped and sealed in light-tight wrappings, and exposed at 0–4 C for 5 weeks. Each sheet of film bore carefully neuroanatomically matched sections from a pair of rats, with slides from experimental and control animals alternating. A tritium standard (^{3}H-Microsales, Amersham) was co-exposed on each sheet of film. After 5 weeks the slides were removed from the film and stained for 1 min in 1% aqueous toluidine blue, washed, dried, dehydrated in acetone, cleared in xylene, and mounted in DPX. The films were developed for 4 min in Ilford Phenisol (20% in distilled water), dipped in distilled water, and fixed for 10 min in Ilford Hypam (also 20%). After a 30 min wash in running water, the films were air-dried.

Neuroanatomical regions were identified on the autoradiographic images by matching them with the stained sections under a low power magnifier. The regions were outlined with a mounted needle to facilitate location under the microscope. Silver-grain densities over each neuroanatomical region, and over the tritium standards, were measured on the films with an Optomax image-analysing system (Synoptics Ltd, 271 Cambridge Science Park, Milton Road, Cambridge, U.K.) mounted on a Nikon microscope fitted with a X40 objective. A graph was drawn of grain density against radioactivity for the tritium standards. The standards as supplied by Amersham International plc were already calibrated in tissue equivalents (Bq/mg brain grey matter). The standard curve was used to convert grain densities from the experimental sections into radioactivity values per mg tissue, once the grain densities from blank sections (incubated with unlabelled ketanserin) had been subtracted. Finally, by referring to the specific activity of the ^{3}H-ligand, the radioactivity values were converted to binding in fmol/mg tissue. Each binding value was derived from measurements over ten sections for each neuroanatomical region in each animal. The final mean is from all six animals in each treatment group (Table 1). As the animals were paired for the binding studies, the data were analyzed by paired statistical tests (paired t-test, and Wilcoxon signed rank test).

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 [12, 13, 15, 16]. As also shown previously, the density of 5-HT$_{2A}$ binding sites in frontal and cingulate cortex was greatest in laminae I, Va and IV [13, 16, 17] (Fig. 1). The density of the 5-HT$_{2A}$ binding sites (Fig. 1; Table 1) corresponds closely with the concentrations of 5-HT$_{2A}$ receptor mRNA in brain [9, 18–20].

Estradiol treatment significantly increased the density of 5-HT$_{2A}$ binding sites in the anterior frontal, anterior cingulate and piriform cortex, the olfactory

Table 1. 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

Brain region	Oil-treated	EB-treated	Percentage change	Statistical significance: Paired *t*-test	Statistical significance: 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 nucleus (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

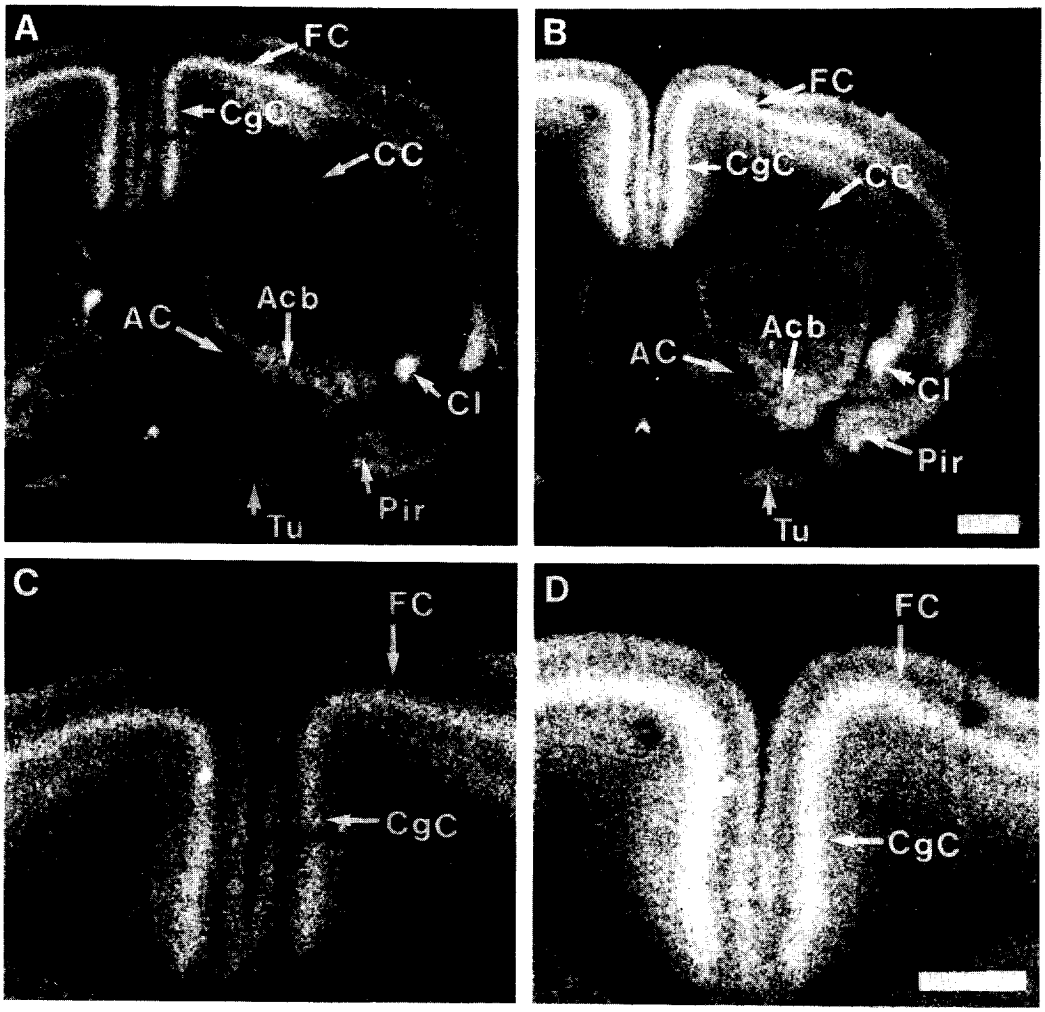

Fig. 1. Dark-field film autoradiographs showing the distribution of 5-HT_{2A} receptors in anterior forebrain (A, B) and cerebral cortex (C, D). The density of 5-HT_{2A} receptors, determined by the binding of [^{3}H]ketanserin in the presence of prazosin, in frontal (FC), cingulate (CgC) and piriform (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.

tubercle, the nucleus accumbens and the lateral dorsal raphe nucleus (DRN) (Table 1; Fig. 1). Preliminary studies had shown that a dosage of 10 μg EB (used in ref. [9]) 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 present, definitive, study, using the 30 μg dose of EB, 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. This could explain how estradiol exerts potent effects on mood and mental state. It is relevant here that 5-HT_{2A} receptors have been implicated in suicide and depression [6] and that 5-HT uptake blockers are effective in treating the psychological disturbances of the premenstrual syndrome [1–3]. The fact that the average age of onset of schizophrenia in women is much later than in men, and that postpartum psychosis is associated with a precipitous drop in plasma estradiol concentrations after birth, underpin the hypothesis that estrogen protects against psychosis in women [4, 8, 21, 22]. The mechanism of this apparent antipsychotic affect 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 [4, 8]. However, recent pharmacological data suggest that as well as playing a pivotal role in affective psychosis, a 5-HT_2 mechanism may also be involved in schizophrenia. Thus, for example, (i) potent 'atypical' antipsychotics such as clozapine and risperidone bind with much greater affinity to 5-HT_2 than to dopamine receptors [23–26], (ii) ritanserin, a 5-HT_{2A} antagonist, is an effective adjunctive treatment for the negative symptoms of schizophrenia [27], and (iii) the therapeutic effect of risperidone in schizophrenia could be due, at least in part, to its 5-HT_2 antagonism [23, 25, 26]. Furthermore, many studies on dopamine receptors have used [^{3}H]spiperone as a ligand [8]; but, spiperone also has a high affinity for 5-HT_{2A} receptors [13].

Nevertheless, it is important to stress that there is no conflict between the dopamine hypothesis of schizophrenia and other major psychoses [4, 8] and our hypothesis that estradiol could also affect mood and mental state by way of 5-HT_{2A} receptors. The two receptor subtypes could both be involved 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_2 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 [23–26], or adjunctive treatment with the 5-HT_{2A} antagonist, ritanserin [27].

The mechanism by which estradiol could exert an antipsychotic effect by increasing the density of 5-HT_{2A} binding sites is not clear in that, since clozapine is a 5-HT_{2A} antagonist, one might predict that the antipsychotic action of estradiol would depend upon a decrease rather than an increase in 5-HT_{2A} binding sites in brain. However, the same line of reasoning also applies to the conventional neuroleptics which are mainly dopamine$_2$ (D_2) receptor antagonists, and, as in the case of 5-HT_{2A} binding sites, D_2 binding sites are also increased significantly by estradiol [8]. So these data point towards the fact that an estrogen-induced increase in 5-HT_{2A} and D_2 binding sites results in increased efficacy of conventional neuroleptics as well as atypical antipsychotics. Now that this principle has been defined, experiments can be designed to determine the precise mechanism involved in the interaction between estrogen and conventional and atypical antipsychotics.

The nucleus accumbens receives major inputs from the amygdala, anterior cingulate cortex and the piriform cortex, and projects, by relay in striatum and thalamus, to the primary motor cortex [28]. The role of 5-HT in the nucleus accumbens is not known, but, as assessed by the ratio of 5-hydroxy-3-indoleacetic acid to 5-HT, estradiol significantly reduces 5-HT metabolism in this nucleus [29]. The 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 1) may also be related to the control of pituitary hormone secretion and mating and motor behavior [11, 30–34]. The 5-HT_{2A} receptor subtype also plays a key role in food intake and sleep mechanisms [6, 30], and also mediates the actions of hallucinogens such as LSD and DOI [6] and some of the actions of cocaine [30].

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 [6, 13, 35], but acute treatment with 10 μg EB using the same temporal protocol as in the present paper did not increase the concentration of 5-HT_{2A} receptor mRNA in brain with the exception of the dorsal raphe nucleus [9]. A similar lack of concordance between the density of receptors and their mRNA was found for D_2 receptors in striatum [8, 36]. There are several possible 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 determine receptor activity (i.e. binding) rather than an increase in actual receptor protein. The potent effect of glucocorticoids on monoamine-synthesizing enzymes is

a precedent for this type of mechanism [37]. 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 a 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.

The clinical and neurobiological significance of our findings depends upon 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 [6, 38], and that in prefrontal cortex 5-HT_{2A} receptor activation counteracts 5-HT_1 receptor mediated inhibition of cell firing [39, 40]. 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 [40]. Since the net effect of 5-HT_{2A} activation in cerebral cortex would appear to facilitate neuronal firing [39], our data 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.

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 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.

Acknowledgements—We thank John Bennie and Sheena Carroll for the LH radioimmunoassay, the National Hormone and Pituitary Program of NIDDK, Baltimore, MD, U.S.A. and the Scottish Antibody Production Unit, Carluke, Scotland, U.K. for radioimmunoassay materials and Lorraine Martin for the careful preparation of the manuscript.

REFERENCES

1. Rapkin A. J.: The role of serotonin in premenstrual syndrome. *Clin. Obstet. Gynec.* **35** (1992) 629–636.
2. Wood S. H., Mortola J. F., Chan Y. F., Moossazadeh F. and Yen S. S. C.: Treatment of premenstrual syndrome with fluoxetine: a double-blind, placebo-controlled, crossover study. *Obstet. Gynec.* **80** (1992) 339–344.
3. O'Brien P. M. S.: Helping women with premenstrual syndrome. *Br. Med. J.* **307** (1993) 1471–1475.
4. Wieck A., Kumar R., Hirst A. D., Marks M. N., Campbell I. C. and Checkley S. A.: Increased sensitivity of dopamine receptors and recurrence of affective psychosis after childbirth. *Br. Med. J.* **303** (1991) 613–616.
5. Meltzer H. Y. and Lowry M. T.: The serotonin hypothesis of depression. In *Psychopharmacology: The Third Generation of Progress* (Edited by H. Y. Meltzer). Raven Press, NY (1987) pp. 513–526.
6. Zifa E. and Fillion G.: 5-hydroxytryptamine receptors. *Pharmac. Rev.* **44** (1992) 401–458.
7. Loranger A. W.: Sex differences in age at onset of schizophrenia. *Arch. Gen. Psychiat.* **41** (1984) 157–161.
8. Di Paolo T.: Modulation of brain dopamine transmission by sex steroids. *Rev. Neurosci.* **5** (1994) 27–42.
9. Sumner B. E. H. and Fink G.: Effects of acute estradiol on 5-hydroxytryptamine and dopamine receptor subtype mRNA expression in female rat brain. *Molec. Cell. Neurosci.* **4** (1993) 83–92.
10. Sumner B. E. H. and Fink G.: Oestradiol-17β 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** (1995) 52P.
11. Dow R. C., Williams B. C., Bennie J., Carroll S. and Fink G.: A central 5-HT_2 receptor mechanism plays a key role in the proestrous surge of luteinizing hormone in the rat. *Psychoneuroendocrinology* **19** (1994) 395–399.
12. Pazos A., Cortés R. and Palacios J. M.: Quantitative autoradiographic mapping of serotonin receptors in the rat brain II. Serotonin-2 receptors. *Brain Res.* **346** (1985) 231–249.
13. 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.: VII. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmac. Rev.* **46** (1994) 157–203.
14. Leysen J. E., Niemegeers C. J. E., Van Nueten J. M. and Laduron P. M.: [^{3}H]Ketanserin (R41 468), a selective [^{3}H]-ligand for serotonin 2 receptor binding sites. *Molec. Pharmac.* **21** (1982) 301–314.
15. Palacios J. M., Waeber C., Hoyer D. and Mengod G.: Distribution of serotonin receptors. *Ann. N.Y. Acad. Sci.* **600** (1990) 36–52.
16. Appel N. M., Mitchell W. M., Garlick R. K., Glennon R. A., Teitler M. and De Souza E. B.: 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. Pharmac. Exp. Ther.* **255** (1990) 843–857.
17. Blue M. E., Yagaloff K. A., Mamounas L. A., Hartig P. R. and Molliver M. E.: Correspondence between 5-HT_2 receptors and serotonergic axons in rat neocortex. *Brain Res.* **453** (1988) 315–328.
18. Sumner B. E. H., Rosie R. and Fink G.: Relative density of 5-hydroxytryptamine receptor subtype mRNAs in female rat neuroendocrine brain determined by *in situ* hybridization histochemistry. *Molec. Cell. Neurosci.* **3** (1992) 215–223.
19. Pompeiano M., Palacios J. M. and Mengod G.: Distribution of the serotonin 5-HT_2 receptor family mRNAs: comparison between 5-HT_{2A} and 5-HT_{2C} receptors. *Molec. Brain Res.* **23** (1994) 163–178.
20. Mengod G., Pompeiano M., Martinez-Mir M. I. and Palacios J. M.: 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** (1990) 139–143.
21. Lewis S.: Sex and schizophrenia: vive la différence. *Br. J. Psychiat.* **161** (1992) 445–450.
22. Seeman M. V. and Lang M.: The role of estrogens in schizophrenia gender differences. *Schizophr. Bull.* **16** (1990) 185–195.
23. Edwards J. G.: Risperidone for schizophrenia. *Br. Med. J.* **308** (1994) 1311–1312.
24. Meltzer H. Y., Matsubara S. and Lee J-C.: Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin$_2$ pK_i values. *J. Pharmac. Exp. Ther.* **251** (1989) 238–246.
25. Janssen P. A. J., Niemegeers C. J. E., Awouters F., Schellekens K. H. L., Megens A. A. H. P. and Meert T. F.: Pharmacology of risperidone (R 64 766), a new antipsychotic with serotonin-S2 and dopamine-D2 antagonistic properties. *J. Pharmac. Exp. Ther.* **244** (1988) 685–693.
26. Leysen J. E., Janssen P. M. F., Gommeren W., Wynants J., Pauwels P. J. and Janssen P. A. J.: *In vitro* and *in vivo* receptor binding and effects on monoamine turnover in rat brain regions of the novel antipsychotics risperidone and ocaperidone. *Molec. Pharmac.* **41** (1992) 494–508.

27. 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.: Ritanserin, a selective 5-HT (2/1C) antagonist, and negative symptoms in schizophrenia. A placebo-controlled double-blind trial. *Br. J. Psychiat.* **163** (1993) 451–455.
28. Zahm D. S. and Brog J. S.: On the significance of subterritories in the "accumbens" part of the rat ventral striatum. *Neuroscience* **50** (1992) 751–767.
29. Shimizu H. and Bray G. A.: Effects of castration, estrogen replacement and estrus cycle on monoamine metabolism in the nucleus-accumbens, measured by microdialysis. *Brain Res.* **621** (1993) 200–206.
30. Levy A. D., Baumann, M. H. and Van de Kar L. D.: Review of the influence of cocaine on the monoaminergic regulation of neuroendocrine function. *Front. Neuroendocr.* 15 (1994) 85–156.
31. Nauta W. J. H.: Central nervous organization and the endocrine motor system. In *Advances in Neuroendocrinology* (Edited by A. V. Nalbandov). University of Illinois Press, Urbana (1963) pp. 5–12.
32. Mendelson S. D. and Gorzalka B. B.: Serotonin type 2 antagonists inhibit lordosis behaviour in the female rat: reversal with quipazine. *Life Sci.* **38** (1986) 33–39.
33. Hunter A. J., Hole D. R. and Wilson C. A.: Studies into the dual effects of serotonergic pharmacological agents on female sexual behavior in the rat: preliminary evidence that endogenous 5-HT is stimulatory. *Pharmac. Biochem. Behav.* **22** (1985) 5–13.
34. James M. D., Lane S. M., Hole D. R. and Wilson C. A.: Hypothalamic sites of action of the dual effect of 5-HT on female sexual behavior in the rat. In *Behavioral Pharmacology of 5-HT* (Edited by P. Bevan, A. R. Cools and T. Archer). Lawrence Erlbaum Associates, Hillsdale, NJ (1989) pp. 73–77.
35. Leysen J. E., Van Gompel P., Verwimp M. and Niemegeers C. J. E.: Role and localisation of serotonin (S_2)-receptor binding sites: effects of neuronal lesions. In *CNS Receptors—From Molecular Pharmacology to Behaviour* (Edited by P. Mandel and F. V. de Feudis). Raven Press, NY (1983) pp. 373–383.
36. Lévesque D., Gagné B., Barden N. and Di Paolo T.: Chronic estradiol treatment increases anterior pituitary but not striatal D_2 dopamine receptor mRNA levels in rats. *Neurosci. Lett.* **140** (1992) 5–8.
37. Weiner R. I. and Ganong W. F.: Role of brain monoamines and histamine in regulation of anterior pituitary secretion. *Physiol. Rev.* **58** (1978) 905–976.
38. Morilak D. A., Garlow S. J. and Ciaranello R. D.: Immunocytochemical localization and description of neurons expressing serotonin$_2$ receptors in the rat brain. *Neuroscience* **54** (1993) 701–717.
39. Aghajanian G. K., Sprouse J. S. and Rasmussen K.: Physiology of the midbrain serotonin system. In *Psychopharmacology: The Third Generation of Progress* (Edited by H. Y. Meltzer). Raven Press, NY (1987) pp. 141–149.
40. Sheldon P. W. and Aghajanian G. K.: 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** (1990) 62–69.