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# Time course of opioid and cannabinoid gene transcription alterations induced by repeated administration with fluoxetine in the rat brain

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**Abstract**

This study examined the time course effects (8, 16 and 31 days) of fluoxetine administration (1 mg/kg, p.o./day) on serotonin transporter (5-HTT), opioid, tyrosine hydroxylase (TH) and cannabinoid $CB_1$ receptor gene expressions in selected regions of the rat brain. Treatment with fluoxetine progressively decreased (35–55%) 5-HTT gene expression in dorsal raphe nucleus at 8, 16 and 31 days. The results revealed that fluoxetine administration decreased (30%) proenkephalin gene expression in nucleus accumbens shell (AcbS) and caudate-putamen (CPu) (31 days) but was without effect in nucleus accumbens core AcbC. A pronounced and time related decrease (25–65%) in prodynorphin gene expression was detected in AcbC, AcbS, CPu, hypothalamic supraoptic and paraventricular nuclei at all time points as well as in proopiomelanocortin gene expression (20–30%) in the arcuate nucleus (ARC) of the hypothalamus. On days 16 and 31, tyrosine hydroxylase gene expression in ventral tegmental area and substantia nigra and cannabinoid $CB_1$ receptor gene expression in the CPu decreased (approximately 45–50% from vehicle). In conclusion, fluoxetine by inhibiting the reuptake of serotonin produced pronounced and time related alterations in genes involved in the regulation of emotional behaviour, suggesting that these neuroplastic changes may be involved, at least in part, in the clinical efficacy of this drug in neuropsychiatric disorders.


*Keywords:* Fluoxetine; Proenkephalin; Tyrosine hydroxylase; Proopiomelanocortin; Opioid; Cannabinoid $CB_1$ receptor; mRNA

## 1. Introduction

Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) extensively used in psychiatry for the treatment of a variety of disorders including depression, anxiety, obsessive compulsive disorder, fibromyalgia, drug dependence, anorexia and bulimia. In many of these disorders, the clinical improvement occurs after several weeks of repeated treatment with the SSRI. This delay in the therapeutic action suggests that the increase of 5-HT concentrations in the brain is not the only neurochemical event responsible for its effects but probably represents the mechanism that initiates or triggers several molecular alterations in key neuropeptides and neurotransmitter genes more closely involved with the clinical efficacy of fluoxetine.

In addition to the advantages of using fluoxetine in psychiatry to regulate emotional behaviour, a large

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body of evidence suggests that this type of drug enhances the actions of opiates in the treatment of pain. Indeed, fluoxetine administration potentiates the antinociceptive action of μ-opiate drugs (Gatch et al., 1998; Nayebi et al., 2001; Singh et al., 2001; Oliva et al., 2002) probably through a mechanism involving central opioid pathways (Singh et al., 2001). Both opioid and cannabinoid endogenous systems are involved in the regulation of pain and emotional behaviours (Manzanares et al., 1999, 2004) and it is possible that the actions of repeated administration of fluoxetine may alter the endogenous activity of opioid peptides and/or cannabinoid $CB_1$ receptors. However, little is known about the role of serotonin reuptake inhibitors and cannabinoid receptor function.

Fluoxetine, as well as other SSRIs are often used to treat depression in movement disorders including Parkinson's disease. Treatment with this drug alleviates symptoms of depression in these patients although it has been reported to increase or exacerbate motor disability (Steur, 1993; Gerber and Lynd, 1998). This alteration in motor behaviour may be related to a reduced dopaminergic activity in nigrostriatal neurons, although this notion needs further investigation.

This SSRI has also been used extensively in the treatment of drug dependence (alcohol). Drugs, by acting directly on dopaminergic terminals or by uninhibiting inhibitory neurons on cell bodies of mesolimbic dopaminergic neurons, stimulate the release of dopamine in the nucleus accumbens (Nestler, 2001; Tanda et al., 1997; Spanagel and Weiss, 1999). Since development of dependence is based on stimulation of dopamine in terminal of nucleus accumbens, the long term beneficial effect of fluoxetine may not only be related to its antidepressant activity but also to its potential ability to alter dopaminergic ventral tegmental neuronal activity.

Considering the multiple therapeutic uses of fluoxetine and the lack of studies on the mechanisms underlying the clinical efficacy of this drug, the purpose of this study was to examine the time course effects of repeated administration of fluoxetine (1 mg/kg p.o.) on serotonin transporter, opioid (proenkephalin, prodynorphin and proopiomelanocortin), tyrosine hydroxylase and cannabinoid $CB_1$ receptor gene expressions in various regions of the rat brain.

## 2. Materials and methods

### 2.1. Animals

Experiments were performed on Wistar adult male rats (85–135 days of age) which were obtained from Harlan Interfauna Ibérica S.A. (Barcelona, Spain). The animals were maintained at a constant temperature of $22 \pm 2$ °C and in a 12-h dark/light cycle (lights on at 0800 h), with free access to food (commercial diet for rodents A04; Panlab, Barcelona, Spain) and water. Animals were housed in standard laboratory cages, each one containing groups of three to four individuals.

All the experiments performed in this study are in compliance with the Royal Decree 223/1988 of 14 March (BOE 18) and the Ministerial Order of 13 October 1989 (BOE 18) about protection of experimental animals, as well as with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

### 2.2. Drugs and treatment

Animals were administered fluoxetine hydrochloride (1 mg/kg p.o.) once a day during 7, 15 and 30 days. Rats were killed on days 8, 16 and 31 (24 h after the last administration). Vehicle-treated rats were killed on day 16.

### 2.3. In situ hybridisation histochemistry (ISHH)

Brain sections were cut at 14 μm at different levels containing the regions of interest: nucleus accumbens (Acb), caudate-putamen (CPu), supraoptic nucleus (SON), paraventricular hypothalamic nucleus (PVN), arcuate nucleus (ARC), hypothalamic ventromedial nucleus (VMN), ventral tegmental area (VTA), substantia nigra pars compacta (SNc) and dorsal raphe nucleus (DR). All these sections were obtained according to Paxinos and Franklin (2001), mounted onto gelatin-coated slides and stored at −80 °C until the day of the assay.

ISHH was performed as described previously by Young et al. (1986) with some modifications, using synthetic oligonucleotide probes complementary to serotonin transporter (5-HTT) gene (bases 77–126 (Fujita et al., 1993), Afigen, Madrid, Spain), to prodynorphin (PDYN) gene (bases 913–960 (Perez-Rosado et al., 2002), Genotek, Barcelona, Spain), to proenkephalin (PENK) gene (bases 388–435, Genotek, Barcelona, Spain), to proopiomelanocortin (POMC) gene (bases 96–134; Advanced Biotechnology Center, Charing Cross and Westminster Medical School, London, England), to tyrosine hydoxylase (TH) gene (bases 1223–1252, Genotek, Barcelona, Spain) and a mixture (1:1:1) of three oligonucleotides complementary to cannabinoid $CB_1$ receptor ($rCB_1$) gene (bases 4–51, 349–396 and 952–999; Genotek, Barcelona, Spain). Oligonucleotide probes were labelled using terminal deoxytransferase (Boehringer, Madrid, Spain) to add a $^{35}$S-labeled deoxyATP (1000 Ci mmol$^{-1}$; Amersham, Madrid, Spain) tail to the 3′ end of the probes. Labelled probes were purified by mini quick spin oligo columns (Roche, Barcelona, Spain). The probe (in 50 μl of hybridisation buffer) was applied to each section and left overnight at 37 °C for hybridisation. Following hybridisation, sections were washed four times