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Auto- and heteromodulation of the rat brain 5-HT system: Involvement in the mechanism of action of antidepressant drugs.

By

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A la mémoire de mon père, à ma mère
A Nadine et petit Ange
A mes frères et soeurs
Et tous mes collègues

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Preface

The studies and the reviews of the present thesis concern the auto- and heteromodulation of the serotonergic and noradrenergic systems, their interactions, and their implications in the mechanism of action of antidepressant treatments.

Option provided in the "Guidelines Concerning Thesis Preparation" required by the Faculty:

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- In the case of manuscripts coauthored by the candidate and others, the candidate is required to make an explicit statement in the thesis of who contributed to such work and to what extent; supervisors must attest to the accuracy of such claims at the Ph.D. Oral defense. Since the task of the examiners is made more difficult in these cases, it is the candidate's interest to make perfectly clear the responsibilities of the different authors of coauthored papers."

The experimental parts of the thesis (chapters II to VII) consist of four original articles that have been already published and two submitted for publication and the overall summary and discussion of the different studies of this thesis are made in chapter VIII.

Abstract

In the last few years, a growing body of evidence revealed the involvement of the serotonin (5-HT) system in the therapeutic effect of antidepressant treatments. Hence, several investigations have suggested that long-term treatments with various classes of antidepressant drugs lead to an enhanced 5-HT neurotransmission. By using electrophysiological paradigms in the rat, the present research endeavour was undertaken to 1) investigate the role of different factors modulating central 5-HT neurotransmission and to assess interactions between the 5-HT and noradrenergic (NA) systems; 2) to further confirm the presence of an enhanced 5-HT neurotransmission following chronic antidepressant treatments.

The study of the automodulation of 5-HT system via $5\text{-HT}_{3/4}$ receptor subtypes suggested that the suppressant effect, observed *in vivo*, of the prototypical 5-HT_3 receptor agonist 2-methyl-5-HT on the firing activity of somatosensory and hippocampus pyramidal neurons was not mediated via 5-HT_3 receptors but rather via 5-HT_{1A} receptors. In contrast, the enhancing effect, observed *in vitro*, of 2-methyl-5-HT on the electrically-evoked release of [^{3}H]-5-HT was mediated via 5-HT_3 receptors. The reducing action of the $5\text{-HT}_{3/4}$ receptor ligand R-zacopride on the endogenous release of 5-HT induced by the electrical stimulation of the ascending 5-HT pathway was mediated via 5-HT_4 receptors. The studies of the heteromodulation of 5-HT system induced by the α_2-adrenoceptor antagonist mirtazapine showed that acute administration of mirtazapine produced a transient increase of the firing activity of dorsal raphe 5-HT and locus coeruleus NA neurons whereas long-term mirtazapine treatment produced a sustained increase of the firing activity of these two types of neurons. Both acute and chronic administration of mirtazapine antagonized the activation of α_2-adrenergic auto- and heteroreceptors in the dorsal hippocampus. The sustained enhancement of the firing activity of 5-HT and NA neurons and the desensitization of α_2-adrenergic heteroreceptors on 5-HT terminals produced by long-term mirtazapine administration have been proposed to underlie the antidepressant activity of this drug. The study of the modulation of the 5-HT system via monoamine oxidase-A (MAO-A) inhibition revealed that short-term administration of the selective and reversible MAO-A inhibitor befloxatone reduced the firing activity of 5-HT neurons which is followed by a recovery after its long-term administration. Given that the firing activity of 5-HT neurons was even above the control value following short-term treatment with both befloxatone and the 5-HT_{1A} receptor antagonist (-)pindolol, it is suggested that this combined treatment could be expected to produce the same enhancing effect on 5-HT neurotransmission as after long-term treatment with befloxatone alone. The study of the interaction between the 5-HT and NA systems revealed that the suppressant effect of the selective 5-HT_{1A} receptor antagonist WAY 100635 on the firing activity of locus coeruleus NA neurons was due to an enhancement of the function of 5-HT neurons mediated via presynaptic 5-HT_{1A} receptors and postsynaptic 5-HT_{2A} receptors. Finally, in the last study, it has been demonstrated that chronic treatments with either befloxatone, mirtazapine, the selective 5-HT reuptake inhibitor paroxetine or with the partial 5-HT_{1A} receptor agonist gepirone enhanced the tonic activation of the postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus, as revealed by the enhancing effect of the administration of WAY 100635 on pyramidal neuronal firing activity. These results constitute further direct evidence that an enhanced 5-HT neurotransmission may underlie the antidepressant response in humans and further emphasize the crucial role played by the 5-HT system in regulation of mood.

Resumé:

Au cours des dernières années, plusieurs études ont indiqué que le système sérotoninergique (5-HT) est impliqué dans l'effet thérapeutique des traitements antidépresseurs. Différents traitements antidépresseurs augmenteraient l'activité de ce système par plusieurs mécanismes adaptatifs. Le but de cette recherche est de déterminer, à l'aide de techniques électrophysiologiques chez le rat, le rôle et la nature de plusieurs facteurs modulant le système 5-HT, d'évaluer certaines interactions entre les systèmes 5-HT et noradrénergique (NA) et aussi de confirmer la présence d'une élévation de l'activité centrale 5-HT produite par différents types de traitements antidépresseurs.

L'étude de l'automodulation du système 5-HT médiée par les récepteurs 5-$HT_{3/4}$ montre que l'activation des récepteurs 5-HT_3 ne s'observe que *in vitro*. Ceci s'est traduit par une augmentation de la libération de [^{3}H]5-HT. En revanche, l'activation des récepteurs 5-HT_4, observé *in vivo*, réduit la libération endogène de 5-HT. Les études sur l'hétéromodulation du système 5-HT induite par la mirtazépine, un antagoniste des récepteurs α_2-adrénergiques, a montré que l'administration aiguë de mirtazépine augmente de façon transitoire la fréquence de décharge spontanée des neurones 5-HT du raphé dorsal et NA du locus coeruleus. Par contre, l'administration chronique de mirtazépine produit cette augmentation de façon soutenue. De plus, l'administration à la fois aiguë et chronique de mirtazépine antagonise l'activation des auto- et hétérorécepteurs α_2-adrénergiques terminaux de l'hippocampe dorsale. Il a été proposé que l'effet antidépresseur de ce médicament résulte de l'augmentation soutenue de la fréquence de décharge des neurones 5-HT et NA ainsi que de la désensibilisation des hétérorécepteurs α_2-adrénergiques localisés sur les terminaisons 5-HT. L'étude de la modulation du système 5-HT par l'intermédiaire de l'inhibition de la monoamine oxidase-A (MAO-A) démontre que l'administration à court-terme de béfloxatone, un inhibiteur sélectif et réversible de la MAO-A, réduit la fréquence de décharge spontanée des neurones 5-HT du raphé dorsal, réduction qui n'était plus observé suite à un traitement à long-terme. Chez des animaux traités à court-terme avec à la fois la béfloxatone et le (-)pindolol (un antagoniste des récepteurs 5-HT_{1A}), l'activité neuronale des cellules 5-HT se situait au dessus du niveau de base observé chez des rats contrôles. Il a donc été proposé que l'effet antidépresseur de cette combinaison pourrait s'avérer aussi efficace qu'un traitement à long-terme avec la béfloxatone. L'étude sur une interaction entre les systèmes 5-HT et NA a montré que l'effet inhibiteur du WAY 100635, un antagoniste sélectif des récepteurs 5-HT_{1A}, sur la fréquence de décharge spontanée des neurones NA du locus coeruleus est due à une augmentation de la fonction des neurones 5-HT médiée par l'activation de récepteurs 5-HT_{1A} présynaptiques et 5-HT_{2A} postsynaptiques. Finalement, dans notre dernière étude, nous avons démontré que des traitements à long-terme avec différentes classes d'antidépresseurs: la mirtazépine, la béfloxatone, la paroxétine (un inhibiteur sélectif de la re-capture de 5-HT) et le gépirone (un agoniste partiel des récepteurs 5-HT_{1A}), augmentent l'activation tonique des récepteurs 5-HT_{1A} postsynaptiques dans l'hippocampe dorsale. En effet, l'administration du WAY 100635 a révélé une désinhibition de l'activité neuronale des cellules pyramidales de la couche CA_3 dans l'hippocampe dorsale seulement chez les animaux traités. Dans l'ensemble, ces résultats constituent une évidence directe supplémentaire que l'élévation de la neurotransmission 5-HT est associée avec la réponse antidépressive.

CHAPTER I: REVIEW OF THE LITERATURE

1. Introduction

With a lifetime prevalence rate of approximately 15-20% in the general population, major depression is one of the most common psychiatric disorders (Kessler et al., 1994). As described in the Diagnostic and Statistical Manual of Mental Disorders (DSM IV), symptoms of the disorder are characterized by depressed mood, attenuated interest (anhedonia), anorexia or hyperphagia, insomnia or hypersomnia, anxiety, worthlessness and failure, and recurrent thoughts of suicide. Although its physiopathology is not fully understood, the development of conventional antidepressants, in the last 3 decades, has been associated with theories based on a deficiency of the catecholamine (noradrenaline, NA) and/or the indolamine (serotonin, 5-HT) systems. Although early antidepressant therapy with tricyclic antidepressants (TCAs) and the monoamine oxidase inhibitors (MAOIs) was efficacious, their marked side effects and toxicity lead to the search for more tolerable and safer antidepressants such as selective NA (SNRIs) or 5-HT (SSRIs) reuptake inhibitors as well as reversible MAO-A inhibitors.

Since Schildkraut (1965) first proposed that depression may result from an underactivity of the central NA system, early clinical evidence in support of the NA hypotheses is based on the efficacy of TCAs and MAOIs, which increase the synaptic availability of NA, and on the mood-lowering effect of reserpine (a monoamine depletor). Furthermore, this hypothesis is supported by the increase of ß-adrenergic receptor binding sites in the brain of suicide victims and by a recent study indicating that the administration of α-methylparatyrosine (a catecholamine synthesis inhibitor) to remitted depressed patients on SNRIs, but not SSRIs, caused a significant return of depressive symptoms (Bunney and Davis, 1965; Mann et al., 1986, 1989; Arango et al., 1990; Miller et al., 1996). There is also preclinical evidence that supports the involvement of NA system in depression. For example, most antidepressant treatments decrease the function of ß-adrenergic receptors (a phenomenon not necessarily associated with a decrease of NA neurotransmission, see section 5.1.2.). Antidepressant treatments that enhance the synaptic NA concentration induce a desensitization of both α_2-adrenergic heteroreceptors located on 5-HT fibers (Mongeau et al. 1994a,b; Blier and Bouchard, 1994) and a desensitization of terminal α_2-adrenergic autoreceptors located on NA neurons (Crews and Smith, 1978; McMillen et al., 1980; Spyraki and Fibiger, 1980; Finberg and Tal, 1985; Lacroix et al., 1991). Finally, the influence of α_2-adrenoceptor antagonists in animal models of anxiety is inconsistent, with results spanning from anxiogenesis to anxiolysis (Handley and Mithani, 1984; File and Johnston, 1987; Cole et al., 1995; Bremner et al., 1996).

On the other hand, various classes of antidepressant treatments enhance 5-HT neurotransmission with a time course that is consistent with their delayed therapeutic effect. The clinical evidence in support of the 5-HT hypothesis has come primarily from the development of SSRIs which enhance synaptic 5-HT level and from the increase of 5-HT_2 receptor binding sites in the brain of suicide victims and in platelets of depressed individuals. In addition, this is

supported by the decreased number of 5-HT transporter binding sites in the brain of suicide victims and depressed patients and in platelets of drug-free depressed patients. More importantly, the reduction of 5-HT levels induced by either parachlorophenylalanine (a 5-HT synthesis inhibitor) or by the depletion of the 5-HT precursor L-tryptophan appears to reverse the antidepressant effects of several drugs (TCAs and SSRIs). Finally, recent studies demonstrates *in vivo* a reduced 5-HT responsivity in the brain of untreated depressed patients (Biegon et al., 1987, 1990; Mann et al., 1989; Van Praag et al., 1990; Arango et al., 1990; Delgado et al., 1990; Cummings, 1993; Owens and Nemeroff, 1994; Mann et al., 1995).

There is also a growing body of preclinical evidence that supports the implication of the 5-HT system in the therapeutic effect of antidepressant treatments (see Table I). It has been shown that TCA and electroconvulsive shock therapy lead to an enhanced 5-HT neurotransmission through a progressive sensitization of the postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus, but the modification of the density of 5-HT_{1A} binding sites is still controversial (de Montigny and Aghajanian, 1978; Welner et al., 1989; Nowak and Dulinski, 1991; Stockmeier et al., 1992; Burnet et al., 1994). Long-term treatment with MAOIs and SSRIs treatments desensitize the somatodendritic 5-HT_{1A} autoreceptors of 5-HT neurons in the dorsal raphe nucleus, thereby allowing their firing rate to recover in the presence of the drugs (Blier et al., 1986b; Chaput et al., 1986). In addition, long-term SSRI treatment desensitizes terminal 5-$HT_{1B/D}$ autoreceptors, whereas long-term MAOI treatment desensitizes terminal α_2-adrenoceptors located on 5-HT fibers (Blier and Bouchard, 1994; Mongeau et al., 1994a,c). The two latter receptor subtypes modulate 5-HT release in the terminal field. Long-term treatment with α_2-adrenoceptors antagonists, such as mianserin, probably enhances 5-HT neurotransmission in two ways. First, increased 5-HT activity through a progressive sensitization of the postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus as well as a result of a sustained increase in 5-HT neuron firing activity. Second, sustained increase in 5-HT function may occurs as a result of decreased function of α_2-adrenergic heteroreceptors located on 5-HT terminals in the dorsal hippocampus (Blier et al., 1984; Mongeau et al., 1994). Long-term treatment with 5-HT_{1A} receptor agonists, such as gepirone, desensitize the presynaptic 5-HT_{1A} receptors on 5-HT neurons but not the postsynaptic 5-HT_{1A} receptors located on CA_3 pyramidal neurons (Blier and de Montigny, 1987). Consequently, in the presence of the 5-HT_{1A} receptor agonists in the brain and a normalized release of endogenous 5-HT, it was hypothesized that long-term treatment with 5-HT_{1A} receptor agonists also leads to an enhanced tonic activation of postsynaptic 5-HT_{1A} receptors. Finally, using animal models, several lines of evidence support the idea that 5-HT_{1A} and 5-HT_3 receptors ligands provide a novel class of anxiolytics (Wieland and Lucki, 1990; Martin et al., 1990; Costall and Naylor, 1991; Barnes et al., 1992; De Vry, 1995; Griebel, 1995; File and Gonzalez, 1996).

The aim of the present research endeavour is to investigate and to assess the nature and the role of different automodulations (via 5-HT auto- and heteroreceptors) and heteromodulations (mediated via NA α-adrenergic auto- and heteroreceptors) of the central 5-HT neurotransmission as well as the nature of the interactions between the 5-HT and the NA systems. Thus, in order to better understand the interactions between brain neuronal systems implicated in the regulation of mood and to further characterize the biological basis of pharmacological treatments of

depression, *in vivo* and *in vitro* electrophysiological studies have been undertaken using as a model the raphe-dorsal hippocampal 5-HT pathway of male Sprague-Dawley rats.

Table I: Effects of long-term administration of antidepressant treatments on 5-HT neurotransmission (reproduced from Blier and de Montigny, 1994, with permission)

	Responsiveness of somatodendritic 5-HT_{1A} autoreceptors	Function of terminal 5-$HT_{1B/D}$ autoreceptors	Function of terminal α_2-adrenergic heteroreceptors	Responsiveness of postsynaptic 5-HT_{1A} receptors	Net effect on 5-HT neurotransmission
SSRI	↓	↓	↔	↔	↑
MAOI	↓	↔	↓	↔ or ↓	↑
5-HT_{1A} agonists	↓	↔	n.d.	↔	↑
TCA	↔	↔	n.d.	↑	↑
ECT	↔	↔	↔	↑	↑

↓: decrease ↔: no change n.d.: not determined ↑: increase

2. Serotonin system.

Vialli and Erspamer (1933, see Cooper et al., 1991) were first to isolate a molecule in the intestinal mucosa that was secreted by the chromaffin cells and thus called enteramine. Then, Rapport et al. (1947) reported the existence of a vasotonic agent in the serum (so called serotonin, 5-HT) for which it has been shown to be identical to enteramine. The chemical synthesis and the brain distribution of 5-HT allowed then to consider 5-HT as a main neurotransmitter in the CNS (see Cooper et al., 1991).

2.1. Metabolic regulation.

5-HT is found in platelets (8%), chromaffin cells of the intestine (90%) and in neurons (2%). The limiting precursor of 5-HT is L-tryptophan, an amino acid which primarily comes from the diet and crosses the blood brain barrier through a non-specific carrier. Only 4% of the circulating tryptophan contributes to 5-HT synthesis in the CNS. The first step of the synthesis consist of hydroxylation of tryptophan by the enzyme tryptophan hydroxylase in presence of 2 cofactors: oxygen and erythrotetrahydrobiopterin. The activity of this enzyme can be antagonized by *para*-chlorophenylalanine. Because tryptophan hydroxylase is not saturated with its substrate under physiological conditions, the hydroxylation of tryptophan constitutes the rate-limiting step in the synthesis of 5-HT. The second step is the decarboxylation of 5-hydroxytryptophan achieved by the enzyme L-amino acid decarboxylase in presence of vitamin B6. Serotonin by itself does not regulate its own synthesis as only the first step as well as the activity of the tryptophan carrier regulate its synthesis.
The catabolism of 5-HT is performed by the enzyme monoamine oxidase (MAO) which results the production of 5-hydroxyindoleacetaldehyde, and that is further oxidized to 5-hydroxyindoleacetic acid (5-HIAA). Catecholamines and 5-HT can be catabolized by the two isoforms of MAO (termed MAO-A and MAO-B), at least under certain conditions (Johnston, 1968), for which selective inhibitors have been developed. The oxidative deamination of 5-HT as well as noradrenaline (NA) and epinephrine is preferentially performed by the MAO-A isoform (abundant in the locus coeruleus), whereas the MAO-B form (abundant in the dorsal raphe) preferentially deaminates phenylethylamine and benzylamine. Dopamine is deaminated by the both forms (Westlund et al., 1985; Denney and Denney, 1985; Youdim and Finberg, 1991; Saura et al., 1992).

2.2. 5-HT receptors.

Gaddum and Picarelli (1957) have proposed the existence of two separate subtypes of 5-HT receptors denoted D and M. The former intervenes in the contraction of smooth muscle and the latter in the depolarization of cholinergic neurons. The Bradley's classification was proposed in 1986 and, on the basis of radioligand binding studies, three receptor subtypes were retained: 5-

HT_1, 5-HT_2 and 5-HT_3. Based on radioligand binding properties, signal transduction and deduced amino acid sequences, the 5-HT receptors are now divided as follows: 5-$HT_{1A,B,D,E,F}$ subtypes are negatively coupled to adenylyl cyclase, 5-$HT_{2A,B,C}$ subtypes are positively coupled to phospholipase C, 5-HT_3 receptor equivalent to the M receptor of Gaddum and Picarelli (1957) incorporates a ligand-gated ion channel, and the 5-$HT_{4,5,6,7}$ subtypes are positively coupled to adenylyl cyclase (Humphrey *et al.*, 1993).

2.2.1. 5-HT_1 receptors.

Binding studies with the non-selective 5-HT receptors antagonist spiperone has been first used to discriminate two subtypes of 5-HT_1 binding sites: the 5-HT_{1A} receptor for which spiperone has high affinity and the low affinity site which corresponds to the 5-HT_{1B} receptor (Pedigo et al., 1981). The 5-HT_{1A} receptor has been selectively labelled with different radioligands: the tetraline derivative 8-OH-DPAT (Gozlan et al., 1983; Arvidsson et al., 1984), the pyrimidinylpiperazines (or azapirones) buspirone or ipsapirone (Traber et al., 1984) and benzodioxanes WB 4101 or spiroxatrine (Norman et al., 1985; Herrick-Davis and Titeler, 1988). Others drugs which are non-selective and interact potently with the 5-HT_{1A} receptor are ergot derivative such as d-LSD, metergoline and various indoles such as 5-HT, 5-carboxytryptamine and RU 24969 (Griebel, 1995). Numerous 5-HT_{1A} receptor agonists are now available but most of these drugs act as partial agonists (Smith and Peroutka, 1986; Martin and Mason, 1987; Gartside et al., 1990; Yocca, 1990; Van den Hooff and Galvan, 1991, Blier and de Montigny, 1987b). Furthermore, 8-OH-DPAT, buspirone and ipsapirone are full agonists inhibiting the production of cAMP in the hippocampus but not in the cortex (Dumuis et al., 1988). On the other hand, most of the 5-HT_{1A} receptor antagonists available are not selective. For instance, spiperone, spiroxatrine, pindolol, propranolol, methiotepin (Griebel, 1995), and phenylpiperazine derivatives BMY 7378 or NAN-190 (Chaput and de Montigny, 1988; Glennon et al., 1988; Rydelek-Fitzgerald et al., 1990) and WAY 100135 (Fletcher et al 1993) act as partial agonists. Few agents seem to be selective and potent 5-HT_{1A} receptor antagonists including S-14063 (Dabire et al., 1991), SDZ 216525 (Sharp et al., 1993a; Hoyer et al.,1994), (S)-UH-301 (Björk et al., 1991) and WAY 100635 (Fletcher et al., 1996). Autoradiographic studies in different species reveal high density of 5-HT_{1A} binding sites in the limbic system including the hippocampus (high density in layer CA_1 but low in CA_3), septum, amygdala and entorhinal cortex as well as on 5-HT neurons of the dorsal and median raphe nuclei (Marcinkiewicz et al., 1984; Pazos and Palacios, 1985; Welner et al., 1989; Hall et al., 1985; Waeber et al., 1989a). Furthermore, a complementary distribution of neurons in the rat brain expressing 5-HT_{1A} mRNA and 5-HT_{1A} receptor binding sites was found in the hippocampus (high in both layers CA_1 and CA_3), septum enthorinal cortex and raphe nuclei (Chalmers and Watson, 1991; Pompeiano et al., 1992). Lesions of the 5-HT system with the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) produces 50% of decrease of the density of 5-HT_{1A} binding sites in the raphe nuclei suggesting that these receptors are not exclusively autoreceptors in these nuclei (Weissman-Nanopoulos et al., 1985; Vergé et al., 1985, 1986). In contrast the kainic, but not 5,7-DHT, lesions produce a marked reduction of the density of 5-HT_{1A} receptor in the hippocampus, suggesting a postsynaptic location of these receptors (Hall

et al., 1985; Vergé et al., 1986). The ultrastructural localization of 5-HT_{1A} receptors in the rat brain reveals that they are found on the dendritic spines in the dorsal hippocampus and frequently associated with synapses, whereas these receptors are located mainly extrasynaptically in both somata and dendrites of 5-HT neurons in the dorsal raphe nucleus (Kia et al., 1996). Molecular biology characterization of 5-HT_{1A} receptors reveals that these receptors are coupled to multiple G-proteins. Preferential coupling is to inhibit the adenylate cyclase via a pertussis toxin-sensitive G_i protein (DeVivo and Maayani, 1985; Dumuis et al., 1988a; Harrington et al., 1988; Okada et al., 1989). It has been shown, using cultured transfected cells, that 5-HT (in nM range) inhibits the forskolin-stimulated adenylyl cyclase activity (Fargin et al., 1989; Albert et al., 1990). Paradoxically, some studies have suggested the existence of coupling to a G_s protein (Shenker et al., 1985; Markstein et al., 1986; Fayolle et al., 1988). Hence, when expressed in cultured cells, HeLa and LZD-7 but not in GH_4ZD_{10} or COS-7, 5-HT_{1A} receptors stimulate IP_3 production and protein kinase C activity. Moreover, in the rat hippocampus, 5-HT_{1A} receptors have been shown to be negatively coupled to a phosphoinositide (PI) phosphodiesterase (Claustre et al., 1988; Raymond et al., 1989; Liu and Albert, 1991). Finally, these receptors also control a G-protein-coupled K^+ channel which is pertussis toxin sensitive (Andrade and Nicoll, 1987; Innis et al., 1988; Newberry, 1992).

As previously noted, binding studies with spiperone have been used for the subdivision of 5-HT_{1A} and 5-HT_{1B} receptors (Pedigo et al., 1981). Because the best correlations were found between the potency of a number of drugs for the terminal 5-HT autoreceptor and their affinity for the 5-HT_{1B} binding sites (Middlemiss et al., 1984b; Engel et al., 1986), it has been proposed that 5-HT autoreceptors on terminals are of the 5-HT_{1B} classes and mediate a negative feedback regulation of the 5-HT release (Engel et al., 1986; Starke et al., 1989; Humphrey et al., 1993). To further classify these subtypes, it has been shown that 8-OH-DPAT does not activate this terminal autoreceptor. Autoradiographic studies of 5-HT_{1B} receptors have been performed with tritiated 5-HT in presence of 5-HT_{1A} and 5-HT_{2C} receptor ligands (Peroutka, 1986), with [^{125}I]iodocyanopindolol in presence of ß-adrenoceptor ligand (Hoyer et al., 1985) or with tritiated CP-96,501, an analog of the 5-HT_{1B} receptors agonist RU 24969 (Koe et al., 1992). There are few selective 5-HT_{1B} receptor agonists such as CP-93,129 or CP-96,501 (but not mCPP, quipazine or TFMPP), that are now available, and good antagonists remain to be developed (Griebel, 1995). Selective lesions of 5-HT system reveal that most of 5-HT_{1B} receptor binding sites (including in the rat hippocampus and locus coeruleus) are located postsynaptically (Fischette et al., 1987; Vergé et al., 1986; Weissmann-Nanopoulos et al., 1985). However, these receptors, also considered as terminal autoreceptors, mediate a negative feedback regulation of 5-HT release (Cerrito and Raiteri, 1979a,b; Martin and Sanders-Bush, 1982; Göthert and Weinheimer, 1979; Maura et al., 1986). As for 5-HT_{1A} subtypes, molecular biology characterization of 5-HT_{1B} receptors have shown that these receptors are negatively coupled to adenylate cyclase. While agonists inhibit the forskolin-stimulated adenylate cyclase activity in the rat substantia nigra (Bouhelal et al., 1988; Schoeffter and Hoyer, 1989), it has been shown in the rat hippocampus that terminal 5-HT autoreceptors are not coupled to G proteins (Blier, 1991), suggesting that different receptor-coupling mechanisms could exist. While 5-HT_{1D} receptors seem to be absent

in rodents but detected in guinea-pig and man, it has been proposed that 5-HT_{1B} receptors are the rodent homologue of 5-HT_{1D} receptors (or 5-$HT_{1D\alpha}$) (Hartig et al., 1992) and subclassified as 5-$HT_{1D\beta}$ (Hartig et al., 1990; Fink et al., 1995). However, recently it has been shown that mRNAs encoding for 5-$HT_{1D\alpha}$ receptors are present in rat brain and these latter receptors modulate the 5-HT release in the rat dorsal raphe nucleus (Hamblin et al., 1992; Piñeyro et al., 1995). Recently, a presynaptic inhibitory 5-$HT_{1D\beta}$-like autoreceptor in the guinea-pig cortex and a new endogenous peptide (5-HT-moduline) selective for 5-$HT_{1B/1D}$ receptors have been described (Bühlen et al., 1996; Massot et al., 1996).

2.2.2. 5-HT_2 receptors.

The molecular biology characterization of 5-$HT_{2A/B/C}$ receptors reveal that these receptors are linked to the PI signalling system and their activation produced inositol triphosphate (IP_3) and diacylglycerol, via a phospholipase C activation (Conn and Sanders-Bush, 1987; Conn et al., 1987; Launay et al., 1994). Several tritiated ligand such as spiperone, ketanserin, mianserin, metergoline or [^{125}I]LSD and [^{125}I]ketanserin, have been used to describe 5-HT_{2A} receptors, as well as agonists such as DOB and DOI (Titeler et al., 1987; McKenna and Peroutka, 1989). Autoradiographic and in situ hybridization studies reveal high level of 5-HT_{2A} binding sites and mRNAs levels, in cortex, but to a lesser extent or even absent in the hippocampus. 5,7-DHT lesions do not reduce the 5-HT_2 receptor density, suggesting that these receptors are mainly located postsynaptically (Hoyer et al., 1986, Pazos et al., 1985; Fischette et al., 1987; Conn et al., 1987; Hoffman and Mezey, 1989; Pompeiano et al., 1994; Wright et al., 1995; Raghupathi et al., 1996). Recently, MDL 100,907 has been identified as a selective and potent 5-HT_{2A} receptor antagonist (Sorensen et al., 1993; Johnson et al., 1996), but the regional distribution of 5-HT_{2A} receptor binding sites with this ligand remains to be performed. Although no selective ligand for the 5-HT_{2C} receptors (previously denoted 5-HT_{1C} receptors) are available, these receptors have been identified with different tritiated ligands (Yagaloff and Hartig, 1985; Sanders-Bush and Breeding 1988; Westphal and Sanders-Bush, 1994) and its mRNA distribution is considerably widespread in the CNS with high density in choroid plexus (Hoffman and Mezey, 1989). However, discrepant results, using specific antisera against 5-HT_{2C} receptors, failed to detect the presence of 5-HT_{2C} receptors in DRN and LC (Molineaux et al., 1989, Pompeiano et al., 1994; Wright et al., 1995; Abramowski et al., 1995). Finally, it is noteworthy that the expression of 5-HT_{2B} receptors could not be detected in rat brain (Pompeiano et al., 1994; Hoyer et al., 1994).

2.2.3. 5-HT_3 receptors.

Previously called by Gaddum and Piccarelli (1957) M receptors, the 5-HT_3 receptor mediates a rapid depolarization of peripheral and central neurons, as well as in neuronal cell lines. Electrophysiological studies have revealed the first current recordings through single-ion channels triggered by 5-HT_3 receptors activation, in excised outside-out membrane patches from guinea pig submucous plexus (Derkach et al,. 1989). Similarly to nicotinic acetylcholine receptors, 5-HT_3 receptors mediate a brief latency response (<10ms in N18 cells) due to an increase in monovalent

cations conductances (Lambert et al., 1989; Bobker and Williams, 1990; Yang, 1990). This response was blocked by (+)tubocurarine in several preparations (Peters et al., 1990; Yakel et al., 1991) and does not involve a G protein (Derkach et al., 1989; Yang, 1990; Kilpatrick et al., 1987). In both whole-cell and outside-out membrane patch studies, 5-HT generates an inward current, for which the reversal potential is close to 0 mV (Derkach et al., 1989; Yakel et al., 1991; Kilpatrick et al., 1987). Differences observed in conductance and permeability predict variability of the desensitization kinetics of 5-HT_3 receptors responses. Almost all 5-HT_3 receptors responses exhibit a rapid desensitization after continuous 5-HT application (Neijt et al., 1988). Thus, in cultured hippocampal neurons, the rate of desensitization decreases with time and is voltage dependent (Yakel and Jackson, 1988; Yakel et al., 1991). In contrast with 5-HT receptors described previously, the development of highly selective drugs has allowed the identification and characterization of 5-HT_3 receptors (Richardson et al., 1985; Richarson and Engel, 1986). Reiser et al. (1989) have demonstrated, in neuronal hybrid cells, that 5-HT_3 receptors activation induces fast and transient rises of both cytosolic Ca^{2+} activity and cGMP level, responses which are blocked by 5-HT_3 receptors antagonists. McNair and Dekin (1992) have demonstrated that 5-HT modulates the repetitive firing activity of vagal motoneurons from the dorsal motor nucleus in guinea pig via a 5-HT_3 receptor acting on Ni^{2+} sensitive conductance, suggesting the involvement of a T-type calcium channel. Since the regulation of this biochemical activity is highly similar to that of the nicotinic receptors, it is conceivable that 5-HT_3 receptors modulate a Ca^{2+}-dependent process leading to a long-term change of cellular activity. As for nicotinic receptors, a Ca^{2+} influx participates in the feedback control of the desensitization process or on the metabolic stability of the receptor (Cachelin and Colquhoun, 1989). Maricq et al. (1991) reported that mRNA for 5-HT_3 receptors could not be detected in the intestine even after polymerase chain reaction amplification. They have thus suggested that, in the intestine, 5-HT_3 receptors could be encoded by a different gene. As with the 5-HT_1 receptors class, on the basis of the different affinity of antagonists (e.g MDL72222 yields a pA2 value of 9.1 on postganglionic nerve fibers in the rabbit heart, but is totally inactive on enteric neurones in the guinea-pig ileum (Richardson et al., 1985), several scientists have proposed that 5-HT_3 receptors could be classified into three subtypes (5-$HT_{3a,b,c}$). Evidence of between-species difference's has also been demonstrated. For instance, the potencies of 5-HT_3 receptor antagonists are different in the rabbit and guinea pig brain (Peroutka, 1988a). Moreover, in superior cervical ganglion from rat, mouse and guinea-pig, the properties of 5-HT_3 receptors response are different (Newberry et al., 1991). The properties of 5-HT_3 receptors have also been found to be different as well as from the state of differentiation of cell line. Shao et al. (1991) even suggested that NG10815 cells can produce different subtypes of receptors. Recently, Ashby et al. (1992) demonstrated that 5-HT suppresses cell firing activity in the medial prefrontal cortex, an effect which is blocked by 5-HT_3 receptor antagonists, but not 5-HT_1 or 5-HT_2 receptor antagonists. Thus, it appears that in this cortical region, the action of 5-HT is mediated (if not exclusively) by 5-HT_3-like receptors. In addition, they have also presented evidence indicating that this response may be coupled to phospholipase C (Ashby et al., 1992). In fact, they have characterized the accumulation of phosphoionositol phosphate induced by the 5-HT_3 receptors agonist 2-methyl-5-HT. This agonist stimulated phosphoinositide

hydrolis, which is Ca^{2+} dependent, coupled with protein kinase C and possibly exerting negative feedback control. It is interesting to note that this unusual suppressant effect of 2-methyl-5HT in the medial prefrontal cortex is markedly enhanced following a 5,7-DHT pretreatment (Ashby et al., 1994). Therefore, this inhibitory response does have the capacity to become supersensitive following its denervation, whereas it is unable to desensitize following a sustained exposure to an agonist. This differential properties of the 5-HT_3 receptor, as compared to those of the cell line NCB20, certainly have a molecular basis which remains to be elucidated. Further experiments are needed to confirm the role of protein kinase and to determine their physiological properties. Autoradiography studies have found a high level of 5-HT_3 receptors in the area postrema and a moderate level mainly in the entorhinal and frontal cortex, hippocampus, amygdala and the thalamus (Kilpatrick et al., 1987; Barnes et al., 1990). More recently, a study on the cellular immunolocalization of the 5-HT_3 receptors in the rat brain reveals intensely immunolabelled in the forebrain (isocortex, olfactory regions, hippocampus and amygdala) and the brainstem (tractus solitary, sensory trigeminal nucleus, hypoglossal nucleus but not in raphe nuclei; Morales et al., 1996a). It has been recently shown that in the rat neocortex and hippocampus, 5-HT_3 receptors are located on GABA interneurons (Morales et al., 1996b). Blier et al. (1993c) have shown that 2-methyl-5-HT increases the K^+ evoked release of [^{3}H]-5-HT from preloaded slices of the guinea-pig hypothalamus and that this effect is blocked by the selective 5-HT_3 receptor antagonist ondansetron. In presence of tetrodotoxin, this enhancement of [^{3}H]-5-HT release induced by 2-methyl-5-HT was no longer present. Moreover, 2-methyl-5-HT did not alter of [^{3}H]-5-HT release in a synaptosomal preparation from guinea-pig hypothalamus, thus indicating that these 5-HT_3 receptors are not located on 5-HT terminals. It has been demonstrated that the lesions of 5-HT fibers in rat hippocampus did not modify the density of 5-HT_3 receptors labelled with [^{3}H](R,S)-zacopride, indicating that 5-HT_3 receptors were not located on 5-HT fibers in this brain region. Moreover, local lesions of the hippocampus with kainic or ibotenic acid reduced 5-HT_{1A} receptor binding sites labelled with [^{3}H]8-OH-DPAT but not the density of 5-HT_3 receptors, suggesting their location on fibers "en passage" (Kidd et al., 1993).

The 5-HT_4 receptors have been characterized by a positive coupling to adenylyl cyclase (Dumuis et al., 1988b; Bockaert et al., 1990). The increase of cAMP, which rapidly desensitizes (Ansanay et al., 1992), is followed by activation of protein kinase A and by the closure of K^+ channels. The resulting depolarization opens Ca^{2+} channels and this increase in intracellular Ca^{2+} triggers an increase in transmitter release (Fagni et al., 1992).

It should be added that other 5-HT receptors (5-$HT_{1E/1F}$ and 5-$HT_{5/6/7}$) have been classified on the basis of molecular cloning and pharmacological studies (Boess and Martin, 1994).

5-HT transporters (5-HTT) remove 5-HT from the synaptic cleft by an ion-dependant reuptake process. 5-HT is taken into the presynaptic terminals where it is metabolised by MAO or sequestered into secretory vesicles by the vesicular transporter. Cloning and sequencing of cDNA encoding 5-HTT revealed two related proteins with twelve transmembrane domains containing the secondary structure required for the substrate translocation, ion and antagonist

binding (Blakely et al., 1991; Hoffman, 1994). Thus, it is hypothetized that the first step involves the binding of 5-HT to the 5-HTT and then a co-transport with Na^+, while the second step involves the translocation of K^+ across the membrane to the outside of the cell. SSRIs bind to the same site of 5-HT itself and the regional distribution of 5-HTT corresponds to discrete regions of rat brain known to contain synaptic terminals, axons and cell bodies of 5-HT neurons (Backström et al., 1989; Hrdina et al., 1990; Mann and Hrdina, 1992).

2.3. Morpho-physiological aspects of 5-HT system.

2.3.1. 5-HT neurons.

Previously divided in nine groups (B1-B9; Dahlström and Fuxe, 1964), the 5-HT cell bodies in the brain are located in the brainstem near or on the midline and are now divided in 2 main groups: superior and inferior brainstem nuclei (see Jacobs and Azmitia, 1992).

The superior group consists of the caudal linear nucleus (B8), median raphe nucleus (MRN; B8 and B5), dorsal raphe nucleus (DRN: divided into medial, lateral and caudal components; B7 and B6), and the lateral B9 nucleus located just dorsal to the medial lemniscus. The inferior group consists of the nuclei raphe osbcurus (B2), raphe pallidus (B1 and B4), raphe magnus (B3), in the ventral lateral medulla (B1/B3) and in the area postrema. The next section will focus mainly on the DRN.

The DRN contains about 50-60% of the total 5-HT neurons in both rat and human CNS, whereas the MRN contains about 5% (Wiklund and Björklund, 1980; Descarries et al., 1982; Baker et al., 1990). In the DRN, about 70% of the cells are 5-HT neurons. Non-5-HT cells include peptidergic neurons: i.e. substance P (Moss, 1983; Magoul, 1986), enkephalin (Glazer et al., 1981; Tanaka et al., 1993), neurotensin (Beitz, 1982) and non-peptidergic neurons: i.e. dopamine (Descarries et al., 1986), GABA (Harandi et al., 1987; Gao et al., 1993) or glutamate (Johnson, 1994).

Efferents 5-HT projections to the forebrain originate mainly from the superior group of raphe nuclei. In the rat, the largest pathway is the medial forebrain bundle which carries fibers from the MRN and DRN to a wide range of target areas in the forebrain. The 5-HT projections from a group or single neuron can innervate several synaptically interconnected target regions. For instance, the DRN innervates the substantia nigra, the corpus striatum, the nucleus accumbens and the amygdala whereas the MRN innervates the cingulate cortex, the septum and the hippocampus. In the hippocampus, 5-HT projections from the MRN arrive via the fasciculus cinguli and the fimbria-fornix bundles, which is constituted of coarse axons with large spherical varicosities whereas the 5-HT projections from the DRN arrive to hippocampus via the amygdaloid bundle, and are made up of very fine axons with small varicosities of different forms (Azmitia and Segal, 1978; Törk, 1990; Jacobs and Azmitia, 1992; Mamounas et al., 1991; Hensler et al., 1994).

Electrophysiological studies in anaesthetized rats, have been performed by Aghajanian et al. (1968, 1970), who describe the first unitary recording from both MRN and DRN. The 5-HT-containing cells exhibit a slow (1-2 Hz) and regular firing rate with a long-duration positive

action potential (Aghajanian, 1978; Aghajanian and VanderMaelen, 1982a,b; VanderMaelen and Aghajanian, 1983). This regular discharge pattern results from a pacemaker cycle attributed to a Ca^{2+}-dependant K^+ outward current. The depolarization is followed by a long afterhyperpolarization (AHP) period, which diminishes slowly during the interspike interval. During the depolarization, extracellular Ca^{2+} enters the neuron via a voltage-dependant Ca^{2+} channel activating a K^+ outward conductance leading to a AHP. Ca^{2+} is then sequestered/extruded and the AHP diminishes slowly. When the membrane potential reaches the low-threshold Ca^{2+} conductance, a new action potential is triggered (Aghajanian and Lakoski, 1984; Burlhis and Aghajanian, 1987; Aghajanian et al., 1990). The firing pattern of 5-HT-containing neurons has been characterized by the combination of unitary recording and fluorescence histochemistry (Aghajanian and Hasher, 1971). In behaving cats, extracellular unitary recordings from DRN have revealed slow and rapid firing cell classes. The slow firing cells exhibit low spontaneous firing during all behavioral states and fire at progressively lower rates from waking (2-4 Hz), slow-wave sleep (1-2 Hz) to rapid eye movement sleep (0.5-1 Hz) (McGinty and Harper 1976; Trulson and Jacobs 1979). Moreover, the rapid firing cells discharge at rates of 15-50 Hz (Aghajanian et al., 1978; Park 1987). It should be added that 50% of DR neurons in the behaving rat discharges synchronously with the theta rhythm (present in the rat during wake-movements and rapid eye movement sleep) of the hippocampus, suggesting that DRN may serve to coordinate the activity of forebrain areas during theta-associated states or behaviours (Kocsis and Vertes, 1992).

Several studies have clearly demonstrated that 5-HT neuron firing activity is dependant on the activity of somatodendritic 5-HT_{1A} autoreceptors and afferent regulation (Rogawski and Aghajanian, 1981; Blier and de Montigny, 1987). **The automodulation of the firing activity** of 5-HT neurons via 5-HT_{1A} autoreceptors is mediated by a membrane hyperpolarization due to an increasing K^+ conductance and the reduction of the high-threshold Ca^{2+} current (Aghajanian and Lakoski, 1984; Sprouse and Aghajanian, 1987; Penington and Kelly, 1990; Penington and Fox, 1994), and seems to be mediated via a G-protein (Innis and Aghajanian, 1987; Penington et al., 1991). Although the inactivation of the $G_{i/o}$ proteins by pertussis toxin reduces the inhibitory action of 5-HT_{1A} receptor agonists, it has been shown that the activation of the 5-HT_{1A} autoreceptors does not involve adenylate cyclase and may involve a direct interaction between a pertussis sensitive G-protein and the inwardly rectifying K^+ channel (Larkman and Kelly, 1995). However, it should be mentioned that pertussis toxin-treatment fails to modify the firing activity of 5-HT neurons for unknown reasons (Blier et al., 1993a; Innis et al., 1988). However, a recurrent axon collateral inhibition of the firing activity of 5-HT neurons has been proposed (Wang and Aghajanian, 1977, 1978; Park et al., 1982). Recently, it has been shown *in vitro* that the stimulation of protein kinase C can selectively uncouple the 5-HT_{1A} receptor of rat DR neurons from the inhibition of Ca^{2+} current and leaving the K^+ current unaltered (Chen and Penington, 1996). The authors proposed that since normally DR neurons are tonically inhibited by 5-HT release at the somatodendritic level and that the released 5-HT affects both membrane potential and its own release, protein kinase activation uncoupling 5-HT receptors from Ca^{2+} but

not K^+ channels might have the following consequences. In DR cells undergoing excessive synaptic activity, Ca^{2+}-dependent stimulation of protein kinase may occur, and during a strong synaptic excitation, a greater Ca^{2+} influx with each action potential may result in a greater 5-HT release within the nucleus, which leads to a more pronounced dampening of neuronal activity due to the 5-HT-mediated opening of K^+ channels. The overall result is that 5-HT would inhibit membrane potential, but be less effective at inhibiting Ca^{2+} influx and transmitter release. Fewer action potentials would be generated and the neuron would release less 5-HT at target neurons (Chen and Penington, 1996).

On the basis of anatomical and electrophysiological studies on afferent inputs in the DRN, the **heteromodulation of the firing activity** of 5-HT neurons has been proposed to be mediated via GABA (from habenula and interneurons; Stern et al., 1979; Gottesfeld et al., 1978), NA (from locus coeruleus and subcoeruleus; Loizou, 1969; Anderson et al., 1977; Baraban and Aghajanian, 1981; Jones and Yang, 1985; Luppi et al., 1995), acetylcholine (from the superior vestibular nucleus (McGeer et al., 1986); epinephrine (paragigantocellular nucleus; Sim and Joseph, 1989; Hebert and Sapert, 1992); dopamine (substantia nigra and ventral tegmental area; Aghajanian and Wang, 1977); glutamate (hypothalamic nuclei; Sakai et al., 1977; Kalen et al., 1985) and neuropeptides such as substance P, ß-endorphin and CCK (Jacobs and Azmitia, 1992).

2.3.2. 5-HT responses in the hippocampus.

The hippocampal formation is characterized by a laminar distribution of cells and fiber layers and composed of the Ammon's horn, the fascia dentata and the subiculum. The Ammon's horn (or hippocampus proper) consists of a number of stratum or layer: the stratum moleculare containing fibers and dendrites, the stratum lacunosum contains bundles of parallel fibers including collateral of pyramidal cells, the stratum radiatum contains sparse cell bodies and fibers including the Schaffer's collateral (axons of CA_3 pyramidal cells in contact with dendrites of CA_1 cells), the stratum pyramidal, the stratum oriens contains basal dendritic arborization of the pyramidal cells and the collaterals of the axons of CA_3 cells that in parallel with the Schaffer collateral and the stratum alveus contains axons of pyramidal cells and incoming fibers. The fascia dentata consists of stratum moleculare and granulosum, hilus fasciae dentate and the subiculum consists of stratum moleculare and pyramidal. The fimbria-fornix bundle arborizes the stratum moleculare and lacunosum of CA_1 as well as the stratum radiatum of CA_3, whereas the amygdaloid bundle projects principally to the stratum molecular of fascia dentata (Chronister and White, 1975; Azmitia and Segal 1978; Oleskevich et al., 1991; Lopes da Silva et al., 1990).

The hippocampal slice preparation has provided the most appropriate starting place for examination of the membrane actions of 5-HT in the CNS. The main membrane conductances of hippocampus pyramidal cells will be described here. Studies of the electrophysiological properties of hippocampal neurons have been undertaken by Kandel and Spencer (1961) showing that the pyramidal cells have a mean resting potential of -60 mV, spike duration of 1.8 ms, and a maximum value of the action potential of 110 mV. A depolarizing afterpotential with an

amplitude of 8.8 mV and a duration of 24 ms has been defined and excitatory and inhibitory postsynaptic potentials have been described, the latter were assumed to result from a Cl^- current and induced by recurrent collaterals (Lopes da Silva et al., 1990). The pyramidal neurons possess Na^+ and K^+ conductances and a Ca^{2+}-regenerative conductance (since they generate TTX-resistant, dendritic action potentials that are Ca^{2+} dependent) (Wong and Prince, 1984; Schwartzkroin and Mueller, 1987). Moreover, they also possess a transient K^+ current (I_A current: rapid onset and decay), a rapidly activating but slowly inactivating K^+ current (I_D current), a K^+ current that is modulated by acetylcholine and suppressed by muscarine (I_M current), and two Ca^{2+}-dependant K^+ currents with a high and low threshold (Schwartzkroin and Mueller, 1987; Numann et al., 1987).

In CA_1 pyramidal cells, 5-HT induces a prominent hyperpolarization associated with a decrease in input resistance due to an increase in K^+ conductance (Segal, 1980; Jahnsen, 1980; Andrade and Nicoll, 1987; Colino and Halliwell, 1987). This hyperpolarization of CA_1-CA_3 pyramidal neurons results in the activation of the postsyanptic 5-HT_{1A} receptors: spiperone or BMY 7378 are effective antagonist. In addition, 8-OH-DPAT acts as a partial agonist and 5-CT as full agonist (Andrade and Nicoll, 1987; Beck, 1989; Collino and Halliwell, 1987; Beck et al., 1992). It has been shown that a pertussis toxin-sensitive protein couples the 5-HT_{1A} receptors to a K^+ channel, which is also coupled to the $GABA_B$ receptor (Andrade et al., 1986; Nicoll, 1988). Andrade et al. (1986) have suggested that this coupling is direct since it does not activate protein kinase C, nor modulate adenylate cyclase nor increase intracellular Ca^{2+} concentration. Microiontophoretic application of 5-HT onto rat CA_1-CA_3 pyramidal neurons produces a suppressant effect of their firing activity and this effect is mediated by postsynaptic 5-HT_{1A} receptors (Blier and de Montigny, 1987; Chaput and de Montigny, 1988; Blier et al., 1993a). 5-HT terminals are almost exclusively located on the dendritic trees of hippocampus pyramidal neurons (Oleskevich and Descarries, 1990) and the endogenous 5-HT, released by the electrical stimulation of the ascending 5-HT pathway, activates the intrasynaptic 5-HT_{1A} receptors located on dendrites of hippocampus pyramidal neurons (Chaput and de Montigny, 1988; Blier et al., 1993b). In the dorsal hippocampus, *in vivo*, pertussis toxin nearly abolish the responsiveness of extrasynaptic 5-HT_{1A} receptors located on the cell body of CA_3 pyramidal neurons, but not of intrasynaptic 5-HT_{1A} receptors located on the dendritic trees of the same neurons (Blier et al., 1993b). Moreover, Hadrava et al. (1994) have demonstrated that the sustained activation of the extrasynaptic, but not of intrasynaptic, 5-HT_{1A} receptors of CA_3 pyramidal neurons, achieved with treatments with 5-HT_{1A} receptors agonists flesinoxan and BMY 42568, prevents their inactivation by pertussis toxin. These data further suggest that these populations of postsynaptic 5-HT_{1A} receptors located on the same neurons are functionally distinct.

5-HT was also reported to depolarize hippocampal neurons (Jahnsen, 1980). Using voltage-clamp techniques it has been shown that 5-HT depolarizes neurons by decreasing the resting or leak K^+ conductance (Andrade and Nicoll, 1987; Colino and Halliwell, 1987). 5-HT decreases the current underlying the slow AHP mediated by a Ca^{2+}-gated K^+ conductance. The decrease of this K^+

conductance has been recently shown to be mediated by 5-HT_4 receptors since the effect of 5-HT was blocked by 5-$HT_{3/4}$ receptor antagonists BRL 24 924, zacopride and cisapride, mimicked by 5-CT or 5-methoxytryptamine (5-$HT_{1/4}$ receptor agonists) but not by the prototypical 5-HT_3 receptor agonist 2-methyl-5-HT (Andrade and Chaput, 1991; Chaput et al., 1990). Recently, it has been shown that 5-HT reduces AHP in the CA_1 region by acting on 5-HT_4 receptors that increase intracellular cAMP levels and activate protein kinase A (Torres et al., 1995, 1996), and these receptors seem to be collocated with 5-HT_{1A} receptors on the same hippocampus pyramidal cell (Roychowdhury et al., 1994). Beck (1992) has described a fast depolarization of pyramidal neurons in presence of spiperone (a 5-$HT_{1A/2A}$ receptor antagonist), which was mimicked by DOI (a 5-$HT_{2A/2C}$ receptor agonist) and antagonized by ketanserin (a 5-$HT_{2A/2C}$ receptor antagonist). It has been suggested that 5-HT increases the subthreshold of excitatory postsynaptic potential amplitude to promote spike firing via 5-HT_{2C} receptors.

As previously described, 5-HT_3 receptor activation produces a rapid depolarization of peripheral and central neurons, including neuronal cell lines. *In vitro*, 2-methyl-5-HT decreased the firing rate of 5-HT neurons in the dorsal raphe and that this inhibitory effect was prevented by 10 μM of the 5-HT_{1A} antagonist l-propranolol (Adrien et al., 1992). In contrast, in rat medial prefrontal cortex, 5-HT and 2-methyl-5-HT suppress neuronal firing activity, but this inhibitory effect is effectively antagonized by 5-HT_3 receptor antagonists (Ashby et al, 1992). Furthermore, the suppressant effect of 2-methyl-5-HT, but not that of 8-OH-PAT, is markedly enhanced following destruction of 5-HT terminals with the 5-HT neurotoxin 5,7-DHT, indicating a selective sensitization of these 5-HT_3-like receptors (Ashby et al, 1994). Using *in vitro* intracellular recordings of CA_1 pyramidal neurons, it has been reported that bath application of 2-methyl-5-HT mimicked the effect of 5-HT, inducing facilitation of unitary inhibitory postsynaptic potentials which were blocked by 5-HT_3 receptor antagonists such as tropisetron and metoclopramide (Ropert and Guy, 1991). These authors have thus proposed that 5-HT activates GABA interneurons via a 5-HT_3 receptor thereby increasing the frequency of inhibitory synaptic events recorded in CA_1 pyramidal cells. In suport of this, there is recent data revealing the presence of 5-HT_3 receptors on hippocampal GABA interneurons (Morales et al., 1996b).

2.3.3. Automodulation of 5-HT release.

5-HT, as for other neurotransmitters, is released from synaptic terminal via exocytosis, where synaptic vesicles fuse with the presynaptic plasma membrane and liberate their content into the synaptic cleft. Exocytosis at synapses differs from other types of exocytosis in the speed and the precise spatial location of the secretion event. The rapid fusion of synaptic vesicles with the plasma membrane of the synaptic terminal during the transmission is triggered by a high concentration of internal Ca^{2+} near the calcium channels at the active zone, and achieved by Ca^{2+}-sensitive vesicle membrane proteins (such as synaptophysin, synaptobrevin and synapsin I) upon depolarization. The synaptic vesicle membrane is rapidly retrieved by endocytosis and reutilized for the formation of new membrane vesicles (Katz and Miledi, 1967, 1968; Augustine et al.,

1985, 1988; Smith and Augustine, 1988; Leveque et al., 1992; Matthews, 1996; Rahamimoff and Fernandez, 1997). One mechanism by which Ca^{2+} entry can be regulated is by changing the amplitude or the duration of the action potential in the presynaptic terminal. If the presynaptic action potential is prolonged by drugs that block voltage-gated K^+ channels, more Ca^{2+} channels are opened for a longer period and more transmitter will be released (Llinas et al., 1981, 1982; Augustine, 1990). This section will focus on the automodulation of the 5-HT release in both somatodendritic and terminal (mainly hippocampus) levels.

Automodulation via 5-HT_{1A} receptors:

Using push-pull cannula perfusion technique in the cat DRN, 5-HT release is blocked by TTX and by low Ca^{2+} level, and increased by high K^+ concentrations (Héry et al., 1982, 1986; Becquet et al., 1990). In contrast, using microdialysis, Adell et al. (1993) showed that local injection of 8-OH-DPAT near the DRN and MRN fails to modify dialysate 5-HT. Although 5-HT release is increased by high K^+ stimulations, the dialysate 5-HT levels are not modified by removal of Ca^{2+} or perfusion of TTX. It has been suggested that the 5-HT release in the midbrain raphe is not regulated by 5-HT_{1A} receptors and may originate from a cytoplasmic pool. In contrast, Bokser et al. (1994) demonstrated that extracellular 5-HT level in the MRN was TTX sensitive and both systemic and local administration of 8-OH-DPAT in the MRN decreased 5-HT release. More recently, Matos et al. (1996) have shown that perfusion of TTX into DRN (but not when the probe was placed outside of DRN) decreases simultaneously DRN and ventral hippocampus 5-HT release, and that systemic but not local administration of the partial 5-HT_{1A} receptor agonist buspirone decreases 5-HT release in both DRN and ventral hippocampus. Furthermore, local DRN perfusion of the ß-adrenoceptor/5-HT_{1A} receptor antagonist (-)pindolol increases both DRN and ventral hippocampus 5-HT release. It has been suggested that 5-HT release in DRN is dependent on the opening of fast activated Na^+ channels and that DR 5-HT_{1A} receptors tonically control somatodendritic and hippocampal 5-HT release (Matos et al., 1996). However, the presence of a tonic activation of somatodendritic 5-HT_{1A} autoreceptors revealed by 5-HT_{1A} receptors antagonists is not fully established in rat. Indeed, *in vitro* and *in vivo*, 5-HT_{1A} receptors antagonists administrations, including spiperone, (-)tertatolol, WAY 100135, WAY 100635 or BMY 7378, produce a slight increase, decrease or no change of the firing activity of 5-HT neurons (Lum and Piercey, 1988; Blier et al., 1993b; Arborelius et al., 1995; Lanfumey et al., 1993; Corradetti et al., 1996). In contrast, Matos et al. (1996) proposed that the latter receptors are under tonic control since they have shown that local perfusion of (-)pindolol increases both DR and hippocampal 5-HT levels, however we should mention that (-)pindolol may also interact with 5-HT_{1B} receptors in the dorsal raphe (Assie and Koek, 1996). It should be also added that local injection of 8-OH-DPAT in the MRN produces a higher decrease of 5-HT release in ventral hippocampus than local injection in the DRN (Bonvento et al., 1992; Kreiss and Lucki, 1994). As previously noted, 5-HT neuron firing activity is negatively regulated by somatodendritic 5-HT_{1A} autoreceptors and their activation reduces both 5-HT neuron firing activity and 5-HT release

in projection areas (Aghajanian, 1990; de Montigny et al., 1984; Bel and Artigas, 1992; Celada and Artigas, 1993). Hence, microdialysis studies have shown that systemic administration of several 5-HT_{1A} receptor ligands such as buspirone, ipsapirone, gepirone, 8-OH-DPAT (also administered locally), NAN-190, SDZ 216-525 or BMY 7378, which reduce 5-HT neuronal firing, decrease the extracellular 5-HT level in the hippocampus of anaesthetized rat and this decrease was antagonized by 5-HT_{1A} receptor antagonists such as pindolol, penbutolol, WAY 100135 and WAY 100635 (Sharp et al., 1989a,c, 1990, 1993; Sharp and Hjorth, 1990; Bonvento et al., 1992; Hjorth and Sharp, 1990, 1993; Hjorth et al., 1995; Routledge et al., 1994, 1995). In *in vitro* superfusion experiments in midbrain raphe slices, it has been shown that 8-OH-DPAT, buspirone and ipsapirone inhibit the electrically-evoked [^{3}H]5-HT release and this effect may be blocked by 5-HT_{1A} receptor antagonists such as NAN-190, WAY 100135 and S-UH-301. Note that WAY 100135 increases the electrically-evoked 5-HT release suggesting tonic activation of 5-HT_{1A} autoreceptors by the endogenous 5-HT (Starkey and Skingle, 1994; Davidson and Stamford, 1995a,b; Piñeyro et al., 1995; Piñeyro and Blier 1996).

Automodulation via 5-$HT_{1B/D}$ receptors:

In *in vivo* voltammetry studies in anaesthetized rat, it has been shown that the systemic administration of the 5-$HT_{1A/B}$ receptor agonists RU 24969 or TFMPP reduce extracellular availability of 5-hydroxindoles in the DRN without altering 5-HT neuronal firing (Blier et al., 1990a; Piñeyro et al., 1995; Piñeyro et al., 1996). Furthermore, the reducing effect of TFMPP on extracellular availability of 5-hydroxindoles is blocked by the α_2-adrenoceptor/5-HT_{1D} receptor antagonist mianserin but not by WAY 100135 (Piñeyro et al., 1995; Piñeyro and Blier, 1996). Using an electrophysiological paradigm, the same group has shown that TFMPP and RU 24969 reduce the duration of suppression of 5-HT neuron firing evoked by the antidromic stimulation of the 5-HT pathway without modifying the basal firing activity of these neurons. The effect of these agonists was blocked by propranolol and mianserin (which has no affinity for 5-HT_{1B} binding sites), further suggesting that 5-HT_{1D} receptors regulate 5-HT release in the DRN. In rodents, where brain 5-HT_{1D} as well as 5-HT_{1B} receptors have been detected, and superfusion studies performed in midbrain raphe slices indicated that 5-HT_{1D} receptors negatively regulate 5-HT release (Hoyer et al., 1985; Waeber et al., 1989a,b, Bruinvels et al., 1993). This somatodendritic release of 5-HT in rodents, regulated by 5-HT_{1D} receptor, is independent of 5-HT neuronal firing. Finally, Piñeyro and Blier (1996) have shown that in the rat midbrain raphe nuclei, 5-HT_{1D} receptors are linked to $G_{i/o}$ proteins, located on 5-HT neurons and may be considered as autoreceptors. On the other hand, in guinea-pigs, only 5-HT_{1D} and not 5-HT_{1B} receptors have been detected (Heuring et al., 1986; Bruinvels et al., 1993). *In vitro* superfusion studies from raphe slices have shown that 5-HT release at the cell body level is also regulated via 5-HT_{1D} receptors since the 5-$HT_{1B/1D}$ receptor antagonist GR 127935 by itself increases the electrically-evoked release of 5-HT in guinea pig (Starkey and Skingle, 1994, El Mansari and Blier, 1996). The 5-HT_{1D} receptor agonist sumatriptan inhibits 5-HT release and this effect is

blocked by GR 127935 which also blocks the inhibitory effect of the non-selective agonist 5-methoxy-tryptamine but not by WAY 100135 and the 5-HT_{1B} receptor agonist CP 93129 has no effect on electrically-evoked release of [^{3}H]5-HT from midbrain guinea-pig slices (Starkey and Skingle, 1994; El Mansari and Blier, 1996).

Terminal 5-HT autoreceptors regulating negatively the 5-HT release have been identified as 5-HT_{1B} receptors in various rat brain areas including hypothalamus (Cerrito and Raiteri, 1979a,b; Martin and Sanders-Bush, 1982), cerebral cortex (Göthert and Weinheimer, 1979; Middlemiss, 1984a,b) and hippocampus (Maura et al., 1986; Feuerstein et al., 1987). In *in vitro* superfusion studies from hippocampal slices, it has been shown that the 5-HT_1 receptors agonist 5-CT decreases the evoked release of [^{3}H]5-HT and this effect is antagonized by the non-selective 5-HT receptor antagonist methiothepin (which by itself increases the evoked release of [^{3}H]5-HT and acts as an inverse agonist, Moret and Briley, 1993), metergoline (which increases the evoked release of [^{3}H]5-HT in presence of SSRIs) and cyanopindolol (which acts as a partial agonist), and other antagonists which possess high affinity for 5-HT_{1B} binding sites. It has been previously demonstrated *in vitro* and *in vivo* that the activation of terminal 5-HT autoreceptors decreases the release of 5-HT (Chaput et al., 1986a, Göthert and Weinheimer, 1979; Blier et al., 1989). *In vivo*, it has been shown that the electrical stimulation of the ascending 5-HT pathway in rat suppresses the firing activity of CA_3 pyramidal neurons by releasing synaptic 5-HT (Chaput et al., 1986a, 1988). The duration of suppression of firing of pyramidal neurons is decresed by the injection of RU 24969, which is antagonized by methiothepin but not by BMY 7378 while methiothepin by itself increases the effectiveness of the stimulation (Chaput et al., 1986, 1988). It has been shown that RU 24969 and TFMPP administered systemically or locally decrease the dialysate 5-HT level from rat frontal cortex and hippocampus (Brazell et al., 1985; Sharp et al., 1989b; Martin et al., 1992). In contrast, Auerbach et al. (1990) have shown that RU 24969 and TFMPP increase the 5-HT release in the hippocampus and it has been suggested that these two ligands, especially at high concentrations, may interact with the 5-HT uptake carrier since their enhancing effect on the 5-HT release is blocked by the 5-HT reuptake inhibitors imipramine and fluoxetine, but not by methiothepin. In fact, TFMPP antagonizes the uptake of [^{3}H]5-HT into synaptosomes (Fuller et al.,1975). On the other hand, the selective 5-HT_{1B} receptor agonist CP-93,129 decreases the dialysate 5-HT level in both presence and absence of citalopram (Hjorth and Tao, 1991).

Automodulation via 5-HT_2 and 5-HT_3 receptors:

As previously mentioned, systemic administration of 5-HT_2 receptor agonist DOI inhibits DR 5-HT firing activity and reduces 5-HT release in rat frontal cortex. In addition, the systemic administration of 5-HT_2 receptor antagonist ritanserin enhances the dialysate 5-HT release in rat nucleus accumbens, suggesting a tonic activation of these receptors by endogenous 5-HT (Wright et al., 1990; Garratt et al., 1991).

In vitro, 2-methyl-5-HT increases the K^+ evoked release of [^{3}H]-5-HT from preloaded slices of guinea-pig hypothalamus and this effect is blocked by selective 5-HT_3 receptor antagonists. In

guinea-pig hypothalamus slices, this enhancement of [^{3}H]-5-HT release induced by 2-methyl-5-HT is abolished in presence of TTX. Moreover, 2-methyl-5-HT does not alter the [^{3}H]-5-HT release in a synaptosomal preparation from guinea-pig hypothalamus, thus indicating that these 5-HT_3 receptors are not located on 5-HT terminals (Blier et al., 1993c). Using intracerebral microdialysis in the rat ventral hippocampus, Martin et al. (1992) reported that 2-methyl-5-HT increased 5-HT levels in the dialysate and that this enhancing effect was abolished by the selective 5-HT_3 receptor antagonist MDL 72222. As shown from *in vitro* and *in vivo* studies (Blier and Bouchard, 1993; Martin et al., 1992), these facilitatory 5-HT_3 receptors are not tonically activated since MDL 72222 did not exert any effect by itself.
The regulation of the 5-HT release in both raphe nuclei and hippocampus by SSRIs and MAOIs will be discussed in sections 5.3 and 5.5.

3. The noradrenaline system.

Firstly called sympathine because it was released by sympathetic nerve terminals and reflecting only the sympathetic innervation of cerebral blood vessels. Noradrenaline (NA) has been established as a neurotransmitter in the CNS using fluorescent techniques. NA is formed in brain, chromaffin cells, sympathetic nerves and ganglia from the amino acid precursor L-tyrosine. L-tyrosine is taken up from the bloodstream and transported actively into the brain. In mammals, tyrosine can be derived from dietary phenylalanine by the enzyme phenylalanine hydroxylase found primarily in liver. The first step of the synthesis is the hydroxylation of L-tyrosine by tyrosine hydroxylase, in presence of O_2, Fe^{2+} and a tetrahydropteridine cofactor. This latter enzyme can be blocked by α-methyltyrosine. This step is the rate-limiting step of the NA biosynthesis. The product formed, 3,4-dihydroxyphenylalanine (DOPA) is decarboxylated by the amino acid decarboxylase, in presence of vitamin B6, to form dopamine (DA). Then, DA is taken up from the cytoplasm to vesicles and hydroxylated by DA-ß-hydroxylase in presence of O_2 and vitamin C to form NA. This enzyme can be blocked by a dithiocarbamate derivative, Fla-63. After its release, NA is taken up into terminals by a specific reuptake carrier. The catabolism of NA is performed, in presence of an aldehyde reductase, by MAO-A which induces the production of 3,4-dihydroxyphenylglycol (DOPEG). This product is excreted and the catechol-O-methyl transferase (COMT) in presence of S-adenosylmethionine induces the formation of 3-methoxy-4-hydroxy-phenylglycol (MHPG) (see Cooper et al., 1991 for review).

3.1. Noradrenergic receptors.

Ahlquist (1948) first proposed the existence of α and ß catecholamine receptors, and on the basis of pharmacological and functional criteria these receptors have been further subdivided

into α_1 and α_2, $ß_1$, $ß_2$ and $ß_3$- adrenoceptors (Langer, 1974; Bylund et al., 1994). Based on radioligand binding properties, signal transduction and deduced amino acid sequences, the adrenoceptors are now divided as follows: $\alpha_{1A,B,D}$ subtypes which are positively coupled to phospholipase C and A_2, $\alpha_{2A/D,B,C}$ subtypes which are negatively coupled to adenylyl cyclase, and $ß_{1,2,3}$ subtypes which are positively coupled to adenylyl cyclase (Bylund et al., 1994). This section will mainly focus on the alpha-adrenoceptors.

3.1.1. α_1-adrenoceptors.

Binding studies have revealed that α_1-adrenoceptors represent a heterogenous population. Three subtypes with a high affinity for the antagonist prazosin (α_{1H} group: α_{1A}, α_{1B} and α_{1D}) and two subtypes with a low affinity for prazosin (α_{1L} group: α_{1L} and α_{1N}) have recently been identified by pharmacological and molecular biological studies (Schwinn and Lomasney, 1992; Hieble et al., 1995; Michel et al ., 1995, Bylund et al., 1994, Ford et al., 1994, Muramatsu et al., 1996). These subtypes have been cloned and pharmacologically characterized (Schwinn et al., 1991; Schwinn and Lomasney, 1992). Compared to the α_{1B}, the α_{1A} subtypes has a 10-100 fold higher affinity for agonists such as methoxamine, oxymetazoline or cirazoline, and for antagonists such as WB4101, phentolamine and (+)-niguldipine (Hieble et al., 1995). In addition KMD-3213 is a selective antagonist for the α_{1A} subtypes whereas agonists for the α_{1B} subtypes include AH 11110A or cyclazosin, and selective antagonists for the α_{1D} subtypes include BMY 7378 (Piascik et al., 1995; Goetz et al., 1995; Giardina et al., 1995; Yamagishi et al., 1996). A binding study with [^{3}H]WB-4101 in the rat cortex revealed an up-regulation of α_1-adrenoceptors following NA fibers dennervation by 6-OHDA suggesting that these receptors are located postsynaptically (U'Prichard et al., 1979). Both autoradiography and *in situ* hybridization studies have demonstrated that α_1-adrenoceptor binding sites and mRNA are widely distributed in the rat CNS. Autoradiography studies revealed low level of α_1-adrenoceptor binding sites in hippocampus, moderate level in raphe nuclei and high level in locus coeruleus (LC) (Unnerstall et al., 1985; Jones et al., 1985; Palacios et al., 1987; Marks et al., 1990; Chamba et al., 1991). A high level of $\alpha_{1A/D}$-adrenoceptor mRNA is found in the hippocampus, a high level of α_{1B}-adrenoceptor mRNA is observed in raphe nuclei whereas no $\alpha_{1A/D}$- and α_{1B}-adrenoceptor mRNAs are detected in LC (Pieribone et al., 1994; Nicholas et al., 1996). However, the distribution of the α_{1A}-adrenoceptor in the rat CNS using both *in situ* hybridization and autoradiography techniques revealed the presence of α_{1A}-receptor mRNA and binding sites in LC but not in raphe nuclei, while in the hippocampus only high mRNA is observed. The authors suggest that the presence of α_{1A}-receptor mRNA and binding sites in the same regions might imply a postsynaptic somatodendritic location in these regions, although a presynaptic location cannot be excluded in the hippocampus (Durkin et al., 1995). The laminar distributions of α_1-adrenoceptors assessed by autoradiography have revealed that high densities in stratum molecular of gyrus dentata and in stratum lacunosum-molecular of CA_1 and CA_2 are found, whereas the hilus and stratum granular of gyrus dentata as well as stratum radiatum of CA_3 show only moderate densities (Zilles et al.,

1991). These authors demontrated, by comparing α_1-adrenoceptors with NA innervation in rat hippocampus, that the density of NA-containing varicosities is much higher and the laminar distribution pattern of hippocampal NA innervation is different from α_1-adrenoceptor distribution, suggesting a possible involvement of postsynaptic α_2- and/or ß-adrenoceptors in the hippocampus NA mediation.

Electrophysiological studies have shown that the activation of α_1-adrenoceptors by NA induce principally excitatory actions of different types of neurons including dorsal motor nucleus of the vagus (Fukada et al., 1987), neonatal LC neurons (Finlayson and Marshall, 1986) and dorsal raphe neurons (Aghajanian, 1985; Yoshimura et al., 1985). This excitation is mainly due to a reduction in resting K^+ conductance and also to a reduction or an enhancement of a Ca^{2+} and/or Ca^{2+}-dependant K^+ current. NA depletion from the terminal by reserpine pretreatment induces a reduction of the neuronal activity suggesting that NA acts as an excitatory neurotransmitter in the DRN (Baraban et al., 1978). The spontaneous firing activity of rat DR neurons is suppressed by the acute administration of α_1-adrenoceptor antagonists such as prazosin, WB4101 or corynanthine, and also by the putative α_2-adrenoceptor antagonists such as yohimbine, rauwolscine or piperoxane (Baraban and Aghajanian, 1980 ; Marwaha and Aghajanian, 1982), and is increased by α_1-adrenoceptor agonist phenylephrine (Vandermaelen and Aghajanian, 1983). In voltage-clamp studies in dorsal raphe neurons, Aghajanian (1985) showed that α_1-adrenoceptors activation produces a depression in the transient outward K^+ current (I_A). Yoshimura et al. (1985) showed that the repetitive focal stimulation to DRN slice surface induces a slow EPSP and that NA pressure ejections caused membrane depolarization. Both the slow EPSP and NA-induced depolarization were inhibited by phentolamine and prazosin but not by yohimbine and propranolol, suggesting the involvement of α_1-adrenoceptors activation. Even though the presence of α_1-adrenoceptor within the LC has been demonstrated (Jones et al., 1985), systemic administrations of α_1-adrenoceptor antagonists (prazosin, WB4101 or corynanthine) do not modify the firing rate of LC NA neurons (Marwaha and Aghajanian, 1982). However, due to the synaptic NA availability, both the α_1-adrenoceptor-mediated excitation (present only in early stage of development) and the α_2-adrenoceptor-mediated collateral inhibition in the regulation of LC neuronal activity have been proposed (Nakamura et al., 1988).

3.1.2. α_2-adrenoceptors

As previously noted, $\alpha_{2A/D,B,C}$-adrenoceptor subtypes have been described. As for 5-HT_{1B} receptors, most of α_2-adrenoceptors are located postsynaptically. Heal et al. (1993) have shown that around 80% of [^{3}H]idazoxan binding sites are unaffected by a NA-lesion with the neurotoxin DSP-4. The decrease in binding sites in different brain regions (such as cortex or hippocampus) clearly demonstrates that these receptors are laso located presynaptically. The classification of α_2-adrenoceptors has been underscored by both pharmacological and molecular studies. The α_{2A}-, α_{2B}- and α_{2C}-adrenoceptor correspond to the human genes α_2-C10, α_2-C2 and α_2-C4 respectively, whereas the rat homologue of α_{2A}-adrenoceptor, the α_{2D} subtypes, corresponds to the rat gene

RG20. Note that all subtypes have been cloned and species differences have been described (see Bylund et al., 1994; MacKinnon et al., 1994). Among the drugs described so far, potent α_2-adrenoceptor antagonists consist of rauwolscine, yohimbine, idazoxan or mianserin and potent agonists consist of clonidine or UK 14304 (see Bylund et al., 1994; MacKinnon et al., 1994). Firstly identified in human platelets, the α_{2A} subtype was characterized as a site with high affinity for the partial agonist oxymatazoline, and the antagonists BRL44408 or rauwolscine; it has a low affinity for prazosin (Chueng et al., 1982). Firstly identified in neonatal rat lung, α_{2B} subtypes is characterized as a site with high affinity for prazosin, antagonists such as ARC 239, and low affinity for oxymatazoline (Bylund, 1985). Firstly found in oppossum kidney, α_{2C} subtypes is characterized as a site with high affinity for prazosin and ARC 239, and has a higher affinity for rauwolscine than the α_{2B} subtype (Murphy and Bylund, 1988). Finally, like the α_{2A} subtypes, α_{2D}-adrenoceptors has a lower affinity for rauwolscine than the other subtypes and is characterized by a low affinity for prazosin and ARC 239. Recently, studies with tritiated RS-15385-197 (MacKinnon et al., 1992) or MK-912 (Uhlen et al., 1992) described the presence of a site in the rat brain having a low affinity for yohimbine or rauwolscine and designated as $\alpha_{2A/D}$-adrenoceptors. However, it should be added that antagonists such as idazoxan, yohimbine, oxymetazoline, rauwolscine, ARC 239 and BRL 44408 recognize 5-HT_{1A} receptor binding sites, suggesting that the α_2-adrenoceptor distribution revealed with such ligands should be taken with caution (Convents et al., 1989; Schoeffter and Hoyer, 1991; Winter and Rabin, 1992; Meana et al., 1996). The rat brain distribution of α_2-adrenoceptors have been investigated using receptor autoradiography (Boyajian and Leslie, 1987, Brüning et al., 1987; Hudson et al., 1992; King et al., 1995), *in situ* hybridization (McCune et al., 1993; Nicholas et al., 1993; Scheinin et al., 1994; Winzer-Serhan et al., 1997a,b) and immunohistochemical techniques (Aoki et al., 1994; Rosin et al., 1996). Wamsley et al. (1992) have proposed that the oxymetazoline-sensitive receptor (α_{2A} or α_{2D}) predominates in the rat LC. In rat brain, among other structures, α_{2A} mRNA labelling was found in LC, dorsal raphe nucleus and CA_3 pyramidal cell layer whereas, in the cerebral cortex, both α_{2A} and α_{2C} mRNAs labelling were observed (Nicholas et al., 1993; Scheinin et al., 1994). On the other hand, Winzer-Serhan et al. (1997a,b) observed high α_{2A} and α_{2C} mRNAs labelling in hippocampus, high α_{2A} mRNA and low α_{2C} mRNA levels in the LC and low levels of both α_{2A} and α_{2C} mRNAs in the DRN.

Many α_2-adrenoceptor ligands bind also to **imidazoline (I) binding sites** in which catecholamines are insensitive. Two classes of I binding sites were defined: I_1 labelled with [^{3}H]clonidine and [^{3}H]para-amino-clonidine have high affinity for imidazolines (clonidine and moxonidine) and oxazoline derivatives (rilmanidine). Agmatine (the decarboxylated arginine) has been proposed to be the endogenous ligand for I_1 (Li et al., 1994a). The I_2 are labelled by [^{3}H]idazoxan or [^{3}H]-RS-450-41-190 and have high affinity for imidazoline derivatives idazoxan or cirazoline (Miralles et al., 1993; Ernsberger et al., 1987; Molderings et al., 1993, 1994; MacKinnon et al., 1995). While autoradiographic studies with such compounds suggested the presence of both I_1 and I_2 in DRN and LC, a recent study from Szabo et al. (1996) rules out the presence of functional I_1 and

I_2 on LC NA neurons. Also, the *in vivo* excitatory effect of I_1 ligands on the firing activity of LC NA neurons previously described by Pineda et al. (1993) appears to be due to a indirect effect, and has recently been proposed to be mediated by I located in the medulla in association with the paragigantocellularis nucleus and modulated by an inhibitory 5-HT mechanism (Ruiz-Ortega et al., 1995; Szabo et al., 1996). This is supported by a recent study showing that agmatine, which recognises α_2-adrenergic binding sites, is devoid of pharmacological activity on these receptors (Pinthong et al., 1995) and fails to modify the firing activity of LC NA neurons *in vitro* (Pineda et al., 1996).

3.2. Noradrenergic neurons.

In the rat brain, half of NA neurons are located in the dorsolateral pontine tegmentum which consists of a tightly packed NA cell group named A6 or locus coeruleus (LC) (Dahlström and Fuxe, 1965; Swansson, 1976; Nygreen and Olson, 1977). With respect to the efferent targets of LC NA neurons, a spatial distribution within the rat LC suggests that projections of both ventral and dorsal hippocampus originate solely from the dorsal segment and those of the hypothalamus originate from the anterior pole of LC (Loughlin et al., 1986a,b). As for 5-HT neurons, the electrophysiological criteria used to identify LC NA neurons include spike waveform and duration (biphasic and longer > 2 ms), discharge frequency (fire spontaneously with a constant rate: 0.2-5 Hz), response to sensory stimulation (burst during nociceptive pinch of the contralateral hind paw) and orthodromic/antidromic activation (Aghajanian, 1978). As for 5-HT neurons, there is evidence that the spontaneous firing of LC neurons correlates in some way with sleep waking cycle (Aston-Jones and Bloom, 1981a,b; Foote et al., 1980; De Sarro et al., 1987). LC NA neurons are endowed with a pacemaker cycle of firing that seems to be intrinsic since it is still present in acutely dissociated and long-term culture preparations (Masuko et al., 1986; Miyake et al., 1989). This tonic pacemaker activity in LC NA neurons depends on endogenous cAMP levels and involves the cAMP phosphorylation pathway. However, the specific substrate that may be phosphorylated by endogenous cAMP via protein kinase A to initiate and maintain tonic firing in LC neurons remains to be identified. Thus, endogenous cAMP appears to induce a persistent Ca^{2+}-independent/TTX-insensitive inward current that depolarizes the cell membrane (Wang and Aghajanian, 1987; Alreja and Aghajanian, 1995). Extracellular recording studies have shown that α_2-adrenoceptor agonists such as NA, clonidine, UK 14304, guanfacine or rilmenidine decrease the firing activity as well as the metabolic activity of LC NA neurons *in vivo* and *in vitro* (Svensson et al., 1975; Cedarbaum and Aghajanian, 1976; Baraban and Aghajanian, 1980; Williams et al., 1985; Dresse and Scuvée-Moreau, 1986; Illes and Norenberg, 1990; Engberg and Eriksson, 1991), whereas α_2-adrenoceptor antagonists such as yohimbine, piperoxan, rauwolscine, idazoxan, mianserin, CP-93-393 or napamezole enhance the firing rate of LC NA neurons, suggesting the presence of a tonic activation of somatodendritic α_2-adrenoceptor by endogenous NA in the LC (Marwaha and Aghajanian, 1982; Freedman and Aghajanian, 1984; Curtis and

Valentino, 1991; Perrone et al., 1990; Reynolds et al., 1995). Hence, the tonic activation of α_2-adrenoceptors by NA release from recurrent axons or/and dendrites may mediate a self-inhibition in the LC. On the other hand, the excitatory α_1-adrenoceptor responsiveness seems to be present only during early stages of development (Williams and Marshall, 1987). Andrade and Aghajanian (1984a,b) have shown that the activation of LC neurons by intracellular current pulses results in a marked AHP which is mediated primarily by the activation of Ca^{2+}-dependent K^+ conductance but not by α_2-adrenoceptor activation. In contrast, intracellular recordings made from LC slices revealed that NA or clonidine (but not phenylephrine or isoprenaline) applications induce membrane hyperpolarizations due to an increase of K^+ conductance, and this hyperpolarization is altered by α_2-adrenoceptor antagonists (Aghajanian and VanderMealen, 1982c; Williams et al., 1985). It has been suggested that these effects are transduced through G_i proteins since permeable analogues of cAMP reverse these effects and pertussis toxin blocks them. In the light of these findings, the opening of K^+ channels by α_2-adrenoceptors (similar with opiate receptor activation on LC NA neurons) seems to be independent of cAMP whereas a cAMP-dependent inward current seems to be affected by the inhibition of adenylate cyclase (Aghajanian and Wang, 1987). Using both extra- and intracellular *in vitro* recordings, Ishimatsu and Williams (1996) have shown that the synchronous activity within LC, which may be the result of firing a cell network, is not synaptically driven but rather the result of electrical interactions between dendrites outside the cell body region. It has been hypothesized that in the resting state, the output from LC would be dependent on the spontaneous activity of individual cells but in the case of the activation of a population of neurons, the electronic coupling between neurons may help regulate activity of cells in the LC to facilitate synchronous increases and decreases in NA release (Ishimatsu and Williams, 1996).

The LC receives wide and divergent afferent projections from the brainstem, spinal cord and forebrain, including the nucleus paragigantocellularis, the prepositus hypoglossi, subregions of the hypothalamus, the periaqueductal gray as well as the mesencephalic reticular formation (Cedarbaum and Aghajanian, 1978; Aston-Jones et al., 1986, 1991a; Luppi et al., 1995). The nucleus paragigantocellularis has been found to provide both inhibitory (NA and enkephalin) and excitatory (glutamate) inputs (Ennis and Aston-Jones, 1988; Aston-Jones et al., 1992), whereas the prepositus hypoglossi provides an inhibitory (GABA) input (Gorea et al., 1991). Although the DR does not provide a robust 5-HT innervation (Aston-Jones et al., 1991), NA neurons of the LC receive dense 5-HT projections (Pickel et al., 1977; Cedarbaum and Aghajanian, 1978; Léger and Descarries, 1978; Segal, 1979; Maeda et al., 1991; Vertes and Kocsis, 1994).
The LC provides a massive, highly divergent efferent axonal network throughout the brain and spinal cord (Segal et al., 1983, Loughlin et al., 1986a,b). NA fibers of the hippocampus originate from LC area via the amygdaloid, the fimbria-fornix and fasciculus cinguli bundles. Immunohistochemical and autoradiography studies of the rat hippocampus have revealed that the highest density of NA varicosities is found in the hilus and stratum lucidum of CA_3 and the lowest densities in all layers of CA_1. The stratum pyramidal and granular of all regions have low

density whereas stratum radiatum and lacunosum-molecular of CA_3 are intermediate (Loy et al., 1980; Milner and Bacon, 1989; Oleskevich et al., 1989).

Electrophysiological studies, using intracellular recordings, showed that NA can produce an hyperpolarization of CA_1 pyramidal neurons and can further block accommodations of neuronal discharges by blocking a Ca^{2+}-activated K^+ conductance responsible for the AHP that follows a train of action potentials (Haas and Konnerth, 1983; Madison and Nicoll, 1982, 1984). Moreover, it can also block the fast transient K^+ current and increases Cl^- conductance (Sah et al., 1985; Langmoen et al., 1981). However, the most prominent effect of NA on hippocampus pyramidal neurons is the blockade of the slow AHP, which alters the firing properties. This effect of NA is mimicked by the $ß_1$-adrenoceptor agonist dobutamine and blocked by the nonselective ß-adrenoceptor antagonist propranolol and the $ß_1$-adrenoceptor antagonist atenolol, but not by the $ß_2$-adrenoceptor antagonist butoxamine (Madisson and Nicoll, 1986). The $ß_1$-adrenoceptors on pyramidal neurons exert their effects by activating adenylate cyclase. In this regard, the effect of NA is mimicked by the cAMP analog 8-BrcAMP or forskolin (an activator of adenylate cyclase) and potentiated by blocking of phosphodiesterase activity (Madisson and Nicoll, 1986). Furthermore, it has been shown in the hippocampus that pressure ejection of NA on pyramidal neurons suppressed their firing activity, an effect blocked by prazosin but not by the α_2-adrenoceptor antagonist rauwolscine (Pang and Rose, 1987). On the other hand, the suppressant effect of microiontophoretically-applied NA onto CA_3 pyramidal neurons is blocked by α_2-adrenoceptor antagonists idazoxan and piperoxan but weakly by prazosin (Curet and de Montigny, 1988a). These authors documented that the suppressant effect of endogenous NA induced by the electrical stimulation of LC on the firing activity of CA_3 pyramidal neurons is increased by clonidine and blocked by prazosin, but not by idazoxan, suggesting that the *in vivo* NA effect on the dorsal hippocampus firing is mediated by extrasynaptic α_2-adrenoceptors and intrasynaptic α_1-adrenoceptors located postsynaptically, while this release is modulated by terminal α_2-adrenergic autoreceptors (Curet and de Montigny, 1988a,b,c). More recently, using *in vitro* whole-cell recordings in rat hippocampus slices, Bergles et al. (1996) have shown that NA potently depolarizes and increases the firing rate of interneurons found in all strata in the CA_1 region, an effect of an α_1-adrenoceptor-dependent reduction in resting K^+ conductance that may contribute to the global inhibitory effects of NA described *in vivo*.

3.3. Modulation of NA release.

Several studies have clearly shown that prejunctional α_2-adrenoceptors are present on nerve endings in both peripheral and central nervous system. These α_2-adrenergic autoreceptors mediate a negative feedback mechanism modulating the Ca^{2+}-dependent stimulation-evoked release of NA (see Starke 1987; Starke et al., 1989, for review). Hence, NA and clonidine decrease the electrically- or K^+-evoked release of [^{3}H]NA from rat and rabbit hippocampus slices. This decrease is antagonized by yohimbine while both yohimbine and idazoxan by themselves

increase the [^{3}H]NA-evoked release, suggesting the existence of a tonic activation of these receptors by endogenous NA (Frankhuijzen and Mulder, 1982; Frankhuijzen et al., 1988; Jackisch et al., 1984; Galzin et al., 1984). Microdialysis studies have shown that the systemic administration of clonidine reduces, whereas yohimbine and idazoxan increase NA levels in the rat hippocampus or cortex (L'Heureux et al., 1986; Dennis et al., 1987; Abercrombie et al., 1988; Thomas and Holman, 1991). Although the molecular mechanism involved in the reduction of the NA release by the α_2-adrenergic autoreceptor activation is still controversial, it has been suggested that the latter inhibit the NA release through a G_i protein-mediated inhibition of adenylate cyclase activity (Markstein et al., 1984; Allgaier et al., 1985, 1986). Since electrical threshold measurements in terminal LC axons have indicated that NA reduces and α_2-adrenoceptor antagonists increase excitability (Nakamura et al., 1981), similarly to the somatodendritic level, it has been proposed that a K^+-conductance increase might contribute to the α_2-adrenergic autoreceptor-mediated inhibition of NA release by indirectly reducing the amount of Ca^{2+} entering at the release site (Williams et al., 1985; Jackisch et al., 1992). Because α_1- and ß-adrenoceptors are generally considered to be postsynaptic receptors, discrepant results have arisen in recent studies. It has been shown *in vitro* that both presynaptic $ß_1$- and $ß_2$-adrenoceptor activations enhance NA release from rat cerebral cortical, hypothalamic and hippocampal slices (Muragaiah and O'Donnell, 1995), and that presynaptic α_1-adrenoceptor activations enhance NA release from rat brain cortical and hippocampal synaptosomes (Pastor et al., 1996). The heteromodulation of NA release via 5-HT system will be discussed in section 4.2.

4. Intermodulations of NA and 5-HT systems.

4.1. Interactions at somatodendritic levels.

As mentioned above, DR neurons receive NA projections from the LC (Loizou, 1969; Anderson et al., 1977; Baraban and Aghajanian, 1981; Jones and Yang, 1985; Luppi et al., 1995). Pharmacological studies suggest that the firing activity of 5-HT neurons in the DRN is dependent on a tonic activation mediated by postsynaptic α_1-adrenergic receptors and also modulated by presynaptic α_2-adrenergic since the reducing or enhancing effects of α_2-adrenoceptor ligands are prevented by neurotoxic NA lesions and the acute administrations of α_1-adrenergic agents modulate the firing activity of DR 5-HT neurons (Svensson et al., 1975; Baraban and Aghajanian, 1980; Clement et al., 1992; Marwaha and Aghajanian, 1982). Moreover, the α_2-adrenoceptor activation reduces 5-HT synthesis in both hippocampus and DRN (Yoshioka et al., 1992a).
On the other hand, several lines of evidence support the notion that the 5-HT system also influences NA neurons. As mentioned above, NA neurons of the LC receive dense 5-HT projections (Pickel et al., 1977; Cedarbaum and Aghajanian, 1978; Léger and Descarries, 1978;

Segal, 1979; Maeda et al., 1991; Vertes and Kocsis, 1994). Electrophysiological and biochemical studies have revealed a tonic inhibitory role of 5-HT on the function of LC NA neurons. Lesion of raphe nuclei or a pretreatment with the 5-HT synthesis inhibitor parachlorophenylalanine increases both tyrosine hydroxylase activity and neuronal firing rate in the LC (Crespi et al., 1980; McRae-Deguerce et al., 1981, 1982; Reader et al., 1986). Although microiontophoretic application of 5-HT_1 receptor agonists does not modify the spontaneous firing activity of LC NA neurons, the activation of 5-HT_1 receptors reduces both glutamate-induced activation and glutamatergic synaptic potentials of NA neurons in the LC (Bobker and Williams, 1989; Aston-Jones et al., 1991b). Partial 5-HT_{1A} receptor agonists administrations, such as buspirone (as well as its analog derivative BMY 7378), gepirone, ipsapirone and their common metabolite 1-(2-pyrimidinyl)-piperazine (1-PP; an α_2-adrenergic antagonist), but not low doses of 8-OH-DPAT, have been shown to increase LC neuron firing activity. This enhancing effects of these partial 5-HT_{1A} receptors agonists have been proposed to be correlated with DA antagonist properties or to their common metabolite 1-PP (Sanghera et al., 1982, 1990; Engberg, 1989; Piercey et al.,1994). Moreover, systemic but not local administration of selective 5-HT_2 receptor antagonists increases the firing activity of LC NA neurons (Rasmussen and Aghajanian, 1986; Gorea and Adrien, 1988) whereas the 5-HT_2 agonist DOI, administered systemically but not locally, decreases the firing activity of LC NA neurons. The latter effect has been proposed to be due to an increased activation of the GABAergic inhibitory input form the prepositus hypoglossal nucleus to the LC (Chiang and Aston-Jones, 1993). Taken together, these data clearly suggest that these two systems might intermodulate their release.

4.2. Interactions at terminal levels.

The presence of α_2-adrenergic heteroreceptors located on brain cortical, hypothalamic and hippocampus 5-HT terminals, which activation reduces 5-HT release, have been well characterized (Göthert and Huth, 1980; Frankhuyzen and Mulder, 1980, Galzin et al., 1984; Starke et al., 1987). In the rat hypothalamus, in contrast with α_2-adrenergic autoreceptors, it has been shown *in vitro* that exogenous NA, but not endogenously released NA, activates α_2-adrenergic heteroreceptors. This suggests that these receptors are not tonically activated by endogenous NA even in the presence of NA reuptake inhibitors (Galzin et al., 1984). On the other hand, Feurstein et al. (1993) have reported a tonic inhibition of 5-HT release via α_2-adrenergic heteroreceptors in human and rat neocortex. *In vitro* studies suggest that the interaction between α_2-adrenergic heteroreceptors and the 5-HT transporter cannot be explained by an increased synaptic availability of NA, but may result from a functional link between these two systems, which depends on the level of activation the presynaptic 5-HT autoreceptor by endogenous 5-HT (Blier et al., 1990a). However, from *in vivo* sudies in the hippocampus, this

statement has to be reconsidered. As the firing activity of 5-HT neurons is modulated via facilitatory α_1-adrenergic and inhibitory α_2-adrenergic influences, one may assume that both α_1-α_2-adrenergic agents modulate 5-HT release *in vivo*, as is the case *in vitro* (Frankhuyzen et al., 1988). In fact, microdialysis studies in the rat ventral hippocampus have shown that systemic administration of α_2-adrenergic agonists reduced the spontaneous release of 5-HT, an effect abolished by idazoxan (Tao and Hjorth, 1992). Furthermore, local administration of clonidine or UK 14304 into the hippocampus decreased the K^+-evoked release of 5-HT in NA-lesioned rats, suggesting that these agonists activate α_2-adrenergic heteroreceptors on terminal 5-HT. These receptors appear to be coupled with G proteins since the effect of UK 14304 is abolished by pretreatment with pertussis toxin (Yoshioka et al., 1992b; Numazawa et al., 1995). Furthermore, using microdialysis in the rat ventral hippocampus, De Boer et al. (1996) have recently shown that while local infusion of TTX decreases the 5-HT release, systemic administration of the α_2-adrenergic antagonist mirtazapine but not mianserin or idazoxan, increase the level of 5-HT. On the other hand, prasozin decreased 5-HT release. In order to distinguish *in vivo* the activations of α_2-adrenergic auto- and heteroreceptors, it has been shown that low doses of clonidine enhance the effectiveness of the electrical stimulation of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus CA_3 pyramidal neurons. In contrast, high doses of clonidine reduce the effectiveness of the stimulation. Both these incremental and decremental effects of clonidine were reversed by the intravenous injection of yohimbine, indicating that these effects are mediated via α_2-adrenoceptors. Furthermore, only the enhancing effect of the low dose of clonidine was abolished in rats pretreated with the NA neurotoxin 6-OHDA. This indicates that the enhancing effect of a low dose of clonidine results from the selective activation of the α_2-adrenergic autoreceptors on NA terminals, thereby reducing the tonic activation by NA of α_2-adrenergic heteroreceptors on 5-HT terminals. On the other hand, the reducing effect of high doses of clonidine could be due to a direct activation of the α_2-adrenergic heteroreceptors on 5-HT terminals (Mongeau et al., 1993). Hence, systemic administration of the selective α_2-adrenergic heteroreceptors antagonist (-)mianserin but not idazoxan nor yohimbine, increase the endogenous release of 5-HT induced by electrical stimulation of the 5-HT pathway, suggesting the *in vivo* existence of tonic activation of α_2-adrenergic heteroreceptors on terminal 5-HT induced by endogenous NA (Mongeau et al., 1993), in conjunction by microdialysis studies in rat frontal cortex and hippocampus (Cheng et al., 1993; De Boer et al., 1996).

Although less compelling than the NA modulation of 5-HT, several studies have demonstrated, *in vivo* and *in vitro*, the presence of a serotonergic modulation of NA release. As previously mentioned, partial 5-HT_{1A} receptor agonists increase whereas 5-HT_2 receptor agonists decrease the firing activity of LC NA neurons, which is in agreement with microdialysis studies from Done and Sharp (1992, 1994). In the hippocampus of anaesthetized rat, these authors showed that the administration of partial 5-HT_{1A} receptor agonists buspirone, gepirone or ipsapirone and their common metabolite 1-PP increase NA levels in the microdialysate, whereas 8-OH-DPAT or MDL 737005EF, which are not metabolized in 1-PP, do not modify NA efflux. Furthermore, as

observed *in vitro* (Mongeau et al., 1994c), the 5-HT_2 receptor agonist DOI or DOB decrease NA release, whereas the 5-HT_2 receptor antagonist ritanserin does not modify NA efflux. In contrast, in the awake rat, the 5-HT_{1A} receptors ligands buspirone, 8-OH-DPAT, MDL 737005EF or NAN-190, as well as the 5-HT_2 receptor ligands ritanserin or ICI 170,809, increase NA efflux. The authors have suggested that there are clear differences in the effects of 5-HT_{1A} and 5-HT_2 receptor activation on NA efflux in hippocampus of anaesthetized versus awake rat. At least in conscious rat, they found that when the tonic 5-HT_{2A} inhibitory influence is removed, either by blocking 5-HT_2 receptors or by decreasing 5-HT release through activation of 5-HT_{1A} autoreceptors, NA release is increased (Done and Sharp, 1992, 1994). However, concerning the 5-HT_{1A} excitatory influence on NA release by 5-HT_{1A} receptors agonists, which seems not to be tonic in awake animal, it has been suggested that this influence may be due to a postsynaptic interaction with the DA and ACh systems, since this effect of 5-HT_{1A} receptors agonists on NA efflux is not abolished in rat pretreated with either the 5,7-DHT neurotoxin or a 5-HT synthesis inhibitor (Piercey et al. 1994; Chen and Reith, 1995; Suzuki et al., 1995; Hajos-Korcsok and Sharp, 1996; Consolo et al., 1996). Furthermore, a 5-HT_3 inhibitory influence on NA release has been described *in vitro* and *in vivo* in both rat hypothalamus and hippocampus, presumably via a indirect action on GABAergic or DAergic systems (Blandina et al., 1991; Matsumoto et al., 1995). In contrast, Mongeau et al. (1994c) described a 5-HT_3 facilitatory influence on the NA release *in vitro* in rat hypothatlamus, hippocampus and cortex. Since this excitatory effect on the NA release was not modified by a 5,7-DHT lesion, and that binding of [^{3}H](S)-zacopride is not affected by a NA dennervation (Kidd et al., 1993), one may assume that 5-HT_3 influences on the NA release are not mediated by 5-HT_3 receptors located on 5-HT or NA terminals. Rather, their location on GABA interneurons may explain these results (Morales et al., 1996b).

Finally, since SSRIs such as fluoxetine or sertraline possess a selective affinity for inhibition of NAT *in vitro* (Koe et al., 1983; Harms, 1983), recent studies showed that local infusion via microdialysis probe of the SSRI fluoxetine increases the NA efflux in the rat frontal cortex of both anaesthetized and conscious rat (Hughes and Stanford, 1996), and also by both citalopram and fluoxetine in the rat VTA or medial prefrontal cortex (Jordan et al., 1994; Chen and Reith, 1994). Moreover, Hughes and Stanford (1996) have also shown *in vitro* that a NA lesion, induced by DSP-4 neurotoxin, reduces the inhibition of cortical synaptosomal [^{3}H]NA uptake induced by both fluoxetine and citalopram. Although not yet defined, it has been hypothesized that the increase of 5-HT by fluoxetine activates 5-HT_{1A}, 5-HT_{2A} or 5-HT_{2C} receptors and increases NA release (Blandina et al., 1991; Done and Sharp, 1994) or occurs via a unknown site such as DA neurons (Wyllie et al., 1985).

5. Effects of antidepressant drugs on NA and 5-HT systems.

5.1. Effects on NA system.

5.1.1. Presynaptic level.

Acute administration of TCA that has potent affinity for the noradrenaline transporter (NAT) (such as desipramine), decrease the firing activity of rat LC NA neurons (Svensson and Usdin, 1978; Scuvée-Moreau and Dresse, 1979; Svensson, 1980; Ceci and Borsini, 1996). Moreover, systemic desipramine administration increases NA efflux in frontal cortex and hippocampus in both anaesthetized and freely moving rats (L'Heureux et al., 1986; Dennis et al., 1987). Pretreatment with α_2-adrenoceptors antagonist rauwolsine or idazoxan potentiates the enhancing effect of desipramine on limbic NA efflux (Palij and Stamford, 1996; D. Nutt, personnal communication). On the other hand, systemic administration of desipramine decreases the effectiveness of rat LC stimulation by increasing the activation of dorsal hippocampus α_2-adrenergic autoreceptors (Curet et al., 1992). While acute administration of the SSRI paroxetine or fluoxetine does not modify the firing activity of LC NA neurons (J-C. Béique, personal communication), it has been shown that acute or short-term treatment with MAO-A inhibitors reduce this firing activity (Blier et al., 1986a,b; Blier and de Montigny, 1987a; Curet et al., 1996). Finally, acute administration of azapirones or α_2-adrenoceptor antagonists increase the firing activity of LC NA neurons (Marwaha and Aghajanian, 1982; Sanghera et al., 1983; Freedman and Aghajanian, 1984; Curtis and Valentino, 1991; Engberg, 1992; Godbout et al., 1991; Piercey et al.,1994) and increase NA levels in the microdialysate (Dennis et al., 1987; Done and Sharp, 1992, 1994; De Boer et al., 1996).

Long-term treatments with TCA (desipramine or imipramine) seem to produce a sustained decrease of the firing activity of LC NA neurons (Huang et al., 1980; Svensson, 1980), although other groups did not find such changes (Valentino et al., 1990; Lacroix et al., 1991). Long-term treatment with MAO-A inhibitors also produce a sustained decrease of the firing activity of LC NA neurons (Blier et al., 1986a,b; Blier and de Montigny, 1987a), whereas chronic treatment with the azapirone tandospirone or α_2-adrenoceptor antagonist mianserin do not modify this firing actvity (Godbout et al., 1991; Curtis and Valentino, 1991). Hence, chronic treatment with MAO-A inhibitor clorgyline, desipramine or electroconvulsive shocks therapy (ECS) increase cortical NA efflux or K^+-evoked synaptosomal [^{3}H]NA release (Finberg et al., 1993; Thomas et al., 1992; Schoffelmeer and Mulder, 1982). However, such treatments fail to modify the effectiveness of the LC stimulation (Blier et al., 1986a; Lacroix et al., 1991). Although, the expression of tyrosine hydroxylase following long-term antidepressant treatments is still controversial and does not seem to be correlated with any modification of firing activity, it appears to be decreased with TCA and ECS (Nestler et al., 1990; Brady et al., 1991; Melia et al., 1992) and increased with the MAO inhibitor phenelzine and SSRI fluoxetine (Brady et al., 1992). The sensitivity of somatodendritic

α_2-adrenoceptors is also variable according to the long-term antidepressant treatments used. TCA's treatments and ECS administrations appear to desensitize these autoreceptors (Scuvée-Moreau and Svensson, 1982; Lacroix et al., 1991), whereas MAO-A inhibitors do not modify the sensitivity of these receptors. Kovachich et al. (1993) reported a down-regulation of α_2-adrenoceptors (assessed with [^{3}H]idazoxan) only in the LC following long-term treatment with mianserin, whereas Piletz et al. (1996) found the same number of binding site in LC following long-term treatment with imipramine. While previous studies have reported an increase of NAT mRNA level in LC, which is not mediated via α_2-adrenoceptor activation (Shores et al., 1994; Szot et al., 1993), more recently, the opposite has been reported. Chronic desipramine treatment has been shown to decrease NAT mRNA levels in LC and this decrease is reversed by a yohimbine pretreatment, suggesting that desipramine, by blocking the NAT and activating α_2-adrenergic autoreceptors, may down-regulate expression of NAT gene itself (Blincoe and Morilak, 1996). Moreover, Bauer and Tejani-Butt (1992) have shown that binding to NAT is reduced in the LC after chronic treatment with desipramine. In contrast, using rat frontal cortical membranes, it has been shown that the number and affinity of NAT (revealed with [^{3}H]nisoxetine) was not altered by long-term treatment of various classes of antidepressant drugs including desipramine, fluoxetine and ECS (Cheetham et al., 1996). Finally, using rat pheochromocytoma (PC12) cells, it has been shown that exposure of desipramine or nisoxetine (3 days) reduce the number, but not the affinity, of NAT binding sites (revealed with [^{3}H]nisoxetine), whereas exposure of NA or citalopram does not. It has been suggested that inhibitors of NAT can down-regulate NAT directly, and are able to do so in the absence of changes in synaptic NA concentration (Zhu and Ordway, 1997).

5.1.2. Postsynaptic level.

There is limited evidence suggesting the existence an enhancement of hippocampal α_1-adrenoceptors sensitivity following chronic antidepressant treatments in different brain regions (Menkes and Aghajanian, 1981; Menkes et al., 1983). Bijak (1989) has shown that long-term treatment with imipramine or mianserin increases the responsiveness of α_1-adrenoceptors to phenylephrine application recorded *in vitro* from CA_1 hippocampal neurons. However, these treatments fail to modify the density of the latter receptors (Nowak and Przegalinski, 1988; Hayakawa et al., 1992). In contrast *in vivo*, treatments with MAO-A inhibitors, desipramine or tandospirone fail to modify the effectiveness of the LC stimulation which is mediated by postsynaptic α_1-adrenoceptors located on CA_3 pyramidal neurons (Blier et al., 1986a; Blier et al., 1991; Lacroix et al., 1991). On the other hand, ECS increases the number of α_1-adrenoceptors in cortex but not in hippocampus (Stockmeier et al., 1987; Nowak and Przegalinski, 1988; Hayakawa et al., 1992). Electrophysiological and binding studies suggest that α_2-adrenoceptors in the dorsal hippocampus are not affected by antidepressant treatments (Blier et al., 1986a,b; de Montigny and Aghajanian, 1978; de Montigny, 1984; Gravel and de Montigny, 1987; Lacroix et al., 1991; Barturen and Garcia-Sevilla, 1992). Finally, chronic treatment with many, but not all,

antidepressants induce a down-regulation of ß-adrenoceptors, a change previously correlated with gradual onset of clinical antidepressant drugs efficacy (Sulser et al., 1983). However, several studies suggest that this ß-adrenoceptor desensitization is not associated with the therapeutic effectiveness of antidepressant drugs. For instance, neither SSRI treatment with paroxetine, fluvoxamine or citalopram, nor the selective NAT inhibitor nisoxetine, down-regulate these receptors (Nelson et al., 1990; Benfield et al., 1986; Hyttel et al., 1984). Furthermore, long-term treatment with desipramine results in a down-regulation of ß-adrenoceptors (Argenti and D'Mello, 1994), but this is also observed following acute treatment (Newman-Tancredi et al., 1996). Finally, the desipramine-induced down-regulation is prevented by a 5,7-DHT or p-chlorophenylalanine pretreatment (Brunello et al., 1982, 1985), suggesting that both 5-HT and NA systems are required for the process of desensitization/down-regulation of central ß-adrenoceptors by antidepressants. More recently, it has been shown that chronic antidepressant treatments (including desipramine, amitriptyline and tranylcypromine) fail to alter the protein and mRNA levels of $G\alpha_s$, $G\alpha_i$ and Gß subunits, suggesting that adaptive changes of rat cortical ß-adrenoceptor-adenylyl cyclase system often seen after chronic treatment with antidepressant drugs are not accompanied by changes in the gene expression of $G\alpha_s$, $G\alpha_i$ and Gß proteins. Rather, the degree of co-translational or post-translational modifications (this includes ADP-ribolysation, phosphorylation which can modify the functional status of these transducing proteins) results in altered couplage efficiency between ß-adrenoceptor and Gs, or between Gs and adenylate cyclase (Emamghoreishi et al., 1996).

5.2. Effects on the 5-HT system.

This section will mainly focus on the acute and chronic effects of different classes of antidepressant drugs on 5-HT neurotransmission (*i.e.* 5-HT neuronal firing, 5-HT release in the raphe nuclei and forebrain as well as on the responsiveness of receptors known to modulate this system).

5.2.1. Partial 5-HT_{1A} receptor agonists.

As previously noted, acute administrations of azapirones (buspirone, gepirone, tandospirone, ipsapirone) suppress the firing activity of 5-HT neurons and reduce 5-HT release (see section 2.3.3). While long-term treatment with gepirone does not modify the effectiveness of the stimulation of the 5-HT pathway (Blier et al., 1987c), paradoxically, the 5-HT release-inhibitory capacity of buspirone is retained despite 10 weeks of repeated treatment with this compound (Soderpalm et al., 1993). Electrophysiological studies have shown that short-term treatments (2 days) with such compounds also reduce the firing activity of 5-HT neurons. However, there is a complete recovery of firing after 2 weeks of treatment. This recovery to normal firing activity is thought to result from the desensitization of the somatodendritic 5-HT_{1A} autoreceptors, since

in vivo and *in vitro*, the reducing effect of 5-HT_{1A} receptor agonists is attenuated after treatments (Blier and de Montigny, 1987b, 1990; Schechter et al., 1990; Godbout et al., 1991; Dong et al., 1996). Mainly studied with ß-adrenoceptors, the mechanisms involved in the desensitization of G-protein-coupled receptors, such as 5-HT_{1A} receptors, are complex and involve at least three distinct processes: uncoupling, sequestration and down-regulation (Hausdorff et al., 1990). It remains unclear whether this desensitisation, occured following azapirone treatments, is associated with a down-regulation of somatodendritic 5-HT_{1A} autoreceptors. Rather, the uncoupling of the receptor from its transduction system may account for this phenomenon (Fanelli et al., 1992; Schechter et al., 1990). However, Newman et al. (1992) have shown that short-term (8 days), but not acute, treatment with either buspirone, ipsapirone or 8-OH-DPAT, reduces 5-HT inhibition of forskolin-stimulated adenylate cyclase activity in rat hippocampal membranes. However, this cannot be transposed to rat midbrain raphe membranes since none of 5-HT_{1A} receptor agonists inhibit the forskolin-stimulated adenylate cyclase activity (Clarke et al., 1996). Hence, as previously mentioned (see section 2.3.2), distinct differences between pre- and postsynaptic (intra- and extrasynaptic) 5-HT_{1A} receptors seem to exist. On the other hand, long-term treatments with azapirones alter neither the responsiveness of terminal hippocampus 5-HT or NA autoreceptors nor change the density or affinity of intra- and extra postsynaptic 5-HT_{1A} receptors located on pyramidal neurons (Blier and de Montigny, 1987; Welner et al., 1989; Schechter et al., 1990; Godbout et al., 1991; Fanelli et al., 1992; Dong et al., 1996). Note that Wieland et al. (1993) have shown that long-term treatment with tandospirone or 1-PP does not change the density of hippocampal 5-HT_{1A} receptors but reduces that of 5-HT_{1A} receptors in the frontal cortex. It has been postulated that long-term treatment with a partial 5-HT_{1A} receptor agonist produced an enhanced tonic activation postsynaptic 5-HT_{1A} receptors resulting from normalized 5-HT release in presence of the exogenous agonist (Blier and de Montigny, 1994).

5.2.2. α_2-adrenoceptor antagonists.

While both α_2-adrenoceptor antagonists increase the firing rate of LC NA neurons, acute administration of idazoxan, but not mianserin, increase the firing activity of 5-HT neurons. However only acute administration of mirtazapine increases 5-HT efflux in the ventral hippocampus (De boer et al., 1996). It has been shown that long-term treatment with mianserin does not alter baseline 5-HT levels from hippocampus microdialysate nor modifies the reducing effect of 8-OH-DPAT on 5-HT release (Kreiss and Lucki, 1995). In addition, long-term treatments with mianserin or idazoxan does not increase the effectiveness of the stimulation of the 5-HT pathway (Mongeau et al., 1994a). However, treatments with the latter α_2-adrenoceptor antagonists induce a desensitization of α_2-adrenergic heteroreceptors located on the 5-HT fibers in the dorsal hippocampus by producing sustained increases in synaptic NA concentrations, leading to the enhancement of 5-HT release. A down-regulation of 5-HT_{2C} receptors and paradoxical up-regulation of 5-HT_{2C} receptor mRNA levels (Sanders-bush, 1990; Hamon et al., 1990), as well as a down-regulation of 5-HT_{2A} receptors without any change in mRNA levels

(Roth et al., 1990), have been shown to occur in rat brain after mianserin treatment. However, none of the above treatments altered the responsiveness of terminal 5-HT autoreceptors, nor postsynaptic 5-HT_{1A} receptors or α_2-adrenoceptors located on pyramidal cell of dorsal hippocampus (Mongeau et al., 1994b). Here again, this desensitization of α_2-adrenergic heteroreceptors is not necessarily associated with a down-regulation of the receptors.

5.2.3. Selective 5-HT reuptake inhibitors.

Acute administrations of SSRIs suppress the firing activity of 5-HT neurons (Chaput et al., 1986b; Jolas et la., 1994; Arborelius et al., 1995; Hajos et al., 1995). This is due to an increase of somatodendritic 5-HT release which activates 5-HT_{1A} autoreceptors and this release is blocked by TTX and reduced by 5-HT_{1A} receptor agonists (Sharp and Hjorth, 1990; Artigas, 1993; Fuller, 1994). In fact, single administration of SSRIs at low doses increase 5-HT levels and this effect is greater in raphe nuclei than in projecting areas (Bel and Artigas, 1992; Invernizzi et al., 1992; Fuller et al., 1994; Malagié et al., 1995). This presumably reflects high densities of 5-HTT located on cell bodies (Hrdina et al., 1990; Vergé et al., 1985). Moreover, the increase in raphe nuclei appears to limit the forebrain response to antidepressant drugs and the 5-HT transmission-enhancing effect of 5-HTT blockade is self-limiting, owing to the concomitant activation of 5-$HT_{1A/1B}$ autoreceptors (Invernizzi et al., 1992; Hjorth, 1993). To demonstrate the involvement of somatodendritic 5-HT_{1A} autoreceptors in the antidepressant effects in 5-HT nerve terminal regions, microdialysis studies have revealed that administration of 8-OH-DPAT a few hours after acute administration of fluoxetine reverses the SSRI-induced increase in 5-HT levels in the striatum and the diencephalon (Rutter and Auerbach, 1993). Furthermore, the effects of a low dose of SSRIs (which do not alter 5-HT levels) were increased by pre-treating animals with 5-$HT_{1A/1B}$ or selective 5-HT_{1A} receptors antagonists. In fact, it has been shown that 5-$HT_{1A/1B}$ receptor antagonists such as (-)penbutol, (-)pindolol or methiothepin as well as selective 5-HT_{1A} receptor antagonists such as UH-301 or WAY 100635 potentiate the ability of SSRIs (including the more selective citalopram and more commonly prescribed fluoxetine) to increase nerve terminal output of 5-HT (Invernizzi et al., 1992; Hjorth, 1993, 1996; 1996; Hjorth and Auerbach, 1994; Artigas et al., 1996; Malagié et al., 1995; Gartside et al., 1995; Romero et al., 19996; Gardier et al., 1996). Short-term treatment with SSRIs also reduces the firing activity of 5-HT neurons. There is a complete recovery of firing after 2 weeks of treatment. This recovery to normal firing activity may result in the desensitization of somatodendritic 5-HT_{1A} autoreceptors since *in vivo* and *in vitro* the reducing effect of 5-HT_{1A} receptor agonists is attenuated after SSRI treatments (see Blier and de Montigny, 1994). As was the case with 5-HT_{1A} receptor agonists, this desensitization of 5-HT_{1A} autoreceptors is not necessarily associated with a down-regulation of the receptors. Li et al. (1994) found that long-term treatment with fluoxetine reduces [^{3}H]8-OH-DPAT binding sites in raphe nuclei whereas other groups using paroxetine, fluoxetine, citalopram or sertraline, found no change in 5-HT_{1A} receptor density (Hensler et al., 1991; Le Poul et al., 1995). Although still unclear, second messenger effects (decrease of $G_{\alpha s}$ mRNA in raphe by

sustained administration of fluoxetine) and/or the effector channels could be candidates for this phenomenon (Lesch et al., 1992; Lesch and Manji, 1992). This assumption is supported a recent showning that the levels of G_o and G_{i2} proteins in the midbrain are reduced by a 3-day treatment with fluoxetine and that this decrease remained at the same level for 22 days of treatment. Because G_o proteins are coupled with 5-HT_{1A} autoreceptors, the decrease of their levels may be associated with the desensitisation of these autoreceptors (Li et al., 1996). However, this reduction of G proteins level is unlikely due exclusively to these autoreceptors, as other receptors coupled to $G_{o/i}$ proteins present in the midbrain could be also involved. For instance, although short-term treatments have not been performed, it has been recently shown *in vitro* that long-term treatment with paroxetine desensitizes somatodendritic 5-HT_{1D} autoreceptors in rat midbrain raphe nuclei, which may contribute to the increase in 5-HT neurotransmission induced by SSRI treatments (Piñeyro and Blier, 1996). Studies of the effects of SSRIs on the postsynaptic 5-HT_{1A} receptor density are controversial. Binding data show that long-term treatment with SSRIs either decreases, increases or does not change 5-HT_{1A} receptor density in the hippocampus (Hensler et al.,1991; Klimek et al., 1994; Le Poul et al., 1995; Yamaguchi et al., 1995; Maj et al., 1996). Moreover, SSRIs treatment do not affect the sensitivity of postsynaptic 5-HT_{1A} receptors in the hippocampus *in vivo* or *ex vivo* (Chaput et al., 1991; Maj and Moryl, 1992, 1993), though a recent *ex vivo* report has shown that paroxetine increases this sensitivity (Maj et al., 1996). On the other hand, treatments with SSRIs (citalopram, zimelidine, fluoxetine and paroxetine) increase *in vivo* the efficacy of 5-HT neurotransmission (enhancing the effectiveness of the stimulation of 5-HT pathway to suppress the firing activity of CA_3 pyramidal neurons) and *in vitro* (paroxetine treatment increases the electrically-evoked release of tritiated 5-HT from preloaded hippocampal slices). Furthermore, SSRIs treatments desensitize terminal 5-$HT_{1B/1D}$ autoreceptors in the hippocampus or hypothalamus as shown both *in vitro* and *in vivo* in rat and guinea-pig. In fact, long-term SSRIs treatments altered the enhancing effect of methiothepin on the effectiveness of the stimulation of 5-HT pathway to suppress the firing activity of CA_3 pyramidal neurons, and the decrease in the effectiveness of the stimulation (upon increasing its frequency) as well as the inhibitory effect of 5-HT_{1B} receptor agonist 5-CT on electrically-evoked release of tritiated 5-HT (Blier and de Montigny, 1983; Blier et al., 1988, 1990; Blier and Bouchard, 1994; Moret and Briley, 1990; Chaput et al., 1986b; El Mansari and Blier, 1996; Piñeyro and Blier, 1996). In contrast, a microdialysis study in rat dorsal hippocampus treated orally with fluvoxamine, found no change in 5-HT_{1B} receptor sensitivity and this study failed to demonstrate any changes in basal and fluvoxamine-induced increases in extracellular 5-HT levels in the MRN or dorsal hippocampus (Bosker et al., 1995). Finally, paroxetine treatment does not affect the sensitivity of rat hippocampus terminal α_2-adrenergic heteroreceptors (Mongeau et al., 1994a), and treatments with SSRIs have been shown to produce mainly decreases or no change of 5-HTT in midbrain raphe, hippocampus and frontal cortex (Cheetham et al., 1993; Dewar et al., 1993; Lesch et al., 1993; Marcusson et al., 1988; Piñeyro et al., 1994). Finally, a recent study in rat revealed that chronic treatments with citalopram or fluoxetine increase the choroid plexus 5-HT_{2C}

receptor density (Laakso et al., 1996). This contrasts with data from Spurlock et al. (1994) in which citalopram or fluvoxamine treatment did not alter rat brain 5-HT_{2C} receptor mRNA levels. However, it has been suggested that chronic SSRIs (but not the TCA desipramine) reduces 5-HT_{2C} receptor responsiveness (Kennett et al., 1994). Since an up-regulation of 5-HT_{2C} receptors in the choroid plexus can be caused by a 5-HT lesion, and a down-regulation by chronic treatment with 5-HT agonists and antagonist (Sanders-Bush and Breeding, 1990; Kidd et al., 1990), one may assume that the up-regulation of 5-HT_{2C} receptors in the choroid plexus observed after citalopram or fluoxetine treatments may not solely be due to a 5-HTT blockade but also via a direct interaction of these SSRIs with 5-HT_{2C} receptors since the drugs possess a high affinity for these receptors (Pälvimäki et al., 1996). We should note here that chronic treatment with ritanserin does not alter the 5-HT release in the rat frontal cortex but reduces the 5-HT_{1A} receptors-mediated autoregulation of 5-HT release (Kidd et al., 1990). As mentioned in section 1, several studies have demonstrated an up-regulation of 5-HT_{2A} receptors in the brain of suicide victim; also we have seen that agonists induce a down-regulation of 5-HT_{2A} receptors but antagonists can produce both an up- and down-regulation of these receptors. Lesions of raphe nuclei produce no compensatory up-regulation (see section 2.2). Chronic treatment with fluoxetine results in increased levels of 5-HT_{2A} receptor mRNA but no increase in binding sites (Butler et al., 1993). Further studies of the relation between 5-HT_{2C} receptor binding sites and 5-HT_{2C} receptor mRNA levels by different antidepressant treatments in various brain region are needed to clarify these discrepancies. Finally, Bijak et al. (1997) have recently shown that long-term treatment with SSRI (citalopram, fuvoxamine and paroxetine), as well as the TCA imipramine, induces a subsensitivity of the excitatory effect of 5-HT_4 receptor activation in the rat CA_1 pyramidal cells.

5.2.4. TCA and dual 5-HT and NA reuptake inhibitors.

The inhibitory effect of TCA on the firing activity of DR 5-HT neurons is correlated with their preferential ability to block 5-HTT rather than NAT. In fact, acute administration of imipramine and amitriptyline (IC_{50} for 5-HT uptake blockade: 80 and 40 nM, respectively) suppress the firing activity of 5-HT neurons, whereas nortriptyline and desipramine (IC_{50} for 5-HT uptake blockade: 160 and 180 nM, respectively) do not (Scuvée-Moreau and Dresse, 1979; Bolden-Watson and Richelson, 1993); note that nortriptyline and desipramine are more potent NA reuptake inhibitors. It has been shown that the firing activity of DR 5-HT neurons and the sensitivity as well as density of DR somatodendritic 5-HT_{1A} autoreceptors remains unchanged following long-term amitriptyline treatment (Blier and de Montigny, 1980; Welner et al., 1989). *In vivo*, it has been shown that long-term imipramine treatment increases the effectiveness of the stimulation of the 5-HT pathway to suppress the firing activity of CA_3 pyramidal neurons, though the sensitivity of terminal 5-HT_{1B} autoreceptors is unchanged (Blier et al., 1987c; Chaput et al., 1991; Sleight et al., 1989). On the other hand, *in vitro*, long-term desipramine treatment fails to alter the K^+-evoked release of tritiated 5-HT from both cortex and hippocampus slices (Schoffelmeer and

Mulder, 1983). In a recent microdialysis study, it has been shown that long-term treatment with desipramine increases baseline 5-HT levels in the striatum but not in the hippocampus (Kreiss and Lucki, 1995). Moreover, Mongeau et al. (1994c) have shown that long-term desipramine treatment desensitized 5-HT_3 receptors modulating the electrically evoked release of tritiated NA from rat hippocampal slices. Most TCA studied *in vivo* and *ex vivo* appear to hypersensitize postsynaptic (both intra- and extrasynaptic) 5-HT_{1A} receptors (de Montigny and Aghajanian, 1978; Gallager and Bunney, 1979; Gravel and de Montigny, 1987; Chaput et al., 1991; Dijcks et al., 1991; Bijak et al., 1997; Maj et al., 1996), though negative results have been reported (Olpe and Schelenberg, 1981; Rowan and Anwyl, 1985; Beck and Halloran, 1989). Perhaps, these negative results are due to the use of 5-HT as an agonist, which can also increase the cell excitability. Most autoradiographic studies have revealed no change of 5-HT_{1A} receptors in the hippocampus following TCA treatments (Watanabe et al., 1993; Hayakawa et al., 1994; Bijak et al., 1997), though increases have been reported (Welner et al., 1989; Burnet et al., 1994). Rather, an increased efficacy of postreceptor transduction mechanisms, such as G-protein coupling to the K^+ channel may be postulated for the sensitization of the inhibitory effects of 5-HT_{1A} receptors agonists in the hippocampus following TCA treatments. However, studies on the alteration in the 5-HT_{1A} receptor-mediated inhibition of forskolin activated adenylate cyclase following TCA treatment are still controversial (Newman and Lerer, 1988, 1989; Varrault et al., 1991; Odagaki et al., 1991). Most of TCA possess a moderate to high affinity for 5-$HT_{2A/2C}$ binding sites and may act as antagonists (Griebel, 1995; Tohda and Nomura, 1991). Few studies have been undertaken to characterize the effect of chronic TCA treatment on the regulation of these receptors. However, it has been shown that long-term TCA treatments appears to reduce 5-HT_{2A} receptor binding sites without changing mRNA levels (Paul et al., 1988; Roth et al., 1990; Spurlock et al., 1994). TCA treatment also down-regulates 5-HT_{2C} receptors (Mizuta and Segawa, 1989) with both no change or increase in 5-HT_{2C} receptor mRNA levels (Spurlock et al., 1994; Tohda and Watanabe, 1996). Further studies on the relation between 5-$HT_{2A/2C}$ receptor and their mRNA levels, as well as the PI-signalling system by different antidepressant drugs, are needed. It has been shown *in vitro* that long-term treatment with desipramine, but not with the TCA trimipramine nor the SSRI fluoxetine, desensitizes 5-HT_3 receptors modulating [^{3}H]NA release from rat hippocampal slices (Mongeau et al., 1994d). It has been shown that systemic administration of desipramine fails to modify the firing activity of 5-HT neurons, but reduces the effectiveness of the stimulation of the 5-HT pathway to suppress the firing activity of CA_3 pyramidal neurons (Mongeau et al., 1993), and long-term administration of desipramine does not change the K^+-evoked release of [^{3}H]5-HT from rat cortex and hippocampus slices. Microdialysis study has reported that long-term treatment with desipramine desensitizes α_2-adrenergic heteroreceptors on 5-HT fibers modulating 5-HT levels from rat hippocampus (Yoshioka et al., 1995). Finally, it has been shown *in vitro* that chronic amitriptyline exposure, in hybrid cell line NG 108-15, reduce the affinity of 5-HT_3 receptors (Shimizu et al., 1996). The reason for such a desensitization is still unknown since amitriptyline possess a moderate affinity for the latter

receptors (Ki: 0.26 μM, Griebel, 1995).

There is sparse data on the *in vivo* characterization of dual NA and 5-HT reuptake inhibitors. As was the case with SSRIs, both acute administration of venlafaxine or duloxetine, which are both preferential 5-HTT inhibitors, reduces the firing activity of DR 5-HT neurons (Kasamo et al., 1996; Béïque et al., 1996). Furthermore, it has been also shown that a 2-day treatment with (-)pindolol reverse the suppressant effect of acute venlafaxine on the firing activity of 5-HT neurons (Béïque, J-C, personal communication). In addition, acute coadministration of duloxetine and a 5-HT_{1A} receptor antagonist LY 206130 (an analogue of pindolol), but not with the ß-adrenergic antagonist metoprolol, potentiates the rat hypothalamic 5-HT basal levels assessed by microdialysis (Engleman et al., 1996). Further studies are needed to assess the responsiveness of other NA and 5-HT receptors, as well as to determine whether such drugs could produce sustained modification of the NA and 5-HT release.

5.2.5. ECS and MAO inhibitors.

Only few studies have examined the effects ECS on 5-HT system. ECS neither alters the firing activity of 5-HT neurons nor modifies the sensitivity of 5-HT_{1A} autoreceptors (Blier and Bouchard, 1992). Furthermore, ECS neither induces a sustained increase of 5-HT release nor modifies the sensitivity of 5-HT_3 receptors modulating the electrically-evoked [^{3}H]5-HT release or that of terminal 5-HT_{1B} autoreceptors in the hippocampus (Chaput et al., 1991; Blier and Bouchard, 1992). On the other hand, it is well established that ECS induces a sensitization of forebrain postsynaptic 5-HT_{1A} receptors (de Montigny, 1984; Chaput et al., 1991) but the correlation with changes in their density is still controversial (Pandey et al., 1991; Nowak and Dulinski, 1991; Stockmeier et al., 1992). Moreover, Pei and Zetterstrom (1996) have recently shown that ECS increases, only in the gyrus dantata and not in other subregions of hippocampus, mRNA levels of the rat voltage-dependent K^+ channel (subunit Kv4.2). However, it has been shown that ECS can reduce the 5-HT_{1A} receptor-mediated inhibition of forskolin activated adenylate cyclase (Newman and Lerer, 1988; Varrault et al., 1991). In contrast with other treatments, ECS has been shown to increase the density and mRNA level of 5-HT_{2A} receptors in rat cortex (Kellar et al., 1981; Butler et al., 1993), but the modification of 5-HT-stimulated PI turnover signalling following ECS is still controversial (Newman and Lerer, 1989; Pandey et al., 1992).

As for SSRIs, acute and short-term administrations of MAOI (pargyline, tranylcypromine, phenelzine, clorgyline, amiflamine) suppress the firing activity of 5-HT neurons (Aghajanian et al., 1970; Blier et al., 1986a,b). This transient decrease in the 5-HT neuronal firing is followed by complete recovery after 3 weeks of MAOI treatment (Blier and de Montigny, 1987a; Blier et al., 1986a,b). This recovery to normal firing activity could result in the desensitization of somatodendritic 5-HT_{1A} autoreceptors since *in vivo* the reducing effect of 5-HT_{1A} receptor agonists is attenuated after MAOI treatments (see Blier and de Montigny, 1994). This desensitization of 5-HT_{1A} autoreceptors is not necessarily associated with a down-regulation of the receptors

(Hensler et al., 1991). Similarly to SSRI, systemic administration of MAOI (tranylcypromine, clorgyline, brofaromine) increase microdialysate 5-HT levels and this effect is greater in raphe nuclei than in projecting areas (Celada and Artigas, 1993; Ferrer and Artigas, 1994; Bel and Artigas, 1995). *In vitro*, it has been also shown that MAOI amiflamine increases the release of [^{3}H]5-HT from rat occipital cortex slices (Ask et al., 1989). Although the sensitivity of terminal 5-HT_{1B} autoreceptors and postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus remains unchanged following long-term MAOI treatments, it has been shown that these treatments increase the effectiveness of the stimulation of the 5-HT pathway to suppress the firing activity of CA_3 pyramidal neurons (Blier et al., 1986 a,b). However, *in vitro*, it has been shown that long-term treatment with befloxatone increases the electrically-evoked release of [^{3}H]5-HT from rat and guinea-pig hippocampus and hypothalamus slices, alhough the sensitivity of terminal 5-$HT_{1B/1D}$ autoreceptors remains unchanged, and a desensitization of terminal α_2-adrenergic heteroreceptors is only observed in hypothalamus (Blier and Bouchard, 1994). *In vivo*, a microdialysis study revealed that long-term treatment with the MAOI MDL 72394 increases 5-HT basal levels in rat frontal cortex without altering the sensitivity of terminal 5-HT_{1B} autoreceptors (Sleight et al., 1989). Finally, Mongeau et al. (1994b) have shown that long-term treatment with befloxatone does not increase the effectiveness of the stimulation of the 5-HT pathway to suppress the firing activity of CA_3 pyramidal neurons but desensitizes terminal α_2-adrenergic heteroreceptors on 5-HT fibers.

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Chapter II: First article

Automodulation of the 5-HT system via 5-$HT_{3/4}$ receptor subtypes:

As reported in section 2.2.3 of the introduction, radioligand binding studies have shown that cortical and limbic regions contain moderate to relatively high density of 5-HT_3 binding sites, and although their cellular location is not yet defined, they are not autoreceptors. Their activation generally mediates a rapid depolarization of peripheral and central neurons but in the rat medial prefrontal cortex, 5-HT and the 5-HT_3 receptor agonist 2-methyl-5-HT can suppress neuronal firing activity.

A first subject of interest was to further pharmacologically characterize, using *in vivo* and *in vitro* electrophysiological paradigms, the central 5-HT_3 receptors mediation. Hence, the effect of the prototypical 5-HT_3 receptor agonist 2-methyl-5-HT on the firing activity of rat somatosensory cortical neurons, dorsal hippocampus pyramidal neurons and dorsal raphe 5-HT neurons was studied. Second the capacity of 5-HT_3 receptor ligands to modify the endogenous release of 5-HT induced by the electrical stimulation of the ascending 5-HT pathway was assessed. Finally, the capacity of 2-methyl-5-HT to modify the electrically-evoked of [^{3}H]5-HT release in preloaded slices of the rat frontal cortex and hippocampus was examined in superfusion experiments.

This article entitle "*Pre-and postsynaptic effects of the 5-HT_3 agonist 2-methyl-5-HT on the 5-HT system in the rat brain.*" by myself and Pierre Blier was published in Synapse (1995, vol. 20, pp. 54-67).

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Pre- and Post-Synaptic Effects of the 5-HT_3 Agonist 2-Methyl-5-HT on the 5-HT System in the Rat Brain

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KEY WORDS 2-Methyl-5-HT, 5-HT_3 receptors, 5-HT_{1A} receptors, 5-HT_4 receptors, [^{3}H]-5-HT release, Zacopride, BRL 46470A, BMY 7378, 8-OH-DPAT, WAY 100135, Renzapride, Tropisetron

ABSTRACT Microiontophoretic applications of 5-HT and of the 5-HT_3 agonist 2-methyl-5-HT produced a current-dependent suppression of firing activity of both hippocampal (CA_1 and CA_3) and cortical neurons in anesthetized rats. Concomitant microiontophoretic applications of the 5-HT_3 antagonists BRL 46470A and S-zacopride, as well as their intravenous injection, did not antagonize the inhibitory effect of 5-HT and 2-methyl-5-HT. In contrast, the 5-HT_{1A} antagonist BMY 7378, applied by microiontophoresis or administered intravenously, significantly reduced the inhibitory action of 5-HT and 2-methyl-5-HT. The firing activity of dorsal raphe 5-HT neurons was also reduced by 5-HT, 2-methyl-5-HT and the 5-HT_{1A} agonist 8-OH-DPAT applied by microiontophoresis. While BRL 46470A (0.1 and 1 mg/kg, i.v.) did not antagonize the inhibitory effect of the three 5-HT agonists on 5-HT neuronal firing activity, only that of 8-OH-DPAT was attenuated by the 5-HT_{1A} antagonist (+) WAY 100135. R-zacopride significantly reduced the duration of suppression of firing activity of CA_3 pyramidal neurons induced by the electrical stimulation of the ascending 5-HT pathway, and this reducing effect was prevented by the three 5-HT_3/5-HT_4 antagonists renzapride, S-zacopride and tropisetron, but not by BRL 46470A. Finally, in in vitro superfusion experiments, both BRL 46470A and S-zacopride antagonized the enhancing action of 2-methyl-5-HT on the electrically-evoked release of [^{3}H]-5-HT in both rat frontal cortex and hippocampus slices. These findings suggest that, in vivo, the suppressant effect of 2-methyl-5-HT on the firing activity of dorsal hippocampus pyramidal, somatosensory cortical, and dorsal raphe 5-HT neurons is not mediated by 5-HT_3 receptors, but rather by 5-HT_{1A} receptors. The attenuating effect of R-zacopride on the effectiveness of the stimulation of the ascending 5-HT pathway is not mediated by 5-HT_3 receptors. In contrast, in vitro, the enhancing action of 2-methyl-5-HT on the electrically-evoked release of [^{3}H]5-HT in both frontal cortex and hippocampus slices is mediated by 5-HT_3 receptors.

INTRODUCTION

Among the multiple subtypes of 5-HT receptors, only the 5-HT_3 receptor incorporates a ligand-gated ion channel (Bobker and Williams, 1990; Derkach et al., 1989; Peters and Lambert, 1989). 5-HT_3 receptors have first been characterized and identified by pharmacological criteria using highly selective ligands (Richardson and Engel, 1986) and has been recently cloned (Maricq et al., 1991). Radioligand binding studies have shown that cortical and limbic regions contain moderate to relatively high density of 5-HT_3 binding sites (Kilpatrick et al., 1987; see, for review, Hoyer, 1990). Their activation generally mediates a rapid depolarization of peripheral and central neurons (Kilpatrick et al., 1987; Peters and Lambert, 1989; Richardson and Engel, 1986). In contrast, Ashby et al. (1992) demonstrated that in the rat medial prefrontal cortex, 5-HT and the 5-HT_3 agonist 2-methyl-5-HT can suppress neuronal firing activity. This inhibitory effect of 5-HT and 2-methyl-5-HT is blocked by 5-HT_3, but not by 5-HT_1 or 5-HT_2 antagonists. This suggests that in this cortical region, the action of 5-HT may be mediated by 5-HT_3-like receptors producing a hyperpolarization. Several studies have also shown that 5-HT_3 receptors can modulate the release of different neurotransmitters like

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noradrenaline (Feuerstein et al., 1986; Mongeau et al., 1994), acetylcholine (Barnes et al., 1989), dopamine (Blandina et al., 1989), cholecystokinin (Paudice and Raiteri, 1991), and 5-HT (Blier and Bouchard, 1992, 1993a; Galzin and Langer, 1991).

The present study was undertaken, first, to pharmacologically characterize, using in vivo electrophysiological paradigms, the effect of 2-methyl-5-HT on the firing activity of rat somatosensory cortical neurons, dorsal hippocampus pyramidal neurons, and dorsal raphe 5-HT neurons and, second, to assess the capacity of 5-HT_3 receptor ligands to modify the endogenous release of 5-HT induced by the electrical stimulation of the ascending 5-HT pathway. Finally, the capacity of 2-methyl-5-HT to modify the electrically-evoked of [^{3}H]5-HT release in preloaded slices of the rat frontal cortex and hippocampus was examined in superfusion experiments.

MATERIALS AND METHODS

The experiments were carried out in male Sprague-Dawley rats, weighing 250 to 300 g, kept under standard laboratory conditions (12:12 light-dark cycle with free access to food and water), and anaesthetized with chloral hydrate (400 mg/kg, i.p.). Supplemental doses were given to maintain the constant anaesthesia and to prevent any nociceptive reaction to a tail pinch.

Recordings from somatosensory cortical neurons, CA_1, and CA_3 dorsal hippocampus pyramidal neurons

Recording and microiontophoresis were performed with five-barrelled glass micropipettes broken back to 8–12 μm under microscopic control (ASI Instruments, Warren, MI). The central barrel was filled with a 2 M NaCl solution and used for extracellular unitary recording. The pyramidal neurons were identified by their large amplitude (0.5–1.2 mV) and long-duration (0.8–1.2 ms) simple spikes alternating with complex spike discharges (Kandel and Spencer, 1961). The side barrels contained three of the following solutions: 5-HT creatinine sulphate (5 mM in 200 mM NaCl, pH 4), 2-methyl-5-HT (10 or 20 mM in 100 mM NaCl, pH 4), quisqualic acid (1.5 mM in 200 mM NaCl, pH 8), glutamic acid (10 mM in 100 mM NaCl, pH 8), BRL 46470A (10 mM in 10 mM NaCl, pH 4), S-zacopride (10 mM in 100 mM NaCl, pH 4), or BMY 7378 (50 mM in 200 mM NaCl, pH 3) and 2 M NaCl used for automatic current balancing. The rats were mounted in a stereotaxic apparatus and the microelectrodes were lowered at 4.2 mm lateral and 4.2 anterior to lambda into the somatosensory cortical layer and the CA_1-CA_3 region of the dorsal hippocampus. Since most somatosensory cortical and hippocampus pyramidal neurons are not spontaneously active under chloral hydrate anaesthesia, a small current of quisqualate (+3 to −7 nA) was used to activate them within their physiological firing range (10–15 Hz; Ranck, 1975). Neuronal responsiveness to the microiontophoretic application of 5-HT and 2-methyl-5-HT were assessed by determining the number of spikes suppressed per nanoampere. The duration of the microiontophoretic applications of the agonists was of 50 s. The same ejection current was always used before and after each intravenous injection of the selective 5-HT_3 antagonist BRL 46470A (0.003, 0.1 and 1 mg/kg; Newberry et al., 1993) and the selective 5-HT_{1A} antagonist BMY 7378 (1 mg/kg; Chaput and de Montigny, 1988).

Recordings of dorsal raphe 5-HT neurons

The microiontophoresis were performed with five-barrelled micropipettes preloaded (R & D Scientific glass CO, Spencerville, MD) with fibreglass filaments in order to facilitate filling and the tip was broken back to 4 to 8 μm. The central barrel was used for recording and filled with a 2 M NaCl solution. The side barrels contained the following solutions: 5-HT creatinine sulphate (20 mM in 200 mM NaCl, pH 4), 2-methyl-5-HT (10 or 20 mM in 100 mM NaCl, pH 4), 8-OH-DPAT (0.5 or 1 mM in 100 mM NaCl, pH 4), and 2 M NaCl used for automatic current balancing. The rats were fixed in a stereotaxic frame and a burr hole was drilled on midline 1 mm anterior to lambda. Dorsal raphe 5-HT neurons were encountered over a distance of 1 mm starting immediately below the ventral border of the Sylvius aqueduct. These neurons were identified using the criteria of Aghajanian (1978): a slow (0.5–2.5 Hz) and regular firing rate and a long-duration (0.8–1.2 ms) positive action potential. The responsiveness of 5-HT neurons to 40 s microiontophoretic applications was always assessed using the same ejection current prior to and following the intravenous injection of the selective 5-HT_3 antagonist BRL 46470A (0.1 or 1 mg/kg) or the selective 5-HT_{1A} antagonist (+) WAY 100135 (1 mg/kg; Fletcher et al., 1993).

Electrical activation of the afferent 5-HT fibers to the hippocampus

A bipolar electrode (NE-110; David Kopf, Tujunga, CA) was implanted on the midline with a 10° backward angle in the ventromedial tegmentum, 1 mm anterior to lambda and 8.3 mm bellow the cortical surface. A stimulator (S8800; Grass Instruments, Quincey, MA) delivered 200 square pulses of 0.5 ms at a frequency of 1 Hz and an intensity of 300 μA. The stimulation pulses and the firing activity of the neuron recorded were fed to an IBM-PC computer equipped with a Tecmar interface. Peristimulus time histograms were generated to determine the duration of suppression of firing activity of the CA_3 pyramidal neuron, measured in absolute silence value (SIL, in ms). This value is obtained by dividing the total number of events suppressed following the stimulation by the mean frequency of firing of the neuron recorded (Chaput et al., 1986). CA_1-CA_3 regions of

the hippocampus receive extensive innervation from 5-HT neurons of raphe nuclei (Hensler et al., 1994). The inhibitory effect of the electrical stimulation of the ascending 5-HT pathway is due to the release of 5-HT into the synaptic cleft (Chaput et al., 1986). Thus, for the CA_3 neurons tested, the effect of the stimulation of the ascending 5-HT pathway was determined first prior to and following the intravenous injection of 1 mg/kg of S-zacopride or BRL 46470A (Newberry et al., 1993; Smith et al., 1988). Then, the effect of the stimulation was assessed prior to and following each of the consecutive injections of R-zacopride (0.1 mg/kg), BRL 46470A (1 mg/kg), and the 5-HT autoreceptor antagonist methiothepin (1 mg/kg; Chaput et al., 1986). Finally, the effect of the stimulation was tested prior to and after each of the consecutive intravenous injections of tropisetron, renzapride, or S-zacopride and R-zacopride (Boess and Martin, 1994).

Superfusion experiments in brain slices

Male Sprague-Dawley rats (250–300 g) were killed by decapitation and their brain rapidly removed and dissected on an ice-cold glass plate. Slices from the frontal cortex and hippocampus of 0.4 mm in thickness were prepared using a MacIlwain chopper and incubated for 30 min at 37°C in Krebs solution containing 20 nM [^{3}H]5-HT creatinine sulphate (specific activity 914 GBq/mmol; NEN Research Products, Mississauga, Canada) and bubbled with a mixture of 95% O_2-5% CO_2. The composition of the Krebs solution in mmol/L concentrations was NaCl 118, KCl 4.7, $CaCl_2$ 1.3, $MgCl_2$ 1.2, NaH_2PO_4 1, $NaHCO_3$ 25, glucose 11.1, EDTA 0.004, and ascorbic acid 0.11. At the end of the incubation period with [^{3}H]5-HT, two frontal cortex or three hippocampus slices were placed into each of the glass chambers and superfused continuously at a rate of 0.5 ml/min with Krebs solution maintained at 37°C and saturated with O_2/CO_2. The slices were superfused 98 min for the frontal cortex and 72 min for the hippocampus before the first stimulation (S1). The two periods of electrical stimulation were delivered 8 min (S1) and 44 min (S2) after the end of the washing period (360 pulses: 2 ms. 20 or 30 mA at 3 Hz for 2 min). 2-Methyl-5-HT (0.01 to 1 μM) was added 8 min before S2 and the 5-HT_3 antagonists BRL 46470A (0.01–0.1 μM) or S-zacopride (0.1 μM) were added 20 min before S1 and maintained in the superfusion medium until the end of the experiments. The amount of tritium released per 4 min sample was expressed as a fraction of the total tritium content of the slices at the onset of the nineteen consecutive fractions collected starting 60 min for hippocampus and 90 min for frontal cortex slices after the beginning of superfusion. At the end of the experiments, the slices were solubilized in 0.5 ml of Soluene 350 (Packard Instruments, Downers Grove, IL) and the radioactivity in the slices and the samples was determined by liquid scintillation spectrometry. The electrically-evoked overflow of tritium was calculated as the total increase in radioactivity above basal outflow (sp1 or sp2) obtained in the sample preceding the stimulation and expressed as the ratio S2/S1. The sp2/sp1 ratios were calculated in order to determine whether the drugs modified the basal outflow of tritium.

Drugs

2-Methyl-5-HT, 8-OH-DPAT (Research Biochemicals, Natick, MA); 5-HT creatinine sulphate, quisqualic acid, glutamic acid (Sigma Chemical Co., St. Louis, MO); methiothepin maleate (Hoffman-Laroche, Montreal); R and S-zacopride (Delalande, Rueil-Malmaison, France); tropisetron (ICS 205-930; Sandoz, Basel, Switzerland); BRL 46470A, renzapride (SmithKline Beecham, Harlow, England); BMY 7378 (Bristol Myers Co., Evansville, IN), and (+) WAY 100135 (Wyeth Research, Berkshire, UK). The concentrations and the doses used for these compounds were chosen on the basis of previous successful experiments carried out in our and other laboratories.

RESULTS

Effects of 5-HT and 2-methyl-5-HT on the firing activity of the somatosensory cortical neurons, CA_1 and CA_3 dorsal hippocampus pyramidal neurons

In order to determine the effect of the 5-HT_3 agonist 2-methyl-5-HT on the firing activity of somatosensory cortical and dorsal hippocampus pyramidal neurons, 5-HT and 2-methyl-5-HT were applied iontophoretically since these two 5-HT agonists do not cross the blood brain barrier. It has been previously demonstrated that, in the medial prefrontal cortex, 5-HT and 2-methyl-5-HT induce an inhibitory effect on the firing activity which were blocked by selective 5-HT_3 antagonists (Ashby et al., 1992). The effect of the 5-HT and of 2-methyl-5-HT was thus tested on somatosensory cortical neurons. The effects of both 5-HT and the 5-HT_3 agonist 2-methyl-5-HT are summarized in Table I. For almost all somatosensory cortical neurons, 5-HT and 2-methyl-5-HT induced a marked reduction of firing activity, generally from 30 to 85%. This inhibitory effect occurred in the absence of alteration of the shape of the action potentials and was current-dependent. Consecutive injections of the selective 5-HT_3 antagonist BRL 46470A (0.1 and 1 mg/kg) did not alter the inhibitory effect of 5-HT and of 2-methyl-5-HT (Fig. 1A). Because of the presence of 5-HT_{1A} receptors in the somatosensory cortex and in the dorsal hippocampus, and because of the the non-negligible affinity of 2-methyl-5-HT for these sites (Ismaiel et al., 1990), we tested the effectiveness of the 5-HT_{1A} antagonist BMY 7378 to block the suppressant effect of 5-HT and of 2-methyl-5-HT. The subsequent i.v. administration of BMY 7378 (1 mg/kg) markedly reduced the inhibitory action of 5-HT and 2-methyl-5-HT (Fig. 1A). The suppressant action of the two 5-HT agonists on seven somatosensory cortical neurons is summarized in Figure 1B. Whereas BRL

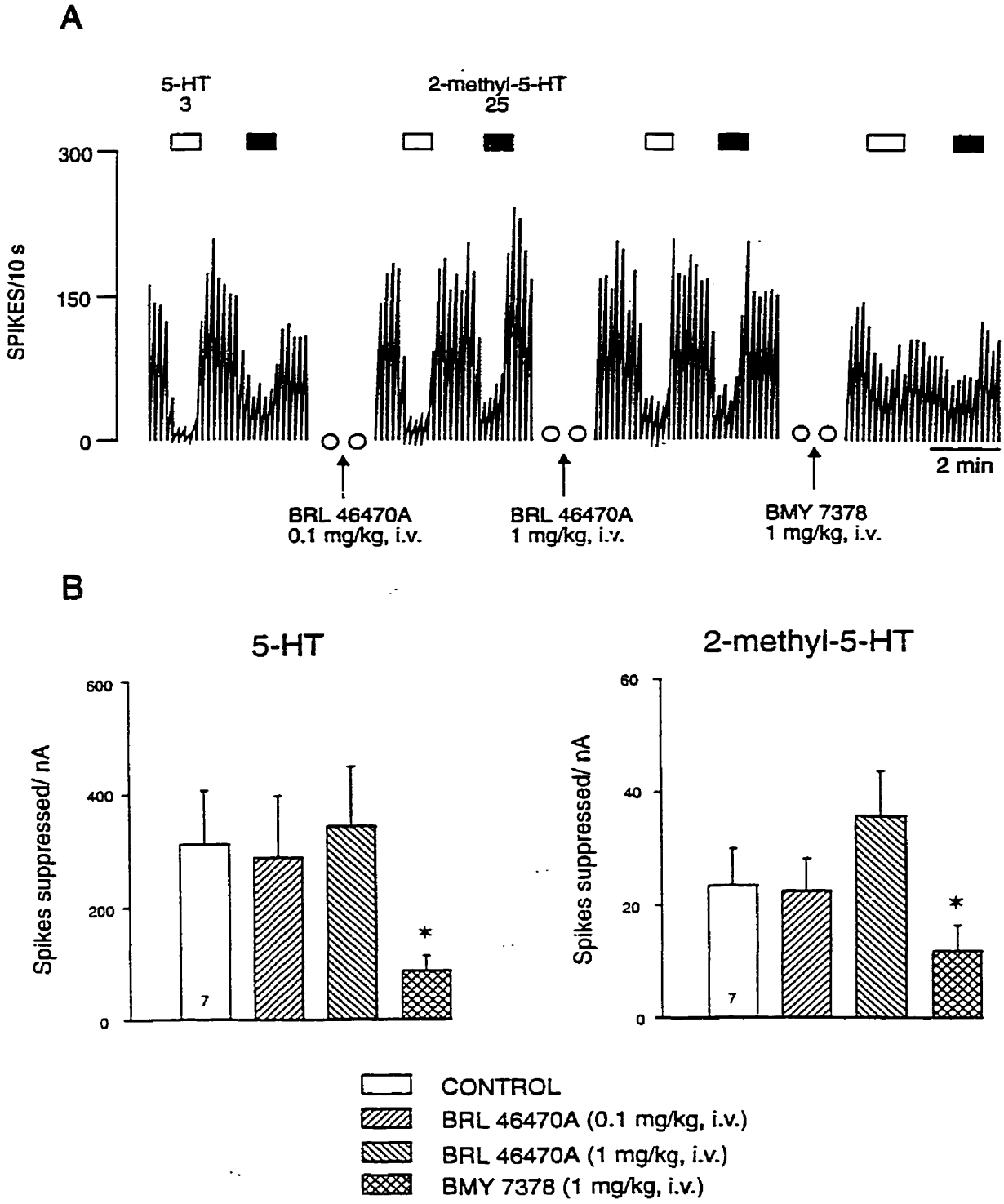

Fig. 1. Integrated firing rate histogram of a somatosensory cortical neuron showing its responsiveness to microiontophoretic application of 5-HT and 2-methyl-5-HT before and after the subsequent injections of BRL 46470A (0.1 and 1 mg/kg) and BMY 7378 (1 mg/kg). This neuron was activated with a quisqualate ejection current of −6 nA. Horizontal bars indicate the duration of the applications for which the current is given in nanoamperes. Note the altered effectiveness of both 5-HT and 2-methyl-5-HT to suppress firing activity after administration of BMY 7378 (A). In B, the responsiveness to 5-HT and 2-methyl-5-HT is expressed as the number of spikes suppressed per nanoamperes, the number of neurons tested is given at the bottom of the columns. $P < .05$ (paired Student's t test).

46470A did not modify the effects of 5-HT and 2-methyl-5-HT, BMY 7378 significantly reduced by 50% the suppressant effect of 2-methyl-5-HT, and by 72% that of 5-HT.

As was the case for somatosensory cortical neurons, 2-methyl-5-HT applied iontophoretically induced an inhibition of the firing activity of CA_1 hippocampus pyramidal neurons (Table I) in a current-dependent man-

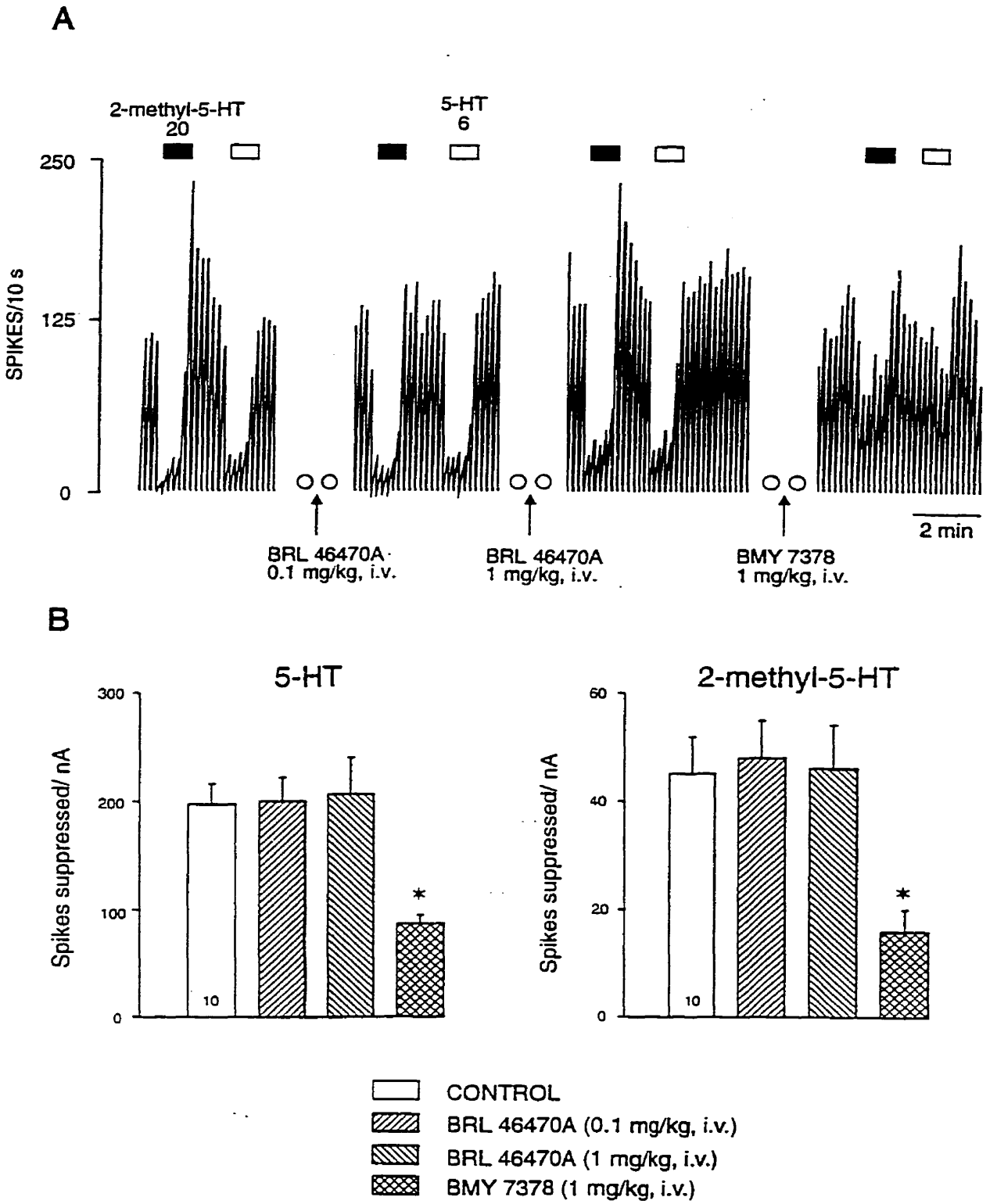

Fig. 2. Integrated firing rate histogram of a dorsal hippocampus CA_1 pyramidal neuron showing its responsiveness to microiontophoretic application of 5-HT and 2-methyl-5-HT before and after the subsequent injections of BRL 46470A (0.1 and 1 mg/kg) and BMY 7378 (1 mg/kg). This neuron was activated with a quisquilate ejection current of −2 nA. Horizontal bars indicate the duration of the applications for which the current is given in nanoamperes. Note the altered effectiveness of both 5-HT and 2-methyl-5-HT to suppress firing activity after administration of BMY 7378 (A). In B, the responsiveness to 5-HT and 2-methyl-5-HT is expressed as the number of spikes suppressed per nanoamperes, the number in the control column indicates the number of neurons tested. $P < .05$ (paired Student's t test).

TABLE I. Effect of 5-HT and 2-methyl-5-HT on the firing activity of somatosensory cortical, hippocampus pyramidal, and dorsal raphe 5-HT neurons[1]

		Inhibition	No change	Excitation
Somatosensory cortex				
	5-HT	95% (18/19)	5% (1/19)	—
	2-methyl-5HT	94% (16/17)	—	6% (1/17)
Hippocampus				
CA1	5-HT	98% (41/42)	2% (1/42)	—
	2-methyl-5HT	79% (41/52)	17% (9/52)	4% (2/52)
CA3	5-HT	99% (78/79)	1% (1/79)	—
	2-methyl-5HT	88% (68/77)	8% (6/77)	4% (3/77)
Dorsal raphe				
	5-HT	76% (57/75)	19% (14/75)	4% (4/75)
	2-methyl-5-HT	65% (45/70)	28% (20/70)	7% (5/70)
	8-OH-DPAT	93% (40/43)	7% (3/43)	—

[1]Numbers in parentheses indicate the ratio between the effect observed and the number of neurons tested.

ner. However, Zhang et al. (1994) have reported that the suppression of the glutamate-activated firing of rat CA_1 hippocampus pyramidal neurons induced by 2-methyl-5-H applied by microiontophoresis was attenuated by BRL 46470A injected intravenously (1–2 μg/kg) or applied by microiontophoresis. In the present study, neither acute injection of the 5-HT_3 antagonists S-zacopride (1 mg/kg, i.v., data not shown) nor BRL 46470A (3 μg/kg, i.v., n = 3, data not shown; 0.1 and 1 mg/kg, Fig. 2) modified the firing activity of the dorsal hippocampus pyramidal neurons nor blocked the inhibitory action of 5-HT and 2-methyl-5-HT applied by microiontophoresis. We also tried to determine if an increasing current of ejection (5 to 40 nA, during 5 min) of 2-methyl-5-HT could be antagonized by the injection of BRL 46470A (2 μg/kg, i.v.) using quisqualate, or glutamic acid, as previously reported by Zhang et al. (1994). Under these conditions, BRL 46470A also did not antagonize the inhibitory effect of 2-methyl-5-HT in six rats (maximal inhibition of glutamate-activated CA_1 hippocampus pyramidal neurons prior to BRL 46470A: 50 ± 6%; following: 57 ± 9%, n = 6; $P = 0.86$). For CA_1 neurons, only the administration of BMY 7378 antagonized the inhibitory effect of 5-HT and 2-methyl-5-HT, by 79% and 58%, respectively (Fig. 2A,B).

In the CA_3 region of the hippocampus, 2-methyl-5-HT decreased the firing activity of pyramidal neurons, and all these neurons were also inhibited by 5-HT applications (Table I). As for somatosensory and CA_1 hippocampus pyramidal neurons, the injection of BRL 46470A (0.1 and 1 mg/kg, i.v.) did not block the inhibitory action of the two 5-HT agonists, whereas that of BMY 7378 (1 mg/kg, i.v.) reduced the inhibitory action of 5-HT and 2-methyl-5-HT by 56% and 64%, respectively (Fig. 3A,B).

The inhibitory effect of 5-HT and 2-methyl-5-HT applied by microiontophoresis onto CA_1 and CA_3 hippocampus pyramidal neurons were determined before and during the microiontophoretic application of S-zacopride and BRL 46470A. As exemplified in Figure 4, concomitant ejection of these selective 5-HT_3 antagonists did not alter the inhibitory effect of 5-HT and 2-methyl-5-HT, and BRL 46470A applied by microiontophoresis did not reverse the inhibitory effect of 5-HT and 2-methyl-5-HT (Fig. 4B). In order to determine whether the antagonistic effect of BMY 7378 was due to a local or distant action, this 5-HT_{1A} antagonist was applied directly by microiontophoresis. As shown in Figure 4C, concomitant ejection of BMY 7378 reduced the inhibitory action of 5-HT and of 2-methyl-5-HT (for CA_1 pyramidal neurons: 2-methyl-5-HT = 50 ± 7 spikes suppressed/nA, and during BMY 7378 application: 2-methyl-5-HT = 27 ± 5 spikes suppressed/nA, n = 7, $P < 0.02$; for CA_3 pyramidal neurons: 2-methyl-5-HT = 34 ± 6 spikes suppressed/nA, and during BMY 7378 application: 18 ± 4 spikes suppressed/nA, n = 8, $P < 0.02$). The inhibitory action of 5-HT and 2-methyl-5-HT promptly reappeared when the BMY application was stopped in all cases (see Fig. 4C).

Effect of 5-HT agonists on dorsal raphe 5-HT neurons

The majority of the dorsal raphe 5-HT neurons were inhibited, in a current-dependent manner, by 2-methyl-5-HT, 5-HT, and the 5-HT_{1A} agonist 8-OH-PAT. In order to characterize this inhibitory effect of 2-methyl-5-HT on 5-HT neurons, a low and high (0.1 and 1 mg/kg, i.v.) dose of BRL 46470A were administered intravenously. As illustrated in Figure 5A, the administration of BRL 46470A (0.1 or 1 mg/kg, i.v.) did not alter the spontaneous firing activity of 5-HT neurons and did not modify the inhibitory effect of 5-HT, 2-methyl-5-HT, and 8-OH-DPAT applied by microiontophoresis (Fig. 5A, Table II). The administration of the potent and selective 5-HT_{1A} antagonist (+)WAY 100135 (1 mg/kg, i.v.) generally reduced by 20–30% the firing rate of 5-HT neurons, but failed to attenuate significantly the reducing effect of 5-HT and 2-methyl-5-HT. However, (+)WAY 100135 did reduce by 76% the inhibitory action of 8-OH-DPAT applied by microiontophoresis (Fig. 5B; Table II).

Electrical activation of the afferent 5-HT fibers to the hippocampus

In order to determine whether the 5-HT_3 receptor ligands could modulate in vivo the efficacy of 5-HT syn-

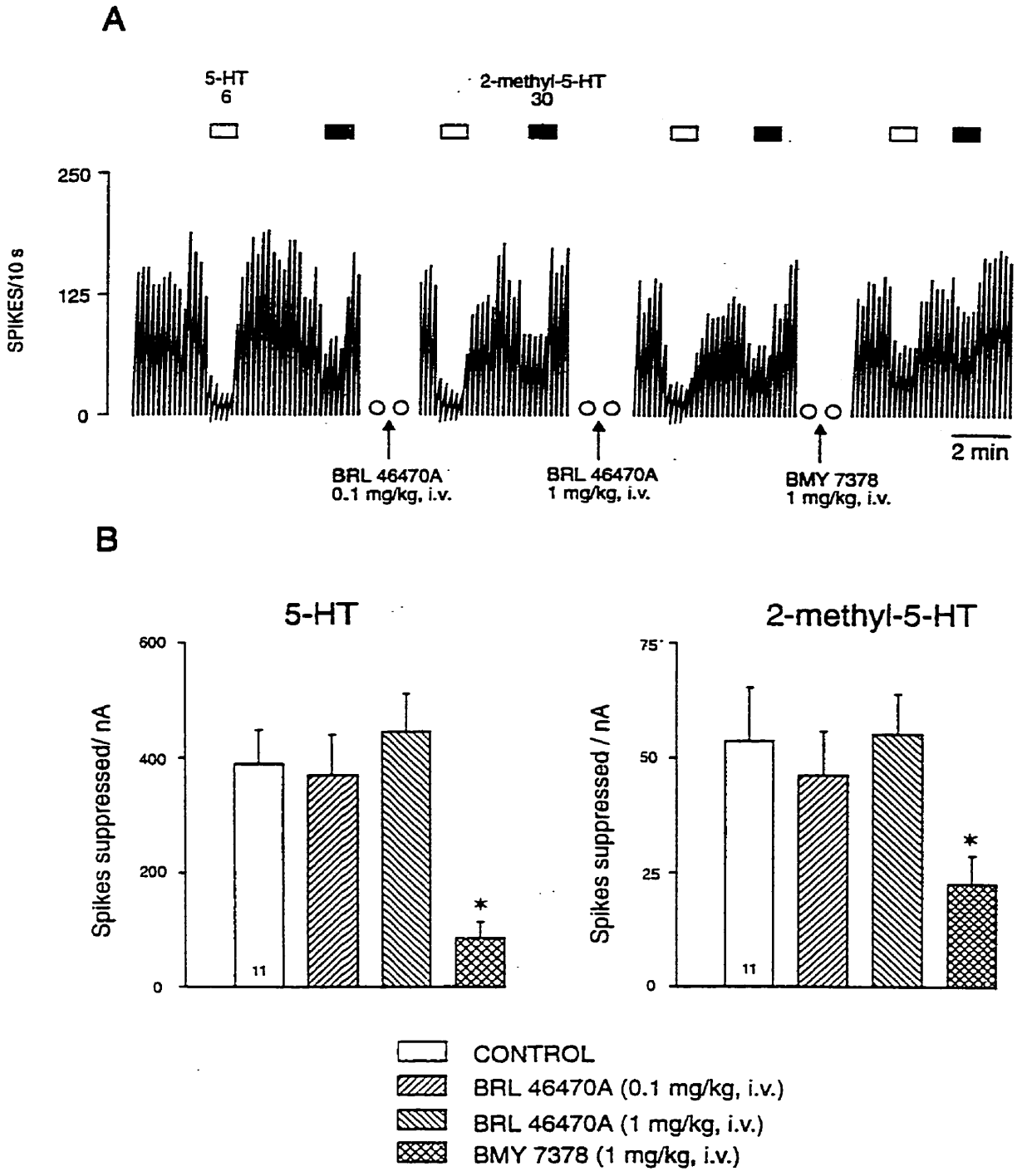

Fig. 3. Integrated firing rate histogram of a dorsal hippocampus CA_3 pyramidal neuron showing its responsiveness to microiontophoretic application of 5-HT and 2-methyl-5-HT before and after the subsequent injections of BRL 46470A (0.1 and 1 mg/kg) and BMY 7378 (1 mg/kg). This neuron was activated with a quisquilate ejection current of −1 nA. Horizontal bars indicate the duration of the applications for which the current is given in nanoamperes. Note the altered effectiveness of both 5-HT and 2-methyl-5-HT to suppress firing activity after administration of BMY 7378 (A). In B, the responsiveness to 5-HT and 2-methyl-5-HT is expressed as the number of spikes suppressed per nanoamps, the number in the control column indicate the number of neurons tested. $P < .05$ (paired Student's t test).

aptic transmission, their capacity to modify the duration of the suppression of the firing activity of CA_3 hippocampus pyramidal neurons produced by the electrical activation of the ascending 5-HT pathway was examined. Neither 2-methyl-5-HT applied by microiontophoresis (5–20 nA continuously), S-zacopride, nor BRL 46470A (both at 1 mg/kg, i.v.) modified the efficacy of the stimulation of the 5-HT pathway (SIL prior to: 66 ± 14 ms and during 2-methyl-5-HT: 67 ± 15 ms, n = 5; prior to: 79 ± 30 ms and after S-zacopride: 69 ± 30 ms, n = 5; prior to: 72 ± 23 ms and after BRL 46470A: 75 ± 24 ms, n = 5). A recent microdialysis study reported that the R-isomer of zacopride reduces 5-HT release in the rat frontal cortex (Barnes et al.,

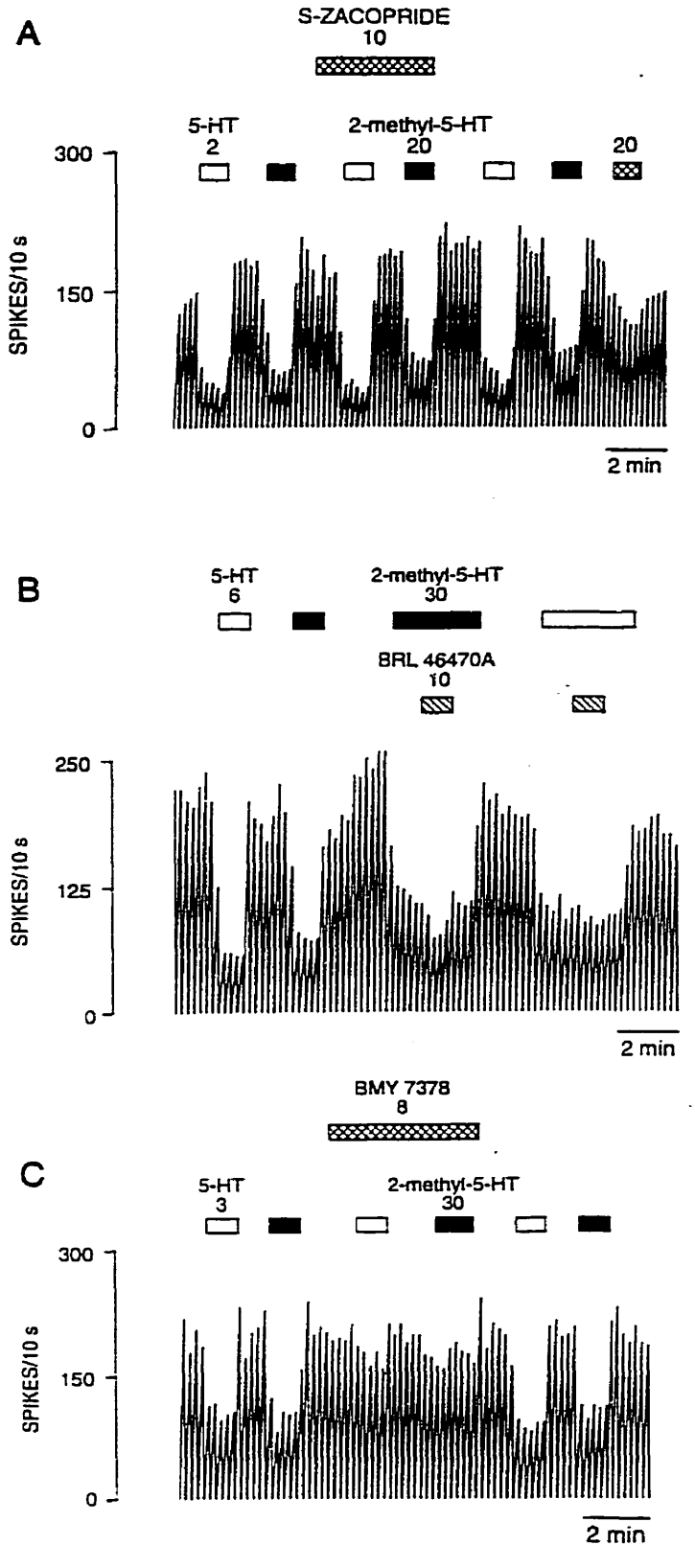

Fig. 4. Integrated firing rate histogram of three dorsal hippocampus CA_3 pyramidal neurons showing its responsiveness to microiontophoretic application of 5-HT and 2-methyl-5-HT before and during the concomitant ejections of S-zacopride (A), BRL 46470A (B), and BMY 7378 (C). Note the blockade of the effectiveness of both 5-HT and 2-methyl-5-HT to suppress firing activity during BMY 7378 application. These neurons were activated with a quisquilate ejection current of −3 (A), −1 (B), and −1 (C) nA. The bars indicate the duration of application for which the ejection currents are given in nanoamperes.

1992). In the present study, R-zacopride (0.1 mg/kg, i.v.) reduced the duration of suppression of the firing activity of CA_3 pyramidal neurons induced by the electrical stimulation of the ascending 5-HT pathway (Fig. 6A,B). This inhibitory effect of R-zacopride was not antagonized by the subsequent injection of the selective 5-HT_3 antagonist BRL 46470A (1 mg/kg, i.v.; Fig. 6B). The apparent further reducing effect following the injection of BRL 46470A was probably due, in fact, to the gradual onset of action of R-zacopride as in two rats, the inhibition produced by R-zacopride progressively increased over 15 min. However, the subsequent intravenous injection of the terminal 5-HT autoreceptor antagonist methiothepin restored to the control value the period of suppression of firing (Fig. 6B).

Recent experimental evidence indicates that 5-HT_4 receptors, which have recently been cloned and are positively coupled to adenylate cyclase, are stimulated by substituted benzamide derivatives such as renzapride, cisapride, and zacopride (Adham et al., 1994; Bockaert et al., 1990; Dumuis et al., 1988). The putative 5-HT_4 antagonist, renzapride (1 mg/kg, i.v.), administered prior to the injection of R-zacopride (0.1 mg/kg, i.v.) prevented the reduction of the period of suppression of firing of CA_3 pyramidal neurons induced by the stimulation of the ascending 5-HT pathway (Table III). S-Zacopride (1 mg/kg, i.v.) injected prior to R-zacopride (0.1 mg/kg, i.v.) also prevented the reduction of the efficacy of the stimulation. Furthermore, the putative 5-HT_4 antagonist tropisetron (1 mg/kg, i.v.) significantly reduced by 21% the efficacy of the stimulation by itself (Table III). However, tropisetron administered prior to R-zacopride (0.1 mg/kg, i.v.) also prevented the reducing effect of R-zacopride.

Superfusion experiments in brain slices

Since the effect of microiontophoretic application of 2-methyl-5-HT did not alter the effectiveness of the stimulation of the 5-HT pathway, as it could have been expected. the capacity of 2-methyl-5-HT to enhance the electrically-evoked overflow of [^{3}H]-5-HT was examined in slices. The electrically-evoked [^{3}H]-5-HT release in rat frontal cortex and hippocampus slices was enhanced in a concentration-dependent manner when 2-methyl-5-HT was added 8 min before S2, without altering the spontaneous outflow of radioactivity (data not shown). As illustrated in Figure 7A, when the 5-HT_3 antagonists BRL 46470A (0.01 μM) or S-zacopride (0.01 μM) were added to the superfusion medium 20 min before S1 until the end of the experiment. They significantly attenuated the enhancing effect of 0.3 μM 2-methyl-5-HT (reductions of 53 and 71%, respectively), and that of 1 μM 2-methyl-5-HT (reductions of 52 and 64%, respectively) on the evoked [^{3}H]-5-HT overflow in frontal cortex slices. In rat hippocampus slices, 2-methyl-5-HT also increased in a concentration-dependent manner the electrically-evoked release of [^{3}H]-5-HT. BRL 46470A (0.1 μM) and S-zacopride (0.1 μM), added 20 min before S1 and main-

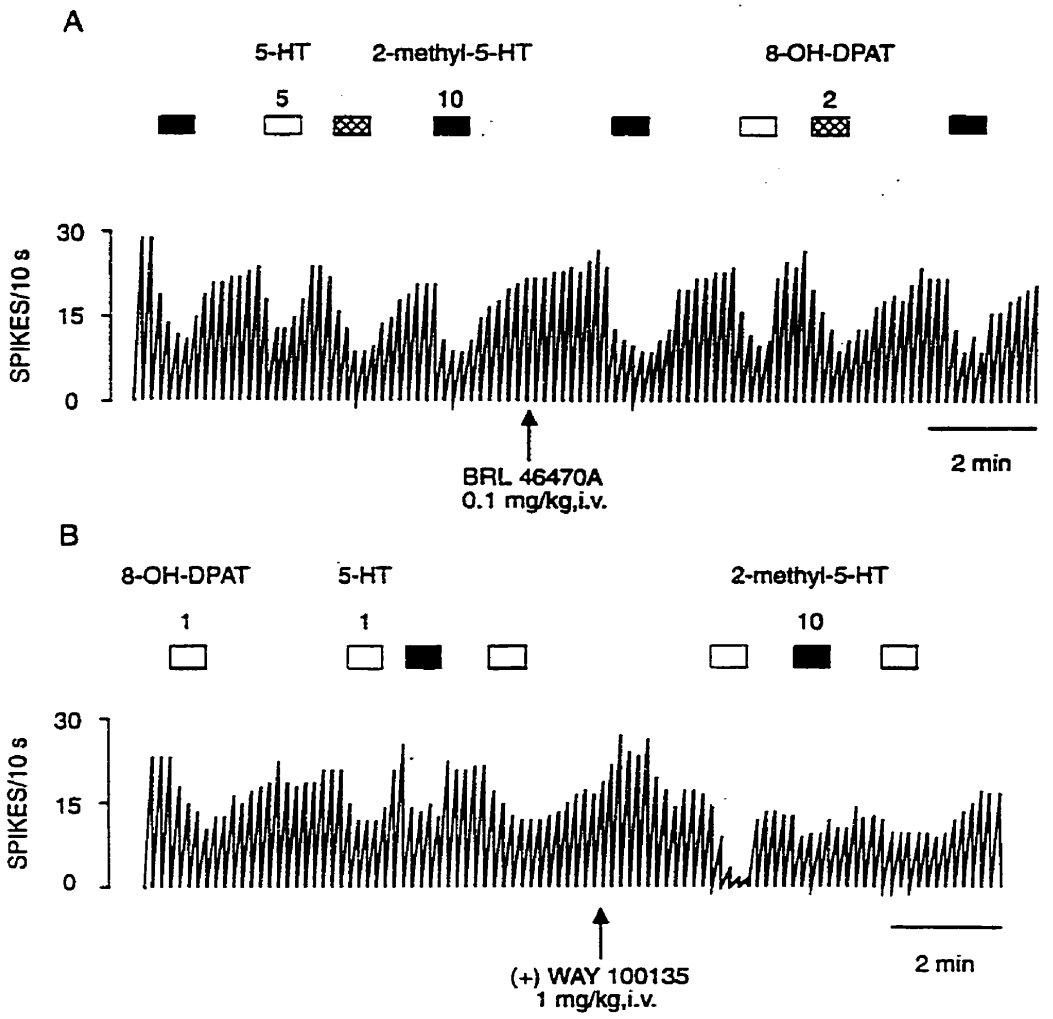

Fig. 5. Integrated firing rate histogram of two dorsal raphe 5-HT neurons showing its responsiveness to microiontophoretic application of 5-HT, 8-OH-DPAT, and 2-methyl-5-HT before and after the injection of BRL 46470A (0.1 mg/kg) (A) and (+) WAY 100135 (1 mg/kg) (B). Horizontal bars indicate the duration of the applications for which the currents are given in nanoamperes. Note the unaltered effectiveness of 5-HT and 2-methyl-5-HT and 8-OH-DPAT to suppress firing activity after administration of BRL 46470A (A), whereas (+) WAY 100135 antagonized the suppressant effect of both 2-methyl-5-HT and 8-OH-DPAT (B).

TABLE II. Effect of microiontophoretically-applied 5-HT, 2-methyl-5-HT, and 8-OH-DPAT on dorsal raphe 5-HT neurons prior to and after administration of either (+) WAY 100135 or BRL 46470A[1]

	5-HT		2-methyl-5-HT		8-OH-DPAT	
	Prior	Following	Prior	Following	Prior	Following
(+) WAY 100135 (1 mg/kg, i.v.)	29 ± 6	29 ± 10 (6)	3 ± 1	2 ± 1 (7)	20 ± 4	5 ± 1* (6)
BRL 46470A (0.1 mg/kg, i.v.)	10 ± 4	10 ± 4 (8)	11 ± 2	8 ± 3 (7)	55 ± 15	54 ± 15 (5)
BRL 46470A (1 mg/kg, i.v.)	15 ± 4	18 ± 6 (8)	4 ± 1	4 ± 1 (8)	34 ± 8	24 ± 5 (7)

[1]The effectiveness of 5-HT, 2-methyl-5-HT, and 8-OH-DPAT is expressed as spikes suppressed /nA (mean ± SEM). Numbers in parentheses indicate the number of neurons tested; only one neuron was tested per rat.
*$P < 0.05$ as determined by Student's t-test for paired data.

tained in the superfusate, attenuated significantly the 0.3 μM of 2-methyl-5-HT-induced increase of tritium overflow (reductions of 40 and 25%, respectively). S-zacopride and BRL 46470A attenuated significantly the 1 μM 2-methyl-5-HT-induced increase of [^{3}H]-5-HT overflow (reductions of 27 and 30%, respectively; Fig. 7B).

The electrically-evoked release of [^{3}H]-5-HT in rat hippocampus slices was significantly enhanced when methiothepin (0.3 μM) was added 8 min before S2 (without methiothepin: S2/S1 = 0.98 ± 0.09 n = 5, with methiothepin: S2/S1 = 1.38 ± 0.12 n = 5) but not modified by R-zacopride (0.1 μM) added 8 min before S2

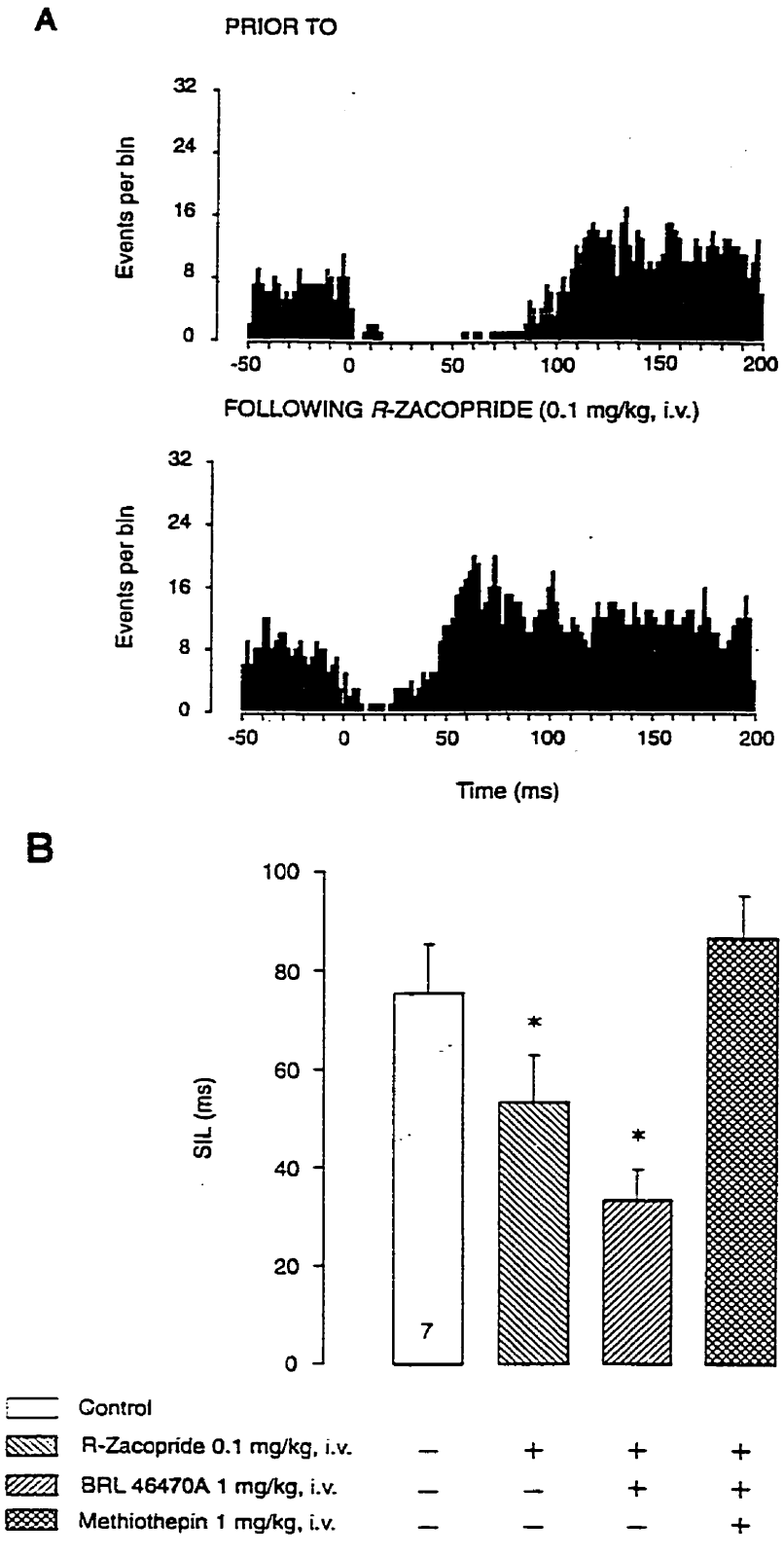

Fig. 6. Peristimulus time histograms showing the effect of the ascending 5-HT pathway at the level of the ventro-medial tegmentum on the firing activity of dorsal hippocampus CA_3 pyramidal neurons prior to and after treatment with R-zacopride (0.1 mg/kg, i.v.) A: Each histogram was constructed from 200 consecutive stimulations (300 μA, 0.5 ms, 1 Hz) and bin width is of 2 ms. In B, the duration of suppression of firing activity (SIL ms ± S.E.M) of CA_3 hippocampus pyramidal neurons, produced by endogenous 5-HT released by the stimulations of the ascending 5-HT pathway, was assessed in rats treated with consecutive i.v. administration of R-zacopride, BRL 46470A, and methiothepin. The number in the control column indicates the number of neurons tested. $P < .05$ using the paired Student's t test.

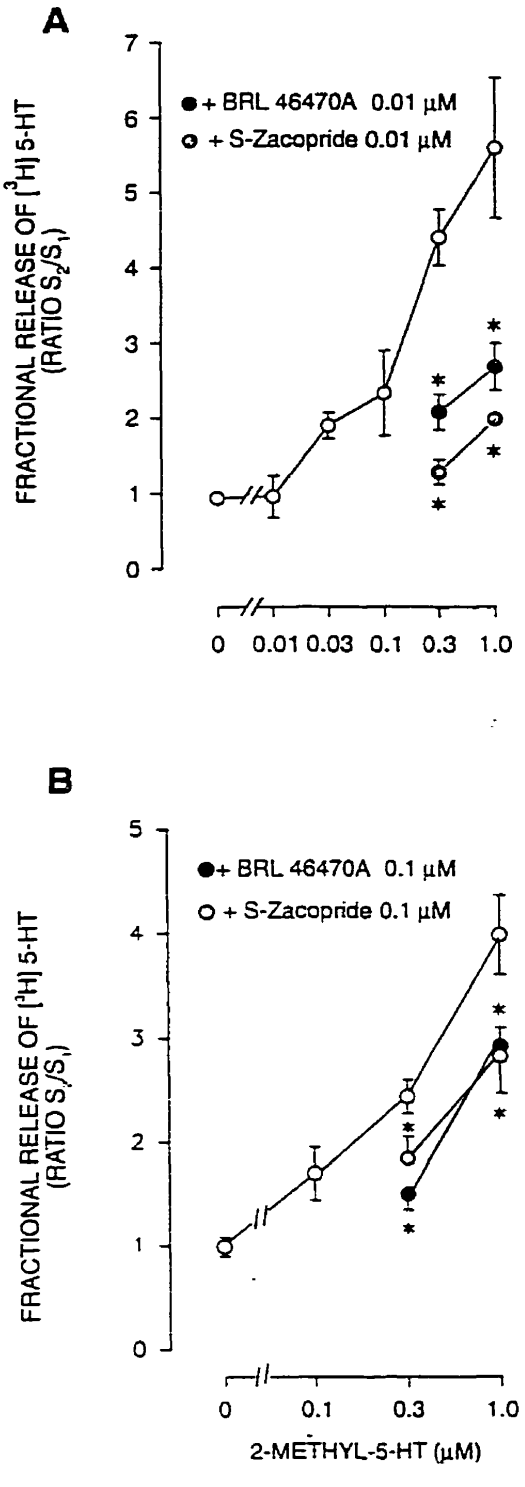

Fig. 7. Effect of 2-methyl-5-HT on the release of tritium induced by the electrical stimulation of frontal cortex (A) and hippocampus (B) slices preloaded with [^{3}H]-5-HT and its modification by two 5-HT$_3$ antagonists. Ordinate scale: fraction of the total tissue radioactivity released by 360 pulses (30 mA, 2 ms, 3 Hz) expressed as the ratio S2/S1 (stimulation with 2-methyl-5-HT vs. without). BRL 46470A and S-zacopride were introduced 20 min before S1 until the end of the superfusion, and 2-methyl-5-HT was introduced 8 min before S2. Each point represents the mean ± S.E.M (vertical bars) of 4–13 experiments. $P < .05$ when compared to the value obtained with 2-methyl-5-HT alone (two-tailed Student's t test).

TABLE III. Duration of suppression of firing activity of CA3 pyramidal neurons by the electrical stimulation of the ascending 5-HT pathway[1]

	Prior	Following	Following R-Zacopride**
Renzapride	63 ± 9 (6)	69 ± 12	66 ± 9
S-Zacopride	71 ± 10 (5)	73 ± 8	76 ± 7
Tropisetron	93 ± 11 (6)	73 ± 14*	91 ± 14

[1]Results are expressed as means ± SEM. Numbers in parentheses indicate the numbers of neurons tested; only one neuron was tested per rat. Renzapride, S-Zacopride, ICS 205-930 (1 mg/kg), and R-Zacopride (0.1 mg/kg) were administered intravenously.
*$P < 0.05$ as determined by Student's t-test for paired data.
**SIL value prior to R-zacopride = 74 ± 8, after R-zacopride (0.1 mg/kg, i.v.) = 55 ± 8 ms, n = 9, $P < 0.02$.

(S2/S1 = 1.10 ± 0.04 n = 5). Moreover, R-zacopride (0.1 μM), added 20 min before S1 and maintained in the superfusate, did not modify the 0.3 μM of methiothepin-induced increase of tritium overflow (with methiothepin: S2/S1 = 1.38 ± 0.12 n = 5, with methiothepin and R-zacopride: S2/S1 = 1.33 ± 0.09 n = 5).

DISCUSSION

The results of the present study show that 5-HT and the prototypical 5-HT$_3$ agonist 2-methyl-5-HT microiontophoretically-applied induce a marked suppression of the firing activity of somatosensory cortical, CA$_1$-CA$_3$ dorsal hippocampus pyramidal and dorsal raphe 5-HT neurons in chloral hydrate anaesthetized rats. (Table I). This inhibitory effect of 2-methyl-5-HT was unaltered by 5-HT$_3$ antagonists but attenuated by the 5-HT$_{1A}$ antagonist BMY 7378. In contrast, Zhang et al. (1994) recently reported that the suppression induced by 2-methyl-5-HT applied by microiontophoresis on glutamate-activated firing of rat CA$_1$ hippocampus pyramidal neurons was attenuated by BRL 46470A. The discrepancy with the present results cannot be due to the dose of the 5-HT$_3$ antagonist BRL 46470A used since when 3 μg/kg of this drug was used, it did not alter the inhibitory action of 2-methyl-5-HT or 5-HT. Zhang et al. (1994) used much longer ejection periods with increasing currents as opposed to 50 s ejection periods with a fixed current in our experiments. Moreover, these authors used glutamate to activate the pyramidal neurons, thus stimulating all subtypes of glutamate sensitive receptors, whereas we used the selective agent quisqualic acid. However, using the same methodological approach as this group, we did not observe an antagonistic property of BRL 46470A against 2-methyl-5-HT on CA$_1$ hippocampus pyramidal neurons.

It may appear paradoxical that the inhibitory effect of 2-methyl-5-HT, observed in the present study, is mediated by 5-HT$_{1A}$ receptors. However, radioligand binding studies have shown that 2-methyl-5-HT has an affinity for brain 5-HT$_{1A}$ sites which is only 30-fold less than that for 5-HT$_3$ binding sites (Ismaiel et al., 1990).

Consistent with these observations, it has been reported that 2-methyl-5-HT elicited contractions of the rabbit renal artery which were not antagonized by selective 5-HT_3 antagonists but rather was mediated via 5-HT_1-like receptors (Tadipartri et al., 1992). Moreover, in vitro, it has been reported that 2-methyl-5-HT decreased the firing rate of 5-HT neurons in the dorsal raphe and that this inhibitory effect of 2-methyl-5-HT was prevented by 10 μM of the 5-HT_{1A} antagonist I-propranolol (Adrien et al., 1992). In contrast, in rat medial prefrontal cortex 5-HT and 2-methyl-5-HT also suppress neuronal firing activity but this inhibitory effect is effectively antagonized by 5-HT_3 antagonists (Ashby et al., 1992). Furthermore, the suppressant effect of 2-methyl-5-HT, but not that of 8-OH-PAT, is markedly enhanced following destruction of 5-HT terminals with the 5-HT neurotoxin 5,7-dihydroxytryptamine indicating a selective sensitization of these 5-HT_3-like receptors (Ashby et al., 1994).

Using in vitro intracellular recordings of CA_1 pyramidal neurons, it has been reported that bath application of 2-methyl-5-HT mimicked the effect of 5-HT, inducing facilitation of unitary inhibitory postsynaptic potentials which were blocked by 5-HT_3 antagonists such as tropisetron and metoclopramide (Ropert and Guy, 1991). These authors have thus proposed that 5-HT activates GABAergic interneurons via a 5-HT_3 receptor, thereby increasing the frequency of inhibitory synaptic events recorded in CA_1 pyramidal cells. Recently, Blier et al. (1993b) have shown that 2-methyl-5-HT increases the K^+ evoked release of [^{3}H]-5-HT from preloaded slices of the guinea-pig hypothalamus and that this effect is blocked by the selective 5-HT_3 antagonist ondansetron. In presence of tetrodotoxin, this enhancement of [^{3}H]-5-HT release induced by 2-methyl-5-HT was not present. Moreover, 2-methyl-5-HT did not alter of [^{3}H]-5-HT release in a synaptosomal preparation from guinea-pig hypothalamus, thus indicating that these 5-HT_3 receptors are not located on 5-HT terminals. In the hippocampus, the localization of the 5-HT_3 receptors has yet to be determined. Recently, it has been demonstrated that the lesions of 5-HT fibers in rat hippocampus did not modify the density of 5-HT_3 receptors labelled with [^{3}H](R,S)-zacopride, thus indicating that 5-HT_3 receptors were not located on 5-HT fibers in this brain region. Moreover, local lesions of the hippocampus with kainic or ibotenic acid reduced 5-HT_{1A} receptors binding sites labelled with [^{3}H]8-OH-DPAT but not the density of 5-HT_3 receptors suggesting their location on fibers "en passage" (Kidd et al., 1993).

In the present study, 5-HT, 2-methyl-5-HT, and 8-OH-DPAT applied by microiontophoresis reduced the firing activity of dorsal raphe 5-HT neurons (Fig. 5). BRL 46470A (0.1 and 1 mg/kg, i.v.) did not modify the inhibitory action of the three 5-HT agonists. Only the inhibitory action of 8-OH-DPAT on dorsal raphe 5-HT neurons was blocked by the 5-HT_{1A} antagonist (+)WAY 100135 injected intravenously (Fig. 5B). Surprisingly, (+)WAY 100135 did not antagonize the inhibitory effect of 5-HT microiontophoretically-applied onto dorsal raphe 5-HT neurons. (Table II). This lack of effect of (+)WAY 100135 against 5-HT itself has also been observed by others. Recent results from our laboratory indicate that the suppression of the firing activity of dorsal raphe 5-HT neurons produced by the antidromic stimulation of the 5-HT pathway, consequently due to the release of the endogenous 5-HT release, was not blocked by the i.v. injection of 0.5 mg/kg (+)WAY 100135 (Piñeyro et al., 1993). Furthermore, the infusion of citalopram, a selective 5-HT reuptake inhibitor, in the dorsal raphe nucleus produced a 50% decrease of 5-HT collected from a microdialysis canula implanted in the striatum which was antagonized by the intraperitoneal administration of the 5-HT_{1A} antagonists (−)pindolol or (−)tertatolol, but not by (+) WAY 100135 (Bel et al., 1994; Artigas, F., personal communication).

It has been previously shown that 2-methyl-5-HT increases the K^+ evoked release of [^{3}H]-5-HT from preloaded slices of the guinea-pig hypothalamus (Blier et al., 1993b). In the present study, 2-methyl-5-HT iontophoretically-applied did not modify the duration of suppression of the firing activity of CA_3 pyramidal neurons induced by the stimulation of the ascending 5-HT pathway. This lack of effect of 2-methyl-5-HT was probably due to the fact that such microiontophoretic applications did not allow 2-methyl-5-HT to reach 5-HT_3 receptors putatively modulating 5-HT release. This explanation is supported by the results of Martin et al. (1992) who reported, using intracerebral microdialysis in the rat ventral hippocampus, that 2-methyl-5-HT increased 5-HT levels in the dialysate and that this enhancing effect was abolished by the selective 5-HT_3 antagonist MDL 72222. These authors have proposed that these facilitatory 5-HT_3 receptors are not tonically activated since MDL 72222 did not exert any effect by itself. Our in vitro results are fully consistent with these data since 2-methyl-5-HT enhanced the electrically evoked release of [^{3}H]-5-HT from preloaded hippocampus and frontal cortex slices and this effect was antagonized by selective 5-HT_3 antagonists.

Unlike the three 5-HT_3 agonists 2-methyl-5-HT, phenylbiguanide, and m-chlorophenylbiguanide, 5-HT_3 antagonists cross the blood brain barrier to a certain extent on the basis of previous physiological experiments (see Ashby et al., 1992). However, the 5-HT_3 antagonists S-zacopride, BRL 46470A did not modify the effectiveness of the stimulations. These data therefore suggest that 5-HT_3 receptors modulating the release of 5-HT were not tonically activated under these in vivo conditions. In contrast, using microdialysis in freely-moving rats, Barnes et al. (1992) have shown that R-zacopride (i.p. or via the microdialysis probe) reduced the extracellular level of 5-HT in the rat frontal cortex, whereas S-zacopride or ondansetron were without ef-

fect. Surprisingly, S-zacopride (injected i.p. or via the microdialysis probe), but not the selective 5-HT_3 receptor antagonist ondansetron, antagonized the reduction of 5-HT extracellular level induced by R-zacopride. Using the same paradigm, Ge et al. (1992) have reported that the 5-HT_3/5-HT_4 receptors antagonist renzapride blocked the reduction in the extracellular level of 5-HT induced by R-zacopride. Consistent with these microdialysis experiments, in the present experiments only the administration of R-zacopride (0.1 mg/kg, i.v.) significantly reduced endogenous 5-HT release induced by the electrical stimulation of the ascending 5-HT pathway. The subsequent administration of BRL 46470A (1 mg/kg, i.v.) did not reverse the inhibitory effect of R-zacopride. However, the reducing effect of R-zacopride was reversed by the 5-HT autoreceptor antagonist methiothepin (Fig. 6). In vitro, methiothepin (0.3 μM) increased the electrically-evoked release of [^{3}H]-5-HT in hippocampus slices but the enhancing action of methiothepin was not reversed by R-zacopride (0.1 μM) added to the superfusion medium (see Results). In vivo, the reducing effect of R-zacopride was prevented by the intravenous administration of 1 mg/kg of renzapride, S-zacopride, and tropisetron which are all considered to be 5-HT_3/5-HT_4 antagonists. Taken together, these data suggest that the effect of R-zacopride was not mediated via 5-HT_{1B} or 5-HT_3 receptors but may be attributable to an activation of a 5-HT_4 receptor, or to another receptor denoted as a R-zacopride-sensitive site (Kidd et al., 1992).

Recently, Gebauer et al. (1993) have suggested that enterochromaffin cells of the guinea-pig ileum are endowed with 5-HT_3 and 5-HT_4 autoreceptors: the activation of the 5-HT_3 receptors would increase 5-HT release, whereas that of the 5-HT_4 receptors would induce a decrease of 5-HT release. Consistent with previous results obtained in the guinea-pig brain (Blier and Bouchard, 1992), the present in vitro data have shown that the electrically-evoked release of [^{3}H]-5-HT in both rat frontal cortex and hippocampus slices was also enhanced, in a concentration dependent-manner, when 2-methyl-5-HT was added to the superfusion medium (Fig. 7). Both BRL 46470A and S-zacopride antagonized significantly the enhancing action of 2-methyl-5-HT (0.3–1 μM) on the electrically-evoked release of [^{3}H]-5-HT in frontal cortex slices (Fig. 7A). The enhancing effect of 2-methyl-5-HT appeared to be more potent in rat than in guinea pig slices, and this is consistent with previous results suggesting that there is interspecies heterogeneity of 5-HT_3 receptors (Newberry et al., 1991).

In conclusion, the results of the present study suggest that, first, the reducing effect elicited by 5-HT and 2-methyl-5-HT applied microiontophoretically on rat somatosensory cortical and CA_1-CA_3 dorsal hippocampus pyramidal neurons activity is mediated via 5-HT_{1A} receptors. Second, the spontaneous firing activity of dorsal raphe 5-HT neurons is reduced by 2-methyl-5-HT applied microiontophoretically but it is not mediated by 5-HT_3 receptors. Third, only the administration of R-zacopride significantly reduced the duration of suppression of the firing activity of CA_3 pyramidal neurons induced by the electrical stimulation of the ascending 5-HT pathway. This reducing effect of R-zacopride appeared to be mediated by 5-HT_4 receptors. Finally, in vitro 5-HT_3 antagonists attenuate the enhancing action of 2-methyl-5-HT on the electrically-evoked release of [^{3}H]-5-HT in both rat frontal cortex and hippocampus slices.

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Chapter III: Second article

Heteromodulations of the 5-HT system via α-adrenoreceptors:

Although very few studies on the interactions between 5-HT and NE systems have been carried out, the characterization of these interactions seems to be crucial to a better understanding of the mechanism of action of antidepressant drugs. Hence, we have interest in the heteromodulation of the 5-HT neurotransmission by noradrenergic agents. Mirtazapine, the 6-aza-analogue of the atypical antidepressant mianserin, is a tetracyclic compound with antidepressant activity in humans. Using *in vivo* electrophysiological paradigms, our second study was undertaken to characterize the effects of acute administrations of (±)mirtazapine on pre- and postsynaptic α_2-adrenoceptors. The effects of (-)mirtazapine were studied because of its purported selectivity for α_2-adrenergic heteroreceptors. First, at the postsynaptic level, the effects of (±) and (-)mirtazapine on the CA_3 dorsal hippocampus pyramidal neurons responsiveness to microiontophoretic applications of NE were assessed. Second, at the level of 5-HT terminals, the effects of (±) and (-)mirtazapine on the endogenous release of 5-HT induced by the electrical stimulation of the ascending 5-HT pathway were assessed, as well as their abilities to antagonize the effects of clonidine on the effectiveness of the stimulation. Finally, the effects of (-) and (±)mirtazapine on the spontaneous firing activity of both dorsal raphe 5-HT and locus coeruleus (LC) NE neurons were investigated.

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Effect of the *Alpha*-2 Adrenoceptor Antagonist Mirtazapine on the 5-Hydroxytryptamine System in the Rat Brain[1]

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ABSTRACT

Mirtazapine [[(±)-MIR], Remeron, ORG 3770) is an *alpha*-2 adrenoceptor antagonist endowed with antidepressant activity in humans. The aim of the present study was to assess the effects of (±)-MIR and of its (−)enantiomer [(−)-MIR] on pre- and postsynaptic *alpha*-2 adrenoceptors and to characterize their putative modulation of 5-HT neurotransmission. (±)-MIR (25 μg/kg i.v.) enhanced the effectiveness of the electrical stimulation of the ascending 5-HT pathway by blocking both *alpha*-2 adrenergic auto- and heteroreceptors. (−)-MIR (10 μg/kg i.v.) enhanced the effectiveness of these stimulations due to a selective action of (−)-MIR on the *alpha*-2 heteroreceptors located on 5-HT terminals. Both (±)- and (−)-MIR (500 μg/kg i.v.) blocked the suppressant effect of microiontophoretically applied norepinephrine (NE) on the firing activity of CA_3 dorsal hippocampus pyramidal neurons, indicating their antagonistic effects on postsynaptic *alpha*-2 adrenoceptors. (±)-MIR (10–250 μg/kg i.v.) enhanced dose-dependently the firing activity of the 5-HT neurons in naive rats, but not in 6-hydroxydopamine-pretreated rats. (±)-MIR also significantly increased the firing activity of locus ceruleus NE neurons. In contrast, (−)-MIR (10–250 μg/kg i.v.) failed to change the firing rate of dorsal raphe 5-HT neurons. In conclusion, these results suggest that both (±)-MIR and (−)-MIR are antagonists at postsynaptic *alpha*-2 adrenergic receptors, that (±)-MIR is an antagonist of somatodendritic as well as terminal *alpha*-2 adrenergic auto- and heteroreceptors, whereas (−)-MIR is a selective antagonist at *alpha*-2 adrenergic heteroreceptors. Furthermore, the inhibitory effect of (−)-MIR on locus ceruleus NE neurons appears to be mediated *via* 5-HT neurons because it is abolished by a 5,7-dihydroxytryptamine pretreatment.

Mirtazapine [(±)MIR] [1,2,3,4,10,14b-hexa-hydro-2-methylpyrazino[2.1-*a*]pyrido[2,3-*c*]benzazepin] (ORG 3770 or Remeron), is a tetracyclic compound with antidepressant activity in humans (Smith *et al.*, 1990; Claghorn and Lesem, 1995; van Moffaert *et al.*, 1995). Its pharmacological profile is characterized by a potent presynaptic *alpha*-2 adrenergic antagonistic activity, weak 5-HT_1 and potent 5-HT_2 and 5-HT_3 antagonistic activities, as well as by a potent H_1 antagonistic activity. It is devoid of anticholinergic activity and has no effect on the reuptake of catecholamines and indolamines (Nickolson *et al.*, 1982; de Boer *et al.*, 1988). The blockade of presynaptic *alpha*-2 adrenoceptors is considered as a possible mechanism for antidepressant activity of MIR (see Pinder and Wieringa, 1993 for review).

By using microdialysis in freely moving rats, it has been shown that (±)-MIR increased 5-HT release in the ventral hippocampus (de Boer *et al.*, 1994). It has been suggested that the indirect *alpha*-1 adrenoceptor-mediated enhancement of 5-HT neuron firing activity and the direct blockade of suppressant action of *alpha*-2 adrenergic heteroreceptors located on 5-HT terminals are responsible for this increase in extracellular 5-HT. In order to distinguish *in vivo* the activations of *alpha*-2 adrenergic auto- and heteroreceptors, previous studies in our laboratory (Mongeau *et al.*, 1993), by using the electrical stimulation of the ascending 5-HT pathway, have shown that low doses of clonidine (2 and 10 μg/kg i.v.) enhance the effectiveness of the electrical stimulation of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus CA_3 pyramidal neurons. In contrast, high doses of clonidine (100 and 400 μg/kg i.v.) reduce the effectiveness of the stimulation. Both the incremental and decremental effects of clonidine were reversed by the i.v. injection of the *alpha*-2 adrenoceptor antagonist yohimbine, indicating that these effects are mediated *via alpha*-2 adrenoceptors. Furthermore, the enhancing effect of the low dose, but not those of the high dose, of clonidine was abolished in rats pretreated with the neurotoxin 6-OHDA. This indicates that the enhancing effect of the low dose of clonidine results from the selective activation of the *alpha*-2 adrenergic auto-

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ABBREVIATIONS: MIR, mirtazapine (Remeron, ORG 3770); 5-HT, 5-hydroxytryptamine (serotonin); 6-OHDA, 6-hydroxydopamine; NE, norepinephrine; LC, locus ceruleus; SIL, silence; 5,7-DHT, 5,7-dihydroxytryptamine; LSD, lysergic acid diethylamide.

receptors on noradrenergic (NE) terminals, thereby reducing the tonic activation by NE of *alpha*-2 adrenergic heteroreceptors on 5-HT terminals. On the other hand, the reducing effect of high doses of clonidine could be attributed to a direct activation of *alpha*-2 adrenergic heteroreceptors on 5-HT terminals (Mongeau *et al.*, 1993).

By using *in vivo* electrophysiological paradigms, the present studies were undertaken to characterize the effects of (±)-MIR on pre- and postsynaptic *alpha*-2 adrenoceptors. The effects of (−)-MIR were studied because of its purported selectivity for *alpha*-2 adrenergic heteroreceptors. First, at the postsynaptic level, the effects of (±)- and (−)-MIR on the CA_3 dorsal hippocampus pyramidal neurons responsiveness to microiontophoretic applications of NE were assessed. Second, at the level of 5-HT terminals, the effects of (±)- and (−)-MIR on the endogenous release of 5-HT induced by the electrical stimulation of the ascending 5-HT pathway were assessed, as well as their abilities to antagonize the effects of clonidine on the effectiveness of the stimulation. Finally, the effects of (−)- and (±)-MIR on the spontaneous firing activity of both dorsal raphe 5-HT and LC NE neurons were investigated.

Materials and Methods

The experiments were carried out in male Sprague-Dawley (Charles River, St. Constant, Québec, Canada) rats weighing 250 to 300 g, which were kept under standard laboratory conditions (12:12 light-dark cycle with free access to food and water), and were anesthetized with chloral hydrate (400 mg/kg i.p.). Supplemental doses were given to maintain constant anesthesia and to prevent any nociceptive reaction to a tail pinch.

Extracellular unitary recording from CA_3 dorsal hippocampus pyramidal neurons. Recording and microiontophoresis were performed with five-barreled glass micropipettes broken back to 8 to 12 μm under microscopic control (ASI Instruments, Warren, MI). The central barrel was filled with a 2 M NaCl solution and was used for extracellular unitary recordings. The pyramidal neurons were identified by their large amplitude (0.5–1.2 mV) and long-duration (0.8–1.2 msec) simple spikes alternating with complex spike discharges (Kandel and Spencer, 1961). The side barrels contained the following solutions: NE (20 mM in 200 mM NaCl, pH 4), quisqualic acid (1.5 mM in 200 mM NaCl, pH 8) and 2 M NaCl used for automatic current balancing. The rats were mounted in a stereotaxic apparatus and the microelectrodes were lowered at 4.2 mm lateral and 4.2 anterior to lambda into the CA_3 region of the dorsal hippocampus. Because most hippocampus pyramidal neurons are not spontaneously active under chloral hydrate anesthesia, a small current of quisqualate (−2 to −7 nA) was used to activate them within their physiological firing range (10–15 Hz) (Ranck, 1975). Neuronal responsiveness to the microiontophoretic application of NE were assessed by determining the number of spikes suppressed per nanoampere. The duration of the microiontophoretic applications of the agonist was 50 sec. The same ejection current was always used before and after each i.v. injection of *alpha*-2 adrenoceptors antagonists (±)- and (−)-MIR (500 μg/kg).

Electrical stimulation of the afferent 5-HT fibers to the hippocampus. A bipolar electrode (NE-110; David Kopf, Tujunga, CA) was implanted on the midline with a 10° backward angle in the ventromedial tegmentum, 1 mm anterior to lambda and 8.3 mm bellow the cortical surface. A stimulator (S8800; Grass Instruments, Quincy, MA) delivered 200 square pulses of 0.5 msec at a frequency of 1 Hz and an intensity of 300 μA. The stimulation pulses and the firing activity of the neuron recorded were fed to an IBM-PC computer equipped with a Tecmar interface. Peristimulus time histograms were generated to determine the duration of suppression of firing activity of the CA_3 pyramidal neuron, measured in absolute silence (SIL) value (in milliseconds) and two determinations of the SIL values were carried out before and after the administration of each drug. This value is obtained by dividing the total number of events suppressed after the stimulation by the mean frequency of firing of the neuron recorded (Chaput *et al.*, 1986). CA_1-CA_3 regions of the hippocampus receive extensive innervation from 5-HT neurons of raphe nuclei (Hensler *et al.*, 1994). The suppressant effect of the electrical stimulation of the ascending 5-HT pathway is due to the release of 5-HT into the synaptic cleft (Chaput *et al.*, 1986). Thus, the effects of the stimulation of the ascending 5-HT pathway were determined before and after the i.v. injection of the *alpha*-2 adrenoceptor agonist clonidine (comparison between prior *vs.* post-clonidine). The effect of the stimulation was then assessed before and after each of the consecutive injections of (±)-MIR (25 μg/kg i.v.) or (−)-MIR (5, 10 or 25 μg/kg i.v.) and clonidine (10 and 100 μg/kg i.v.). Finally, the effect of the stimulation was tested before and after each of the consecutive i.v. injections of the 5-HT autoreceptor antagonist metergoline (1 mg/kg), (−)-MIR (25 and 500 μg/kg) and clonidine (100 μg/kg). The duration of the effects of all drugs studied was greater than 30 min. The effects of consecutive drug injections on the effectiveness of the stimulation were compared using the SIL values before and after each drug administration.

Unitary extracellular recording from dorsal raphe 5-HT and LC NE neurons. Unitary extracellular recordings were performed with single-barreled glass micropipettes preloaded with fiberglass filaments to facilitate filling. The tip was broken back to 1 to 4 μm and filled with a 2 M NaCl solution saturated with Fast Green FCF. The rats were placed in a stereotaxic frame and a burr hole was drilled on midline 1 mm anterior to lambda. Dorsal raphe 5-HT neurons were encountered over a distance of 1 mm starting immediately below the ventral border of the Sylvius aqueduct. These neurons were identified by using the criteria of Aghajanian (1978): a slow (0.5–2.5 Hz) and regular firing rate and long-duration (0.8–1.2 msec) positive action potentials. The responsiveness of 5-HT neurons was assessed before and after the i.v. injection of clonidine (10 μg/kg) and both (−)- and (±)-MIR (10–250 μg/kg) for the determination of the dose-response curves. The effect of (±)-MIR on the firing rate of dorsal raphe 5-HT neurons were also determined in 6-OHDA-lesioned rats and (−)-pindolol-pretreated rats. Lesion of NE neurons were performed under chloral hydrate anesthesia by injecting 6-OHDA i.c.v. (120 μg of free base in 20 μl of 0.9% NaCl and 0.1% ascorbic acid) 1 hr after the injection of the 5-HT reuptake blocker fluoxetine (10 mg/kg i.p.) to protect 5-HT neurons. The rats were tested 10 days later. The same volume of vehicle was injected in control rats. Two-day treatment with (−)-pindolol (15 mg/kg/day) was performed with minipumps (Alza, Palo Alto, CA) inserted s.c.

LC NE neurons were recorded with single-barreled glass micropipettes lowered at −0.7 mm interaural and 1.1 to 1.4 mm lateral (Paxinos and Watson, 1982). The NE neurons were identified by regular firing rate (1–5 Hz), long-duration (0.8–1.2 msec) positive action potentials and their characteristic burst discharge in response to nociceptive pinch of the contralateral hind paw (Aghajanian, 1978). The responsiveness of LC NE neurons was assessed before and after the i.v. injection of clonidine (0.5–5 μg/kg) alone and after 250 μg/kg of (±)-MIR for the determination of the dose-response curves. The responsiveness of LC NE neurons was assessed after (−)-MIR (250 μg/kg i.v.). The effect of (−)-MIR on LC NE neurons firing activity was assessed after the i.v. injection of the 5-HT_{1A} antagonist BMY 7378 (1 mg/kg) (Chaput and de Montigny, 1988). The effect of (−)-MIR (250 μg/kg i.v.) on the spontaneous responsiveness of NE neurons was also assessed after pretreatment with 5,7-DHT. Lesioning of the 5-HT system was performed under chloral hydrate anesthesia by injecting 5,7-DHT i.c.v. (200 μg of free base in 20 μl of 0.9% NaCl and 0.1% ascorbic acid) 1 hr after the injection of desipramine (25 mg/kg i.p.) to protect NE neurons from the neurotoxic action of 5,7-DHT and the rats were tested 10 days later. The same volume of the vehicle was injected in control rats.

Drugs. (±)-MIR and (−)-MIR (Organon; Oss); clonidine, NE bitartrate, quisqualic acid, 6-OHDA HCl and 5,7-DHT creatinine sulfate (Sigma Chemical Co.); BMY 7378 (Bristol Meyers Co., Evansville, IN); and metergoline (Farmitalia, Milano, Italia); (−)-pindolol and desipramine HCl (Research Biochemicals); and fluoxetine (Eli Lilly, Indianapolis, IN). The concentrations and the doses used for these compounds were chosen on the basis of previous successful experiments carried out in our and other laboratories.

Results

Effects of (±)- and (−)-MIR on CA_3 dorsal hippocampus pyramidal neuron responsiveness to NE. It has been demonstrated previously that the microiontophoretic application of NE onto rat dorsal hippocampus pyramidal neurons produces a suppressant effect on their firing activity, and that this effect is mediated by postsynaptic *alpha*-2 adrenoceptors (Curet and de Montigny, 1988a). For all CA_3 hippocampus pyramidal neurons, NE (10–30 nA) induced a reduction of firing activity, ranging from 30 to 80%. This suppressant effect of NE occurred in the absence of alteration of the shape of the action potentials and was current-dependent. (±)-MIR and (−)-MIR (0.5 mg/kg i.v.) reduced the suppressant effect of NE. (±)-MIR reduced the suppressant effect of NE by 55% and (−)-MIR by 50% (fig. 1).

Effects of (±)- and (−)-MIR on the response of CA_3 dorsal hippocampus pyramidal neurons to the electrical stimulation of the ascending 5-HT pathway. Previous studies in our laboratory have shown that low doses of clonidine (2 and 10 μg/kg i.v.) enhance the effectiveness of the electrical stimulation of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus CA_3 pyramidal neurons. In contrast, high doses of clonidine (100 and 400 μg/kg i.v.) reduce the effectiveness of the stimulation (see Introductory section). In the present study, clonidine (10 μg/kg i.v.) increased by 35% the duration of suppression of the firing activity of CA_3 pyramidal neurons induced by the electrical stimulation of the ascending 5-HT pathway (fig. 2, A and B). In contrast, subsequent injections of clonidine of 100 and 400 μg/kg i.v. reduced by 30 and 65%, respectively, the duration of suppression of the firing activity of CA_3 pyramidal neurons induced by the electrical stimulation of the ascending 5-HT pathway (SIL before, 59 ± 7 msec; after 10 μg/kg of clonidine, 81 ± 11 msec, $n = 13$, $P < .05$; after 100 μg/kg of clonidine, 45 ± 9 msec, $n = 9$, $P < .05$; and after 400 μg/kg of clonidine, 26 ± 4 msec, $n = 6$, $P < .05$, fig. 2B).

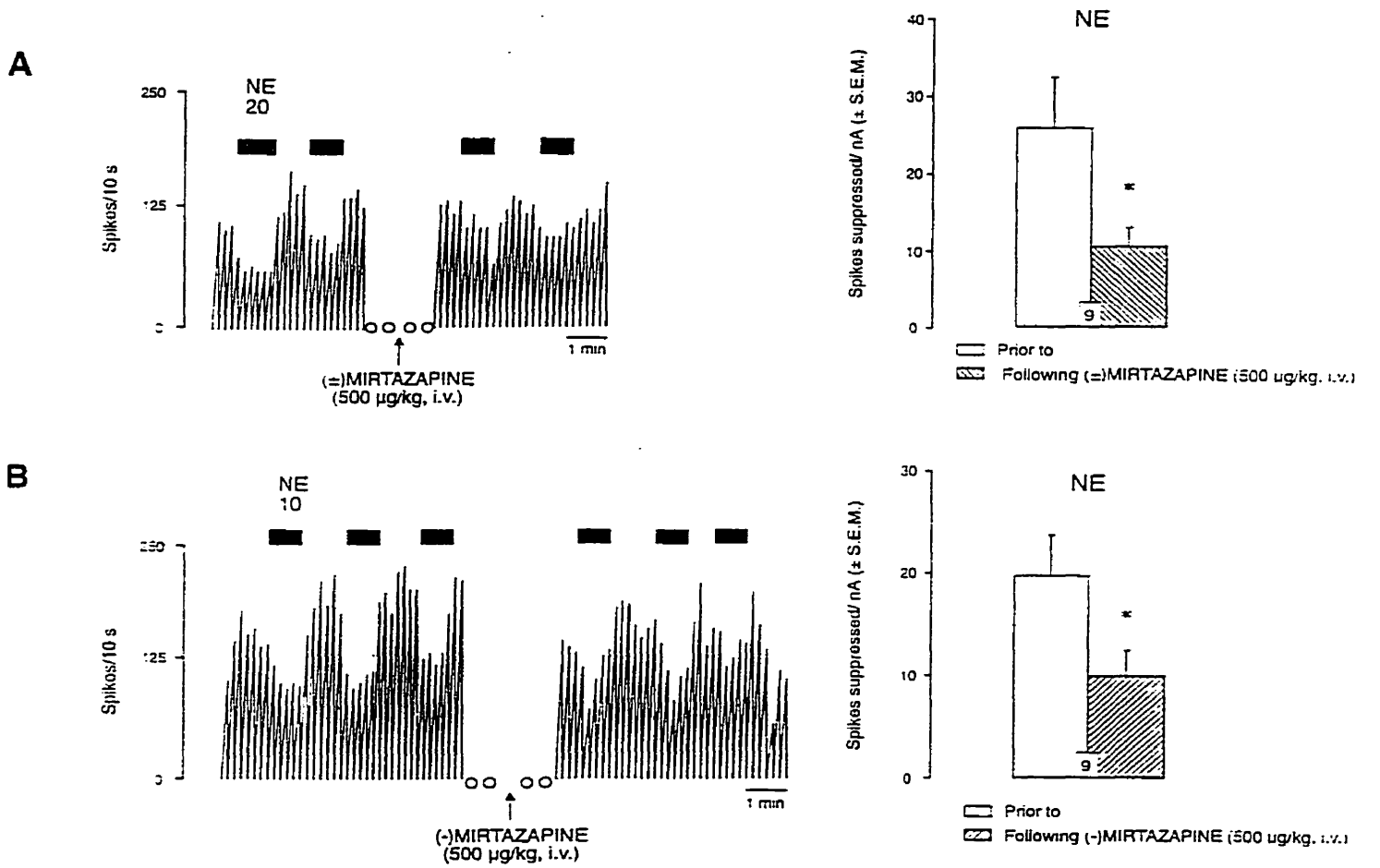

Fig. 1. Integrated firing rate histograms of two dorsal hippocampus CA_3 pyramidal neurons showing their responsiveness to microiontophoretic application of NE before and after the administration of (±)-MIR (A) and (−)-MIR (B). These neurons were activated with quisqualate ejection currents of −1 and −3 nA, respectively. Horizontal bars indicate the duration of the applications for which the current is given in nanoamperes. Corresponding results (means ± S.E.M.) are presented on the right. Numbers at the bottom of the columns indicate, the number of neurons tested. *$P < .05$, using the paired Student's *t* test).

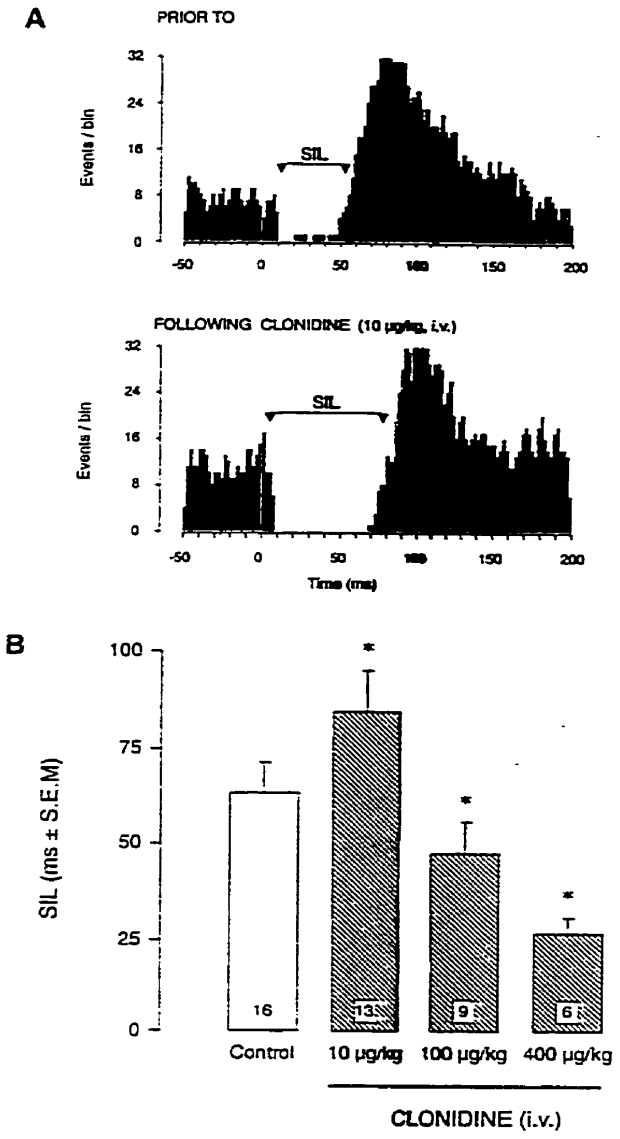

Fig. 2. A. peristimulus time histograms illustrating the effect of the electrical stimulation of the ascending 5-HT pathway at the level of the ventro-medial tegmentum on the firing activity of dorsal hippocampus CA_3 pyramidal neurons before and after clonidine. SIL represents the duration of suppression of firing (see "Materials and Methods"). Each histogram was constructed from 200 consecutive stimulations (300 µA, 0.5 msec, 1 Hz) with a bin width of 2 msec. B, duration of suppression of firing activity (means ± S.E.M) of CA_3 hippocampus pyramidal neurons produced by the stimulation of the ascending 5-HT pathway, before and after the administration of clonidine (cumulative doses of 10, 100 and 400 µg/kg). Numbers in the columns, the number of neurons tested. *P < .05, using the paired Student's *t* test.

de Boer *et al.* (1994) have shown, using microdialysis, that (±)-MIR increases the release of 5-HT in the rat ventral hippocampus. In order to determine whether (±)- and (−)-MIR could modulate *in vivo* the efficacy of 5-HT synaptic transmission and antagonize the effects of the *alpha*-2 adrenoceptor agonist clonidine, their capacity to modify the duration of the suppression of the firing activity of CA_3 hippocampus pyramidal neurons produced by the electrical stimulation of the ascending 5-HT pathway was examined. (±)-MIR (25 µg/kg i.v.) increased by 20% the efficacy of the stimulation of the 5-HT pathway (SIL before, 40 ± 5 msec; and after (±)-MIR, 48 ± 7 msec, $n = 8$; $P < .05$), suggesting a possible blockade of *alpha*-2 adrenergic heteroreceptors. (±)-MIR (25 µg/kg) antagonized the effects of subsequent i.v. injections of both a low and a high dose of clonidine (SIL before, 48 ± 7 msec; after 10 µg/kg of clonidine, 53 ± 15 msec, $n = 6$; and after 100 µg/kg of clonidine, 60 ± 12 msec, $n = 8$, fig. 3A). Several doses of (−)-MIR were used to study its specificity. (−)-MIR (5 µg/kg i.v.) did not modify by itself the efficacy of the stimulation of the 5-HT pathway (SIL before, 47 ± 6 msec; and after (−)-MIR, 47 ± 10 msec, $n = 6$). However, 5 µg/kg of (−)-MIR antagonized only the effect of subsequent i.v. injection of a high dose of clonidine (SIL before, 47 ± 10 msec; after 10 µg/kg of clonidine, 60 ± 11 msec, $n = 6$, $P < .05$; and after 100 µg/kg of clonidine, 50 ± 7 msec, $n = 8$). However, 10 µg/kg of (−)-MIR increased by 10% the efficacy of the stimulation of the 5-HT pathway (SIL before, 42 ± 5 msec; and after (−)-MIR, 46 ± 5 msec, $P < .05$, $n = 10$). (−)-MIR (10 µg/kg i.v.) also antagonized the effect of

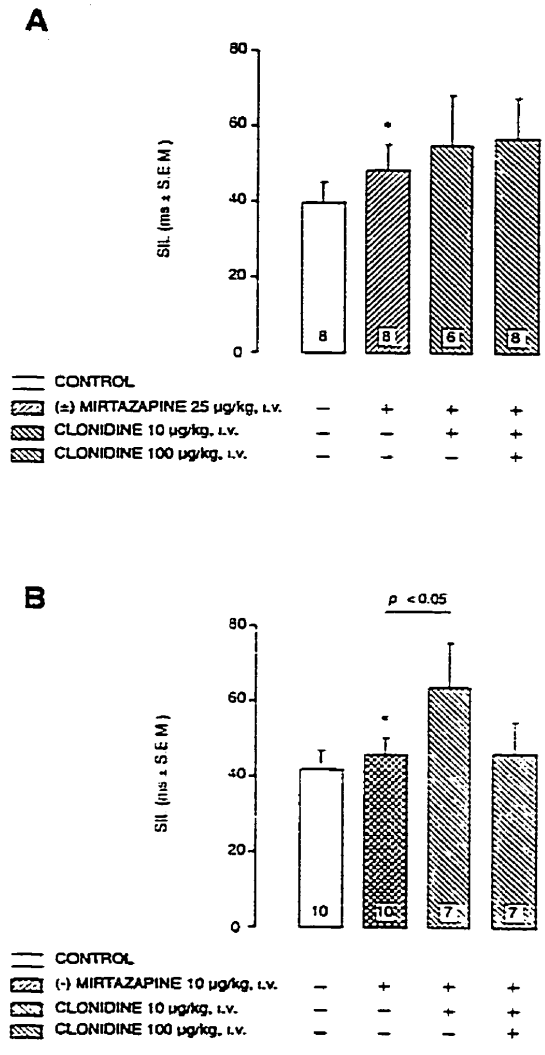

Fig. 3. Histograms illustrating the effects of the i.v. injections of (±)-MIR (A) and (−)-MIR (B) on the efficacy of the stimulation of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus pyramidal neurons and the effects of subsequent consecutive administrations of a low (10 µg/kg) and a high (100 µg/kg) dose of clonidine (means ± S.E.M.). Number in the columns indicates the number of neurons tested. *P < .05, using the paired Student's *t* test.

subsequent i.v. injection of a high dose of clonidine (SIL before, 46 ± 5 msec; after 10 μg/kg of clonidine, 64 ± 12 msec, $n = 7$, $P < .05$; and after 100 μg/kg of clonidine, 46 ± 9 msec, $n = 7$, fig. 3B), indicating its specificity for the *alpha*-2 adrenergic heteroreceptors. Unexpectedly, 25 μg/kg i.v. of (−)-MIR reduced by 23% the efficacy of the stimulation of the 5-HT pathway (SIL before, 33 ± 5 msec; and after (−)-MIR, 26 ± 2 msec, $n = 6$, $P < .05$); and antagonized only the effect of subsequent i.v. injection of a high dose of clonidine (SIL before, 26 ± 2 msec; after 10 μg/kg of clonidine, 31 ± 1 msec, $n = 6$, $P < .05$; after 100 μg/kg of clonidine, 29 ± 2 msec, $n = 6$; and after 400 μg/kg of clonidine, 24 ± 2 msec, $n = 6$, fig. 4A).

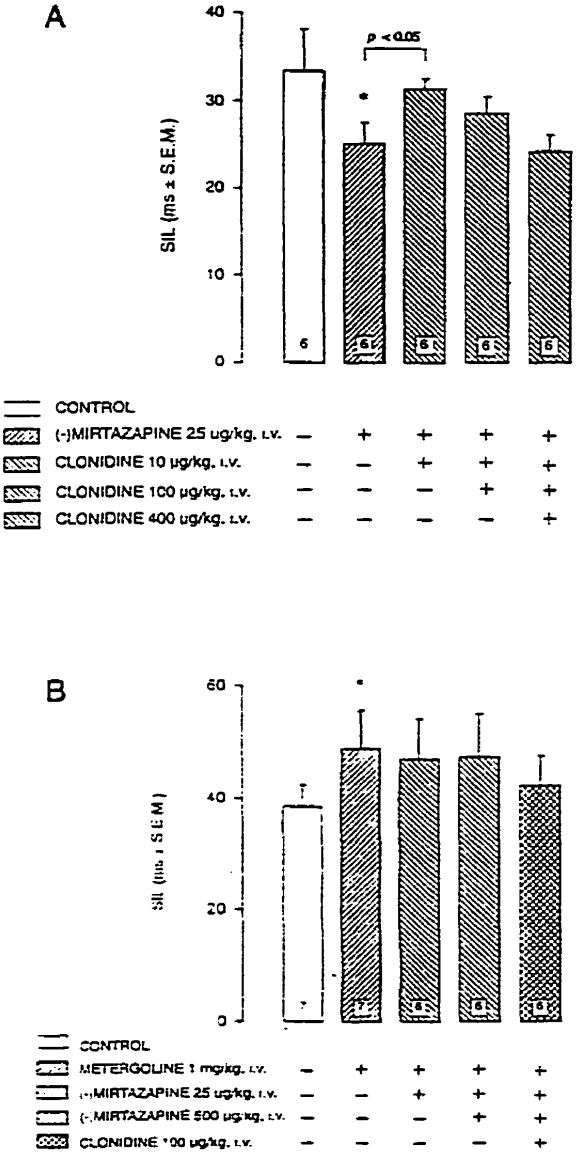

Fig. 4. A. effects of (−)-MIR on the efficacy of the stimulation of the ascending 5-HT pathway of consecutive administrations of 10, 100 and 400 μg/kg of clonidine (means ± S.E.M.). B. effects of metergoline (1 mg/kg) and consecutive injections of (−)-MIR (25 and 500 μg/kg) and clonidine (100 μg/kg) on the efficacy of the stimulation of the ascending 5-HT pathway (means ± S.E.M.). Numbers in the columns indicate. the number of neurons tested. *P < .05, using the paired Student's *t* test.

The reducing effect of the 25-μg/kg dose of (−)-MIR was investigated further by using the 5-HT autoreceptor antagonist metergoline. The 5-HT autoreceptor antagonist metergoline (1 mg/kg i.v.) increased by 27% the efficacy of the stimulation of the 5-HT pathway (SIL before, 38 ± 4 msec; and after metergoline, 49 ± 7 msec, $n = 7$, $P < .05$). Metergoline prevented the effect of subsequent injections of (−)-MIR (SIL before, 49 ± 7 msec; after 25 μg/kg i.v. of (−)-MIR, 47 ± 7 msec, $n = 6$; and after 500 μg/kg i.v. of (−)-MIR, 47 ± 8 msec, $n = 6$) and the effect of the high dose of clonidine was abolished (SIL before, 47 ± 7 msec; after 100 μg/kg of clonidine, 42 ± 5 msec, $n = 6$, fig. 4B).

Effects of (±)- and (−)-MIR on the firing activity of dorsal raphe 5-HT neurons. The firing activity of the dorsal raphe 5-HT neurons tested was increased, in a dose-dependent manner, by (±)-MIR (10–250 μg/kg i.v.) reaching a plateau at an 80% increase from the dose of 100 μg/kg (figs. 5A and 6). Moreover, the reducing effect of clonidine (10 μg/kg i.v.) was reversed or prevented by (±)-MIR (250 μg/kg i.v., $n = 6$, fig. 5C). On the other hand, (−)-MIR (10–250 μg/kg i.v.) did not modify the spontaneous firing activity of the dorsal raphe 5-HT neurons (figs. 5B and 6). In order to

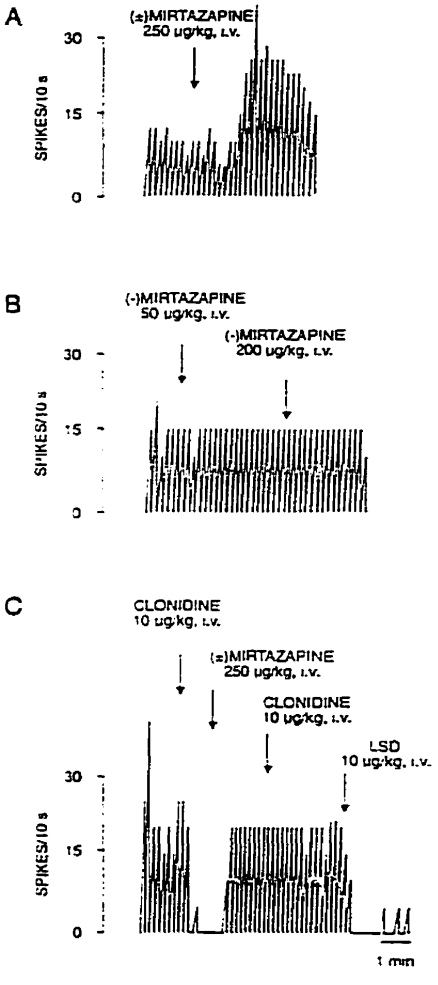

Fig. 5. Integrated firing rate histogram of dorsal raphe 5-HT neurons showing their responses to (±)-MIR (250 μg/kg; A), (−)-MIR (50 and 200 μg/kg; B) and clonidine (10 μg/kg, before and after 250 μg/kg of (±)-MIR and after 10 μg/kg of lysergic acid diethylamide (LSD) to confirm the 5-HT nature of the neuron recorded (C).

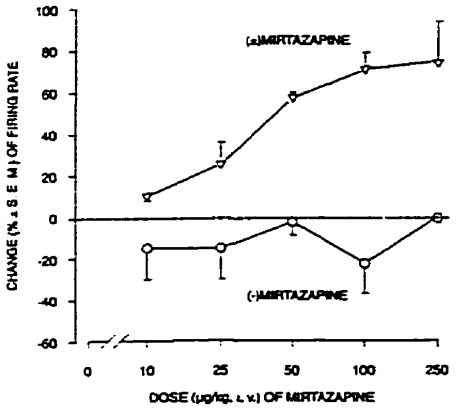

Fig. 6. Dose-response curves of the effects of (±)-MIR (▽) and (−)-MIR (○) on the spontaneous firing activity of dorsal raphe 5-HT neurons. One dose was administered to one rat while recording from one neuron. Each point represents the mean ± S.E.M. of the effects in two to four rats.

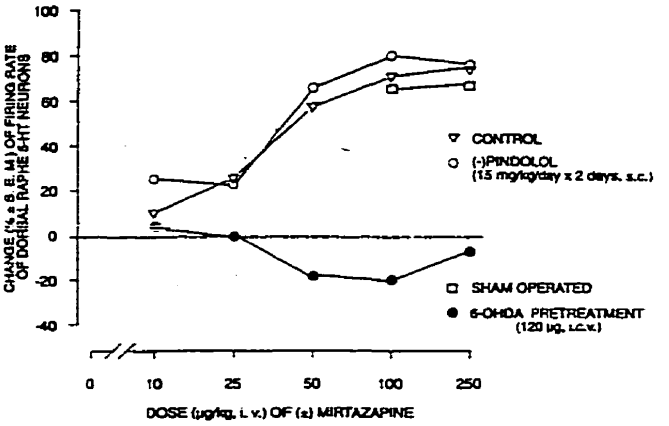

Fig. 8. Dose-response curves of the effects of (±)-MIR in control, (−)-pindolol-pretreated rats, in sham-operated and 6-OHDA-pretreated rats on the spontaneous firing activity of dorsal raphe 5-HT neurons. One dose was administered to one rat while recording from one neuron.

determine whether this enhancing effect of (±)-MIR on 5-HT neurons was mediated by NE neurons, the effect of (±)-MIR was assessed in 6-OHDA-pretreated rats. As illustrated in figure 7B, in 6-OHDA-pretreated rats, the administration of (±)-MIR (25–50 µg/kg i.v.) no longer modified the firing activity of 5-HT neurons (figs. 7B and 8), suggesting that the activating effect of (±)-MIR is mediated by an *alpha*-2 autoreceptor blockade. A 2-day treatment with the 5-HT_{1A} antagonist (−)-pindolol (15 mg/kg/day × 2 days), in order to block the somatodendritic 5-HT_{1A} receptors (Blier *et al.*, 1994), did not modify the dose-response curve of (±)-MIR on dorsal raphe 5-HT neurons (figs. 7C and 8). Thus, it would appear that the increase of the firing rate induced by (±)-MIR does not result in a physiologically significant activation of somatodendridic 5-HT_{1A} autoreceptors under these experimental conditions.

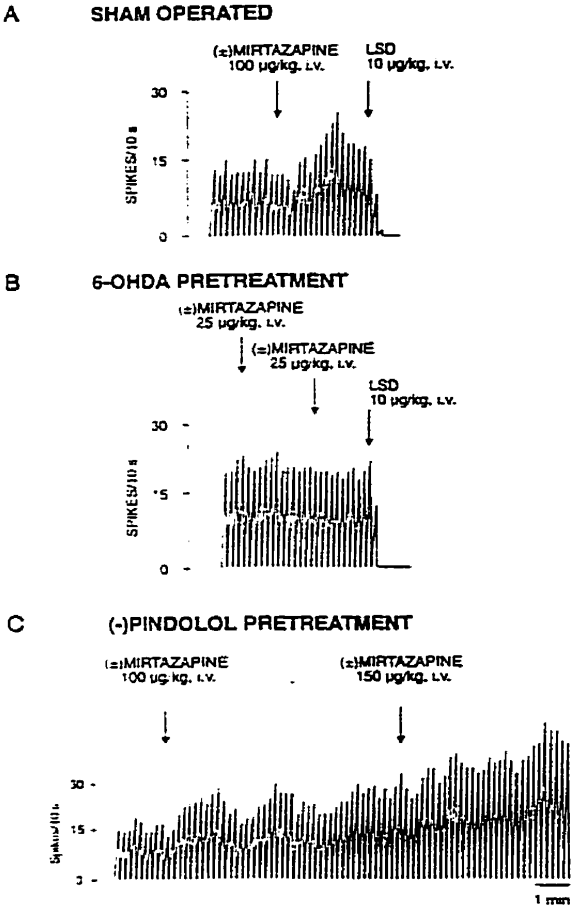

Fig. 7. Integrated firing rate histogram of dorsal raphe 5-HT neurons showing their response to (±)-MIR in sham-operated rats (A), in a 6-OHDA-pretreated rat (B) and in a rat pretreated with (−)-pindolol (15 mg/kg/day s.c. × 2 days) (C). LSD was used to confirm the 5-HT nature of the neuron recorded.

Effects of (±)- and (−)-MIR on the firing activity of LC NE neurons. It has been shown previously that the selective *alpha*-2 adrenoceptor antagonist, idazoxan, increases the spontaneous firing activity of LC NE neurons, whereas clonidine decreases it (Baraban and Aghajanian, 1980). (±)-MIR (250 µg/kg i.v.) increased the firing activity of LC NE neurons by 36% ± 5 (n = 7, fig. 9A). Moreover, the reducing effect of clonidine was prevented by (±)-MIR: IC_{50} value for control, 1.9 ± 0.3 µg/kg i.v.; IC_{50} value after (±)-MIR, 3.8 ± 0.5 µg/kg i.v. ($P < .05$, using confidence limits method (CL), fig. 9, A, C and D). Furthermore, (−)-MIR did not affect the reducing effect of clonidine on the firing rate of LC NE neurons (fig. 10C). In contrast, (−)-MIR (250 µg/kg i.v.) suppressed the firing activity of LC NE neurons (figs. 9B and 10D, n = 9). This effect of (−)-MIR on the firing activity of LC NE neurons was completely prevented by the selective 5-HT_{1A} antagonist BMY 7378 (Chaput and de Montigny, 1988) (1 mg/kg i.v., n = 6, fig. 10B). Hence, in order to determine whether this reducing effect of (−)-MIR on the firing activity of LC NE neurons was mediated *via* 5-HT neurons, the effect of (−)-MIR was examined in rats pretreated with 5,7-DHT. In the seven pretreated rats, the reducing effect of (−)-MIR on the firing activity of LC NE neurons was no longer present (fig. 10, C and D).

Discussion

The present electrophysiological studies show first that only (±)-MIR dose-dependently increased the firing activity of dorsal raphe 5-HT neurons and antagonized the suppressant effect of clonidine on the firing activity of 5-HT neurons, indicating its capacity to block the *alpha*-2 adrenergic auto-

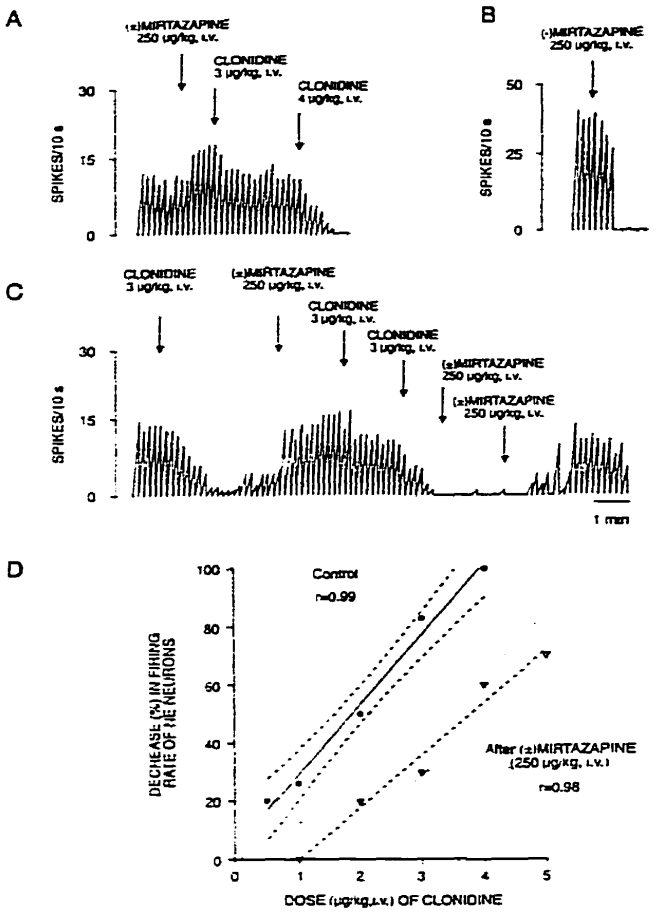

Fig. 9. Integrated firing rate histograms of LC NE neurons showing their response to (±)-MIR (A), (−)-MIR (B) and to clonidine before and after (±)-MIR (C). D, relationship between the dose of clonidine and the degree of decrease in firing rate of LC NE neurons of control rats (●) and after (±)-MIR (250 µg/kg i.v.) injection (▼). Each point represents the response of one LC neuron to one dose of clonidine administered to one rat, except for the dose of 5 µg/kg corresponding to the mean of two neurons. Curved lines, the S.E. for the regression lines. The shift to the right was statistically significant (IC_{50} value for control, 1.9 ± 0.3 µg/kg i.v.; IC_{50} value after (±)-MIR, 3.8 ± 0.5 µg/kg i.v., $P < .05$, using CL).

receptors. Second, (±)-MIR acted as an antagonist at both terminal *alpha*-2 adrenergic auto- and heteroreceptors, whereas (−)-MIR, depending to the dose used, acted selectively at the of terminal *alpha*-2 adrenergic heteroreceptors and possibly as a 5-HT autoreceptor agonist. Third, both (±)- and (−)-MIR blocked the suppressant effect of microiontophoretically applied NE on the firing activity of CA_3 hippocampus pyramidal neurons, which is mediated by postsynaptic *alpha*-2 adrenoceptors (Curet and de Montigny, 1988a). Finally, whereas (±)-MIR increased the firing activity of LC NE neurons and antagonized the suppressant effect of clonidine, (−)-MIR suppressed the firing activity of these neurons.

The comparative pharmacological profiles of (±)- and (−)-MIR have been reviewed by de Boer *et al.* (1988). (±)-MIR does not inhibit the uptake of [^{3}H]NE in rat hypothalamus synaptosomes. (±)-MIR antagonizes the effects of NE, which is mediated by *alpha*-2 adrenoceptors, on the evoked-release of both [^{3}H]NE or [^{3}H]-5-HT from preloaded cortical synaptosomes, whereas (−)-MIR antagonizes only that of [^{3}H]-5-HT. Furthermore, both (±)- and (+)-MIR facilitate the evoked-release of [^{3}H]NE in parietal cortex slices. In radioligand binding studies, (±)-MIR has about 10-fold higher affinity for *alpha*-2 than *alpha*-1 adrenoceptors. As mentioned in the Introductory section, MIR has weak 5-HT_1 and potent 5-HT_2 and 5-HT_3 antagonistic activities, as well as a potent H_1 antagonistic activity (de Boer *et al.*, 1988). However, several observations allow us to rule out the involvement of these latter antagonistic properties of this drug in the effects observed in the present studies. By using the same *in vivo* electrophysiological model, we have shown previously that selective 5-HT_3 receptor agonists and antagonists do not modify the endogenous release of 5-HT induced by the stimulation of the ascending 5-HT pathway (Haddjeri and Blier, 1995). It has been shown in the rat that 5-HT_2 receptors are mainly located postsynaptically (Fischette *et al.*, 1987). Moreover, the selective 5-HT_2 receptor antagonist, ketanserin, reduced the firing activity of dorsal raphe 5-HT neurons and failed to modify the suppressant effect of 5-HT applied microiontophoretically (Lakoski and Aghajanian, 1985). It has also been shown that the inhibition of cortical 5-HT release and the suppression of dorsal raphe 5-HT firing activity induced by the 5-HT_2 receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, was not mediated via 5-HT_2 receptor activation (Kidd *et al.*, 1991). In the LC, selective 5-HT_2 receptor antagonists increase (Rasmussen and Aghajanian, 1986) whereas 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, administered systemically but not locally, decreases the firing activity of LC NE neurons. This latter effect has been proposed to be due to a tonic activation of the GABA input to the LC (Chiang and Aston-Jones, 1993). The studies of the role of the histaminergic system on monoaminergic release are still conflicting. In conscious rats, Beaier (1993) has shown that histamine increases NE release in the paraventricular nucleus/anterior hypothalamus *via* the activation of H_1 receptors. However, in rat hypothalamus slices, the enhancing action of histamine on NE release has been reported to be mediated *via* H_2 receptors (Blandina *et al.*, 1989). Furthermore, Schliker *et al.* (1992) have reported that histamine inhibits NE release from mouse brain cortex slices *via* H_{3A} receptors. Finally, Sleight *et al.* (1989) have found that long-term treatment with the antidepressant drug amitriptyline, which has high affinity for H_1 receptors, does not modify the *in vivo* 5-HT release.

The presence of *alpha*-1 and *alpha*-2 adrenoceptors in the rat hippocampus is well documented (Young and Kuhar, 1980; Bylund and U'Prichard, 1983) and terminal *alpha*-2 adrenergic autoreceptors have been shown to be of the *alpha*-2D subtype in the rat (Trendelenburg *et al.*, 1993). Microiontophoretic application of NE onto rat dorsal hippocampus pyramidal neurons produces a suppressant effect on their firing activity mediated by postsynaptic *alpha*-2 adrenoceptors (Curet and de Montigny, 1988a). However, the suppressant effect of endogenous NE, released by the stimulation of LC, is mediated by *alpha*-1 adrenoceptors (Curet and de Montigny, 1988b). In the present study, both (±)- and (−)-MIR (500 µg/kg i.v.) reduced, to an extent similar to that of the selective *alpha*-2 adrenoceptors antagonist, idazoxan (Curet and de Montigny, 1988a), the suppressant effect of NE applied microiontophoretically on the firing activity of CA_3 hippocampus pyramidal neurons, suggesting that they are

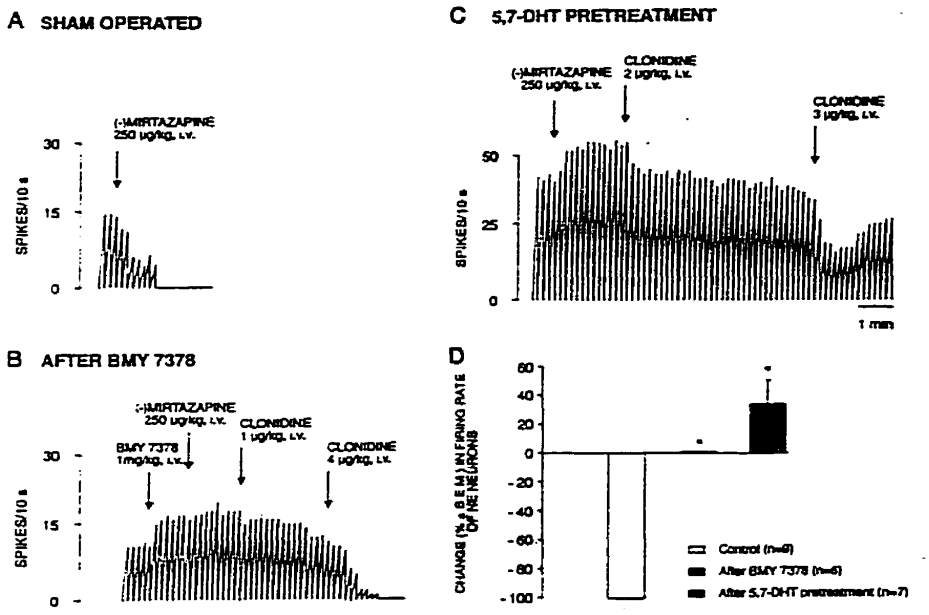

Fig. 10. Integrated firing rate histogram of NE neurons recorded in the LC showing their responses to (−)-MIR (250 µg/kg i.v.) in a sham-operated rat (A), in another rat after BMY 7378 (1 mg/kg, i.v.) and before clonidine (B) and in a 5,7-DHT-pretreated rat (C). D, responsiveness of NE neurons to (−)-MIR (250 µg/kg i.v.) in control, in BMY 7378- and 5,7-DHT-pretreated rats (means ± S.E.M.) *P < .001, using the unpaired Student's *t* test.

equipotent antagonists at the postsynaptic *alpha*-2 adrenoceptors (fig. 1).

Using microdialysis in freely moving rats, de Boer *et al.* (1994) have shown that (±)-MIR increased the ventral hippocampal 5-HT release by 80% at a dose of 2 mg/kg s.c. An indirect *alpha*-1 adrenoceptor-mediated enhancement of 5-HT neuronal firing and a direct blockade of suppressant *alpha*-2 heteroreceptors located on 5-HT terminals have been proposed to account for this increase in extracellular 5-HT. Previous studies from our laboratory have shown that the *in vivo* activation of *alpha*-2 autoreceptors on NE terminals by a low dose of clonidine enhances the effectiveness of the electrical stimulation of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus CA_3 pyramidal neurons, whereas a high dose of clonidine activates directly the *alpha*-2 heteroreceptors on 5-HT terminals, thereby reducing the effectiveness of the stimulation. Both the incremental and decremental effects of clonidine were reversed by the i.v. injection of the *alpha*-2 adrenoceptor antagonist, yohimbine, indicating that these effects are mediated *via alpha*-2 adrenoceptors. Furthermore, the enhancing effect of the low dose, but not that of the high dose, of clonidine was abolished in rats pretreated with the NE neurotoxin 6-OHDA, indicating that the enhancing effect of the low dose of clonidine results from the selective activation of the *alpha*-2 adrenergic autoreceptors on NE terminals, thereby reducing the tonic activation of NE at *alpha*-2 adrenergic heteroreceptors on 5-HT terminals. In contrast, the reducing effect of the high dose of clonidine is due to a direct activation of the *alpha*-2 adrenergic heteroreceptors on 5-HT terminals (Mongeau *et al.*, 1993). By using this *in vivo* model, the injection of (±)-MIR (25 µg/kg i.v.) significantly enhanced the effectiveness of the stimulation, reflecting an enhanced endogenous release of 5-HT, and prevented the effects of subsequent i.v. administration of both low and high doses of clonidine (fig. 3A). Whereas 10 µg/kg i.v. of (−)-MIR enhanced the effectiveness of the stimulation, it did not prevent the enhancing effect of the low dose of clonidine, but antagonized the reducing effect of the high dose of clonidine, showing its selectivity for *alpha*-2 adrenergic heteroreceptors (fig. 3B). Unexpectedly, 25 µg/kg i.v. of (−)-MIR significantly reduced the endogenous release of 5-HT and prevented only the effect of the high dose of clonidine (fig. 4A). This reducing effect of 25 µg/kg of (−)-MIR was prevented by the 5-HT autoreceptor antagonist metergoline (1 mg/kg i.v.; fig. 4B), which is devoid of affinity for *alpha*-2 adrenergic receptors (Fuxe *et al.*, 1975) and increases by itself the efficacy of the stimulation. Taken together, these data suggest that (±)-MIR is an antagonist at terminal *alpha*-2 adrenergic auto- and heteroreceptors, whereas (−)-MIR is a selective antagonist at terminal *alpha*-2 heteroreceptors and, according to the dose used, may act as an agonist of terminal 5-HT autoreceptors. Because (−)-MIR has low affinity for 5-HT_1 receptors (de Boer *et al.*, 1988), further studies are required to determine the 5-HT receptor subtype involved.

Dorsal raphe 5-HT neurons receive NE projections from the LC (Loizou, 1969; Anderson *et al.*, 1977; Baraban and Aghajanian, 1981; Jones and Yang, 1985; Luppi *et al.*, 1995). Pharmacological studies have suggested that the firing activity of 5-HT neurons in the dorsal raphe is dependent on a tonic activation by its adrenergic input (Svensson *et al.*, 1975; Baraban and Aghajanian, 1980). Yoshioka *et al.* (1992) have shown that *alpha*-2 adrenoceptor activation reduces 5-HT synthesis in both hippocampus and dorsal raphe nucleus. Moreover, it has been shown that the *alpha*-2 adrenergic agonist clonidine suppressed the firing activity of 5-HT neurons of the dorsal raphe; this reducing action was suggested to be due to the activation of *alpha*-2 adrenergic autoreceptors decreasing the endogenous NE excitatory input on *alpha*-1 adrenergic receptors located on 5-HT neurons (Svensson *et al.*, 1975; Clement *et al.*, 1992). Accordingly, the *alpha*-2 adrenergic antagonist, idazoxan, enhances the firing activity of dorsal raphe 5-HT neurons (Freedman and Aghajanian, 1984; Garrat *et al.*, 1991). In the present study, whereas (−)-MIR did not modify by itself the firing activity of dorsal raphe 5-HT neurons (figs. 5B and 6), (±)-MIR dose-dependently increased the spontaneous firing activity of these neurons (figs. 5A and 6) and antagonized the suppres-

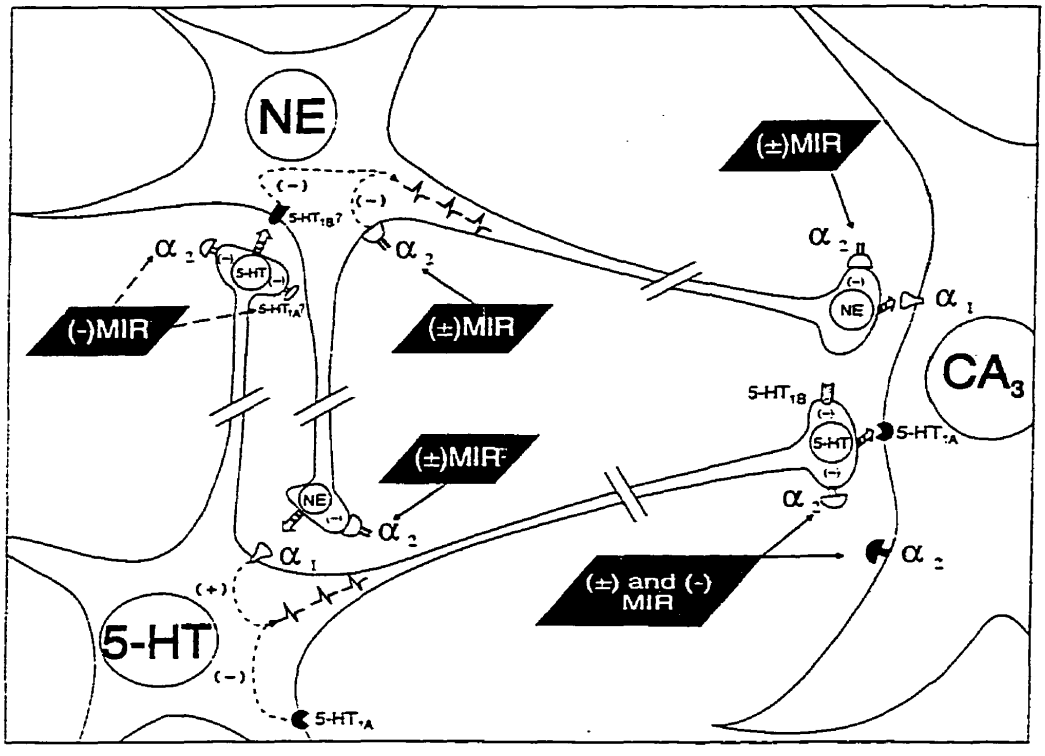

Fig. 11. Hypothetical scheme of the anatomical connections and functional interactions of dorsal raphe nucleus (5-HT), LC (NE) and dorsal hippocampus (CA_3) in the rat. The activity of dorsal raphe 5-HT neurons is dependent on the tonic activation by the adrenergic input from LC *via alpha*-2 adrenergic autoreceptors on NE terminals (although there is no definite evidence that *alpha*-1 adrenoceptors are located at the somatodendritic level of dorsal raphe 5-HT neurons) and tonic autoinhibition by somatodendritic $5\text{-}HT_{1A}$ autoreceptors. The activity of LC NE neurons is dependent mainly on the tonic autoinhibition by somatodendritic *alpha*-2 adrenergic autoreceptors and the tonic inhibitory input from 5-HT fibers. At the level of dorsal hippocampus, the amount of NE and 5-HT is regulated negatively by terminal *alpha*-2 adrenergic and $5\text{-}HT_{1B}$ autoreceptors, respectively, and endogenous NE dampens 5-HT release through the tonic activation of *alpha*-2 adrenergic heteroreceptors.

sant effect of clonidine (fig. 5C). The enhancing effect of (±)-MIR on the firing activity of 5-HT neurons was abolished by a 6-OHDA-pretreatment (figs. 7B and 8), indicating that this effect of (±)-MIR was due to blockade of the *alpha*-2 adrenergic autoreceptors and not to a direct activation of *alpha*-1 adrenergic receptors located on 5-HT neurons. It has been shown that a 2-day treatment with the $5\text{-}HT_{1A}$ antagonist, (−)-pindolol, blocked the somatodendritic $5\text{-}HT_{1A}$ receptors of dorsal raphe 5-HT neurons (Blier *et al.*, 1994) and potentiated the efficacy of selective 5-HT reuptake inhibitors (Bel *et al.*, 1994). Such a treatment did not modify the dose-response curve of (±)-MIR on dorsal raphe 5-HT neurons (figs. 7C and 8). Under these experimental conditions, it seems therefore that the increase of the firing rate dorsal raphe 5-HT neurons induced by (±)-MIR does not result in a physiologically significant activation of somatodendridic $5\text{-}HT_{1A}$ autoreceptors, because pretreatment with (−)-pindolol failed to enhance the effect of (±)-MIR on dorsal raphe 5-HT firing activity (figs. 7C and 8).

Alpha-2 adrenoceptor antagonists increase the firing activity of NE neurons, whereas clonidine decreases it, both actions being mediated *via alpha*-2 adrenergic autoreceptors (Baraban and Aghajanian, 1980; Marwaha and Aghajanian, 1982; Curtis and Valentino, 1991). In the present study, the administration of (±)-MIR significantly increased the firing activity of LC NE neurons (fig. 9A and "Results"), presumably by blocking the somatodendritic *alpha*-2 adrenergic autoreceptors. In support of this contention, (±)-MIR caused a parallel shift to the right of the dose-response curve of clonidine on the firing activity of NE neurons (fig. 9D). In the present study, the (−)-MIR (250 μg/kg i.v.) suppressed the firing activity of LC NE neurons (figs. 9A and 10D). This effect of (−)-MIR on the firing activity of LC NE neurons was completely prevented by the $5\text{-}HT_{1A}$ antagonist BMY 7378 (fig. 10, B and D). BMY 7378 has been shown to block the firing activity of dorsal raphe 5-HT neurons (Chaput and de Montigny, 1988; Cox *et al.*, 1993), reduced ventral hippocampus 5-HT release (Hjorth *et al.*, 1995) and in the present study increased by itself the firing activity of LC NE neurons (fig. 10B). NE neurons of LC receive a dense 5-HT projection (Pickel *et al.*, 1977; Cedarbaum and Aghajanian, 1978; Léger and Descarries, 1978; Segal, 1979; Maeda *et al.*, 1991; Vertes and Kocsis, 1994). Hence, in order to determine whether this suppressant effect of (−)-MIR on the firing activity of LC NE neurons was mediated *via* 5-HT neurons, the effect of (−)-MIR was examined in rats pretreated with 5,7-DHT. This pretreatment not only abolished the suppressant effect of (−)-MIR on the firing activity of LC NE neurons (fig. 10, C and D), but increased their spontaneous firing activity and failed to prevent the reducing effect of clonidine mediated by *alpha*-2 adrenergic autoreceptors. (fig. 10D). The mechanisms underlying this latter incremental effect of (−)-MIR have yet to be determined.

In conclusion, this electrophysiological study reveals the actions of (±)- and (−)-MIR at three levels. First, both (±)- and (−)-MIR reduced the suppressant effect of microiontophoretically applied NE on the firing activity of CA_3 hippocampus pyramidal neurons through blockade of postsynaptic *alpha*-2 adrenoceptors. Second, (±)-MIR acted as an antagonist at both the terminal *alpha*-2 adrenergic auto- and heteroreceptors, whereas (−)-MIR, depending on the dose used, acted as an antagonist at terminal *alpha*-2 adrenergic heteroreceptors and as a terminal 5-HT autoreceptor agonist. Third, only (±)-MIR increased dose-dependently the firing activity of dorsal raphe 5-HT neurons and antagonized the suppressant effect of clonidine on the firing activity of 5-HT neurons mediated *via alpha*-2 adrenergic autoreceptor activation. Finally, whereas (±)-MIR markedly increased the firing activity of NE neurons of the LC and antagonized the suppressant effect of clonidine, (−)-MIR inhibited the firing

activity of these neurons, presumably *via* an activation of terminal 5-HT receptors or *alpha*-2 adrenergic heteroreceptors (fig. 11), thus providing a novel functional evidence for an important role of 5-HT neurons in controlling LC NE neurons firing activity.

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Chapter IV: Third article

Previous studies from our laboratory have shown that antidepressant treatments that enhance the NE synaptic concentration induce a desensitization of α_2-adrenergic heteroreceptors located on 5-HT fibers leading presumably to an enhanced 5-HT neurotransmission. In a subsequent study, using the same *in vivo* electrophysiological paradigms, we assessed the effects of long-term treatment with (±)mirtazapine on pre- and postsynaptic α_2-adrenoceptors and on the modulation of 5-HT neurotransmission.

This article entitle "*Effect of long-term treatment with the α_2-adrenoceptor antagonist mirtazapine on the 5-HT neurotransmission.*" by myself, Pierre Blier and Claude de Montigny was published in Naunyn-Schmiedeberg's Archives of Pharmacology (1997, vol. 355, pp. 20-29.

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ORIGINAL ARTICLE

Nasser Haddjeri · Pierre Blier · Claude de Montigny

Effects of long-term treatment with the α_2-adrenoceptor antagonist mirtazapine on 5-HT neurotransmission

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Abstract Mirtazapine (ORG 3770, Remeron®) is a non-selective α_2-adrenoceptor antagonist with antidepressant activity in major depression. The aim of the present study was to assess, using an in vivo electrophysiological paradigm, the effect of long-term treatment with mirtazapine on pre- and postsynaptic α_2-adrenoceptors and on 5-HT neurotransmission in male Sprague-Dawley rats. A 21-day treatment with mirtazapine (5 mg/kg/day, s.c., using osmotic minipumps) increased the spontaneous firing activity of locus coeruleus noradrenaline (NA) neurons. Their firing activity was back to normal 48 h after removing the minipump. However, this treatment did not modify the dose-response curve of the suppressant effect of the α_2-adrenoceptor agonist clonidine on the firing activity of NA neurons. The spontaneous firing activity of dorsal raphe 5-HT neurons was also markedly increased in mirtazapine-treated rats, and was back to normal 48 h after removing of the minipump. The dose-response curve of the suppressant effect of clonidine on the firing activity of 5-HT neurons was altered in mirtazapine-treated rats. Furthermore, it was further shifted to the left after a 48-h washout. Long-term mirtazapine treatment did not modify the suppressant effects of microiontophoretically-applied NA and 5-HT on the firing activity of CA_3 dorsal hippocampus pyramidal neurons. However, this mirtazapine treatment antagonized both the enhancing effect of a low dose (10 µg/kg, i.v.) and the reducing effect of a high dose (100 µg/kg, i.v.) of the α_2-adrenoceptor agonist clonidine on the effectiveness of the electrical stimulation of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus CA_3 pyramidal neurons. After a 48-h washout, only the effect of the high dose of clonidine was attenuated, suggesting a desensitization of the terminal α_2-adrenergic heteroreceptor, but not of the terminal α_2-adrenergic autoreceptor. The decrease in the effectiveness of the stimulation upon increasing its frequency from 1 to 5 Hz (due to the activation of terminal 5-HT autoreceptors) was unaltered after the long-term mirtazapine treatment. In conclusion, the tonic activation of postsynaptic 5-HT receptors is enhanced by a 21-day treatment with mirtazapine, as a result of a sustained increase in 5-HT neuron firing activity in the presence of decreased function of α_2-adrenergic heteroreceptors located on 5-HT terminals in the dorsal hippocampus.

Key words Mirtazapine · α_2-Adrenoceptor · Hippocampus · Dorsal raphe · Locus coeruleus · Serotonin

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Introduction

Mirtazapine, 1,2,3,4,10,14b-hexa-hydro-2-methylpyrazino[2,1-*a*]pyrido[2,3-*c*]benzazepin (ORG 3770 or Remeron®), is a tetracyclic compound with antidepressant activity in humans (Smith et al. 1990; Claghorn and Lesem 1995; van Moffaert et al. 1995). Its pharmacological profile is characterized by α_2-adrenergic, 5-HT_2, 5-HT_3 and H_1 antagonistic activities. It is devoid of anticholinergic activity and has no effect on the reuptake of 5-HT or catecholamines (Nickolson et al. 1982; De Boer et al. 1988). Among these neurochemical effects, the blockade of presynaptic α_2-adrenoceptors has been proposed to be a possible substratum for its antidepressant activity (see: Pinder and Wieringa, 1993 for review). Using microdialysis in freely moving rats, De Boer et al. (1994, 1996) have shown that mirtazapine increases extracellular 5-HT and DOPAC (used as an index of presynaptic noradrenergic activity) in the ventral hippocampus. The indirect α_1-adrenoceptor-mediated enhancement of 5-HT neuron firing and the direct blockade of inhibitory α_2-adrenergic heteroreceptors located on 5-HT terminals may be responsible for this increase in extracellular 5-HT (Haddjeri et al. 1996). In order to distinguish in vivo the activations of α_2-adrenergic auto- and heteroreceptors, previous studies in our laboratory (Mongeau et al. 1993; Haddjeri et al. 1996), using the electrical stimulation of the ascending 5-HT pathway, have

shown that low doses of the α_2-adrenoceptor agonist clonidine (2 and 10 μg/kg, i.v.) enhance the effectiveness of the electrical stimulation of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus CA_3 pyramidal neurons. In contrast, high doses of clonidine (100 and 400 μg/kg, i.v.) reduce the effectiveness of the stimulation. This indicates, on the one hand, that the enhancing effect of a low dose of clonidine results from the selective activation of the α_2-adrenergic autoreceptors on NA terminals, thereby reducing the tonic activation by NA of α_2-adrenergic heteroreceptors on 5-HT terminals. On the other hand, it indicates that the reducing effect of high doses of clonidine is due to a direct activation of the α_2-adrenergic heteroreceptors on 5-HT terminals (Mongeau et al. 1993; Haddjeri et al. 1996). Furthermore, antidepressant treatments that enhance NA synaptic concentration induce a desensitization of both α_2-adrenergic heteroreceptors located on 5-HT fibers (Mongeau et al. 1994; Blier and Bouchard 1994) and of terminal α_2-adrenergic autoreceptors located on NA neurons (Crews and Smith 1978; McMillen et al. 1980; Spyraky and Fibiger, 1980; Finberg and Tal 1985; Lacroix et al. 1991).

Using in vivo electrophysiological paradigms in anesthetized rats, we have previously characterized the acute effects of racemic (±)mirtazapine and of its (–)enantiomer on pre- and postsynaptic α_2-adrenoceptors. In this study, we concluded that the main effect of mirtazapine is mainly mediated via α_2-adrenoceptors. First, (±)mirtazapine and (–)mirtazapine antagonize postsynaptic α_2-adrenergic receptors; second, (±)mirtazapine is an antagonist of somatodendritic as well as of terminal α_2-adrenergic auto- and heteroreceptors, whereas (–)mirtazapine is an antagonist of α_2-adrenergic heteroreceptors (Haddjeri et al. 1996).

The aim of the present study was to assess the effects of long-term treatment with mirtazapine on pre- and postsynaptic α_2-adrenoceptors and on the efficacy of 5-HT neurotransmission, using an in vivo electrophysiological paradigm.

Materials and methods

The experiments were carried out in male Sprague-Dawley rats weighing 250 to 300 g which were housed under standard laboratory conditions (12:12 light-dark cycle with free access to food and water). They were treated for 21 days with 5 mg/kg per day of mirtazapine or vehicle, delivered by an osmotic minipump (ALZA, Palo Alto, Calif., USA) inserted subcutaneously. Experiments were carried out either with the minipump in place or after a 48-h washout (i.e. the minipumps were removed 48 h before the experiment to allow the elimination of the drug). The animals were anesthetized with chloral hydrate (400 mg/kg, i.p.). Supplemental doses were given to maintain constant anesthesia preventing any nociceptive reaction to a tail pinch.

Extracellular recordings from locus coeruleus (LC) NA neurons. Unitary extracellular recordings were performed with single-barrelled glass micropipettes preloaded with fibreglass filaments in order to facilitate filling. The tip was broken back to 1 to 4 μm and filled with a 2 M NaCl solution saturated with Fast Green FCF. LC NA neurons were recorded at the following stereotaxic coordinates: –0.7 mm posterior to lambda and 1.1 to 1.4 mm lateral (Paxinos and Watson 1982). Noradrenaline neurons were identified by their regular firing rate (1–5 Hz), long-duration (0.8–1.2 ms) positive action potentials and their characteristic burst discharge in response to nociceptive pinch of the contralateral hind paw (Aghajanian 1978). In each rat, the spontaneous firing activity of LC NA neurons was determined from four to five electrode descents through this nucleus. Their spontaneous firing activity was assessed and dose-response curves of the suppressant effect of clonidine (0.5–5 μg/kg, i.v.) were constructed by injecting one dose to one rat while recording from one LC NA neuron.

Extracellular recordings from dorsal raphe 5-HT neurons. Serotonergic neurons were also recorded with single-barrelled glass micropipettes. The burr hole was drilled 1 mm anterior to lambda on midline. Dorsal raphe 5-HT neurons were encountered over a distance of 1 mm starting immediately below the ventral border of the Sylvius aqueduct. These neurons were identified using the criteria of Aghajanian (1978): a slow (0.5–2.5 Hz) and regular firing rate and long-duration (0.8–1.2 ms) positive action potentials. In each rat, the spontaneous firing activity of dorsal raphe 5-HT neurons was determined from five to six electrode descents through this nucleus. Dose-response curves of the suppressant effect of clonidine (0.25–5 μg/kg) were constructed by injecting one dose to one rat while recording from one dorsal raphe 5-HT neuron.

Recordings from CA_3 dorsal hippocampus pyramidal neurons. Recording and microiontophoresis were performed with five-barrelled glass micropipettes broken back to 8–12 μm under microscopic control (ASI Instruments, Warren, Mi., USA). The central barrel was filled with a 2 M NaCl solution and used for extracellular unitary recordings. Pyramidal neurons were identified by their large amplitude (0.5–1.2 mV) and long-duration (0.8–1.2 ms) simple spikes alternating with complex spike discharges (Kandel and Spencer 1961). The side barrels contained the following solutions: NA (20 mM in 200 mM NaCl, pH 4); 5-HT creatinine sulphate (5 mM in 200 mM NaCl, pH 4); quisqualic acid (1.5 mM in 200 mM NaCl, pH 8); and, 2 M NaCl used for automatic current balancing. The microelectrode was lowered at 4.2 mm lateral and 4.2 anterior to lambda into the CA_3 region of the dorsal hippocampus. Since most hippocampus pyramidal neurons are not spontaneously active under chloral hydrate anesthesia, a leak or a small ejection current of quisqualate (+2 to –6 nA) was used to activate them within their physiological firing range (10–15 Hz; Ranck 1975). Neuronal responsiveness to the microiontophoretic application of NA were assessed by determining the number of spikes suppressed per nA. The duration of microiontophoretic applications of 5-HT and NE was always of 50 s.

Electrical activation of the afferent 5-HT fibers to the hippocampus. A bipolar electrode (NE-110; David Kopf, Tujunga, Calif., USA) was implanted on the midline with a 10° backward angle in the ventromedial tegmentum, 1 mm anterior to lambda and 8.3 mm below the cortical surface. A stimulator (S8800; Grass Instruments, Quincey, Mass., USA) delivered 200 square pulses of 0.5 ms at a frequency of 1 or 5 Hz and an intensity of 300 μA. The stimulation pulses and the action potentials of the neuron recorded were fed to an IBM computer equipped with a Tecmar interface. Peristimulus time histograms were generated to determine the duration of suppression of firing activity of the CA_3 pyramidal neuron, measured in absolute silence value (SIL, in ms). This value is obtained by dividing the total number of events suppressed following the stimulation by the mean frequency of firing of the neuron recorded (Chaput et al. 1986). The CA_3 region of the hippocampus receive extensive innervation from 5-HT neurons of dorsal and median raphe nuclei (Hensler et al. 1994). The brief suppressant effect (≈50 ms) resulting from the electrical stimulation of the ascending 5-HT pathway is due to the release of 5-HT and is mediated by postsynaptic 5-HT_{1A} receptors (Chaput et al. 1986). The effect of the stimulation of the ascending 5-HT pathway was determined prior to and following each of two intravenous injections of the α_2-adrenoceptor agonist clonidine (10 and 100 μ/kg) in control rats and in mirtazapine-treated rats with minipumps on board or after a 48-h washout. In order to determine the function of the terminal 5-HT autoreceptors, two series of stimulations (1 and 5 Hz) were carried out, while recording the same neurons, since it has been pre-

viously demonstrated in vitro and in vivo that the activation of terminal 5-HT autoreceptors decreases the release of 5-HT and that increasing the frequency of stimulation from 1 to 5 Hz results in a greater activation of terminal 5-HT autoreceptors (Chaput et al. 1986; Göthert 1980; Blier et al. 1989).

Statistics. All results are expressed as means ± SEM. For the dose-response curves, the ED_{50} and the Pearson's r values were calculated by simple linear regression analysis and used as index of the shift of curves. Statistical comparisons between groups were carried out by using either unpaired or paired Student's *t*-tests, and either the two way analysis of variance or covariance.

Drugs. Mirtazapine (Organon, Oss, The Netherlands); clonidine, NA bitartrate, 5-HT creatinine sulphate, quisqualic acid (Sigma Chemical Co., St. Louis, Mo., USA). All the drugs have been solubilized in water except mirtazapine (in 50% ethanol and 50% water). The concentrations and the doses used for these compounds were chosen on the basis of previous experiments carried out in our and other laboratories.

Results

Effect of long-term mirtazapine treatment on the firing activity of locus coeruleus NA neurons

The acute administration of mirtazapine increases the spontaneous firing activity of LC NA neurons and antagonizes the suppressant effect of clonidine on the firing activity of LC NA neurons (Haddjeri et al. 1996). In the present study, mirtazapine (5 mg/kg/day × 21 days) significantly increased (by 30%) the firing activity of NA LC neurons (Fig. 1B and D). Their firing activity was back to normal after a 48-h washout (Fig. 1C and D). Although there was a trend for a sensitization to clonidine when the experiments were carried out with the minipump in place, this treatment did not modify significantly the dose-response curve of the suppressant effect of clonidine on LC NA neuron firing activity (Fig. 2B–D).

Effect of long-term mirtazapine treatment on the firing activity of dorsal raphe 5-HT neurons

The acute administration of mirtazapine (10–250 µg/kg, i.v.) increases, in a dose-dependent manner, the firing activity of the dorsal raphe 5-HT neurons and this enhancing action of mirtazapine is abolished in 6-hydroxydopamine-lesioned rats (Haddjeri et al. 1996). As illustrated in Fig. 3B and C, long-term treatment with mirtazapine produced a marked increase (by 75%) of the spontaneous firing activity of dorsal raphe 5-HT neurons, which was back to normal after the 48-h washout (Fig. 3D). Clonidine (0.5–5 µg/kg, i. v.) dose-dependently suppressed the firing activity of dorsal raphe 5-HT neurons (Fig. 4B and D). The long-term mirtazapine treatment altered this effect when the experiment was carried out with the minipump in place (Fig. 4B and D). After a 48-h washout, there was a marked shift to the left of the dose-response curve of the suppressant effect of clonidine on dorsal raphe 5-HT neurons (Fig. 4C and D).

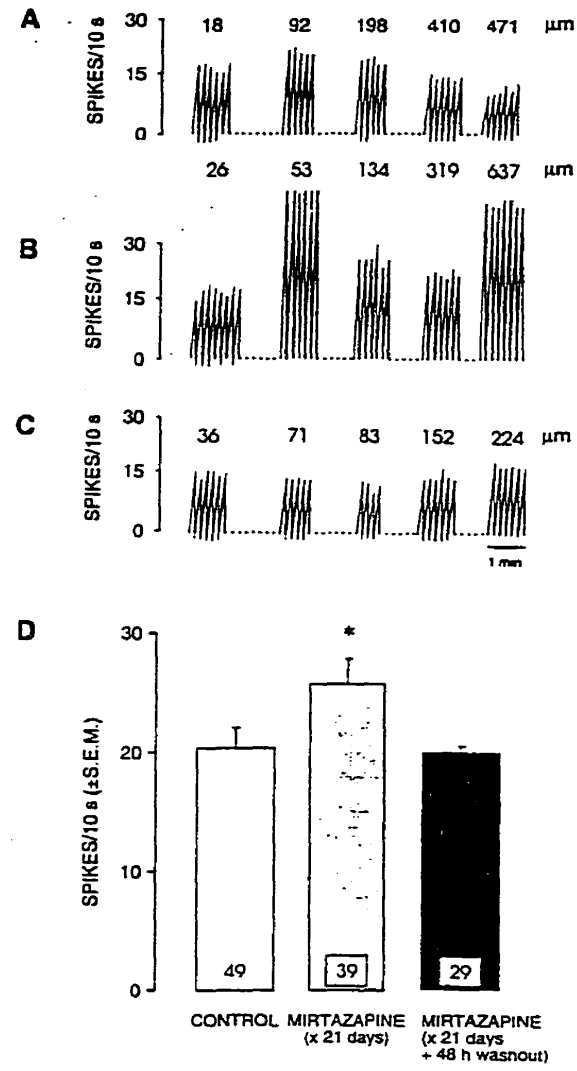

Fig. 1 Integrated firing rate histograms of LC NA neurons showing their spontaneous firing activity in a control rat (**A**), in mirtazapine-treated rats with the minipump in place (**B**) and after a 48-h washout (**C**). The *number above each neuron* indicates the depth from the floor of the fourth ventricle at which it was recorded. Corresponding results (means ± SEM) are presented on **D**. The *numbers at the bottom of the columns* indicates the number of neurons tested in 6 to 8 rats per group. *$P<0.05$ (unpaired Student's *t*-test)

Effect of long-term mirtazapine treatment on the CA_3 dorsal hippocampus pyramidal neurons responsiveness to NA and 5-HT

The microiontophoretic application of NA onto rat dorsal hippocampus pyramidal neurons in vivo produces a suppressant effect on their firing activity which is mediated by postsynaptic α_2-adrenoceptors (Curet and de Montigny 1988a), and that of 5-HT also induces an inhibitory effect on the firing activity which is mediated by postsynaptic 5-HT_{1A} receptors (Blier and de Montigny 1987; Chaput and de Montigny 1988). For almost all CA_3 hippocampus pyramidal neurons, NA (5, 10 and 20 nA) and 5-HT (5 and 10 nA) induced a current-dependent reduction of their firing activity, ranging from 30 to 100% (Fig. 5A and C).

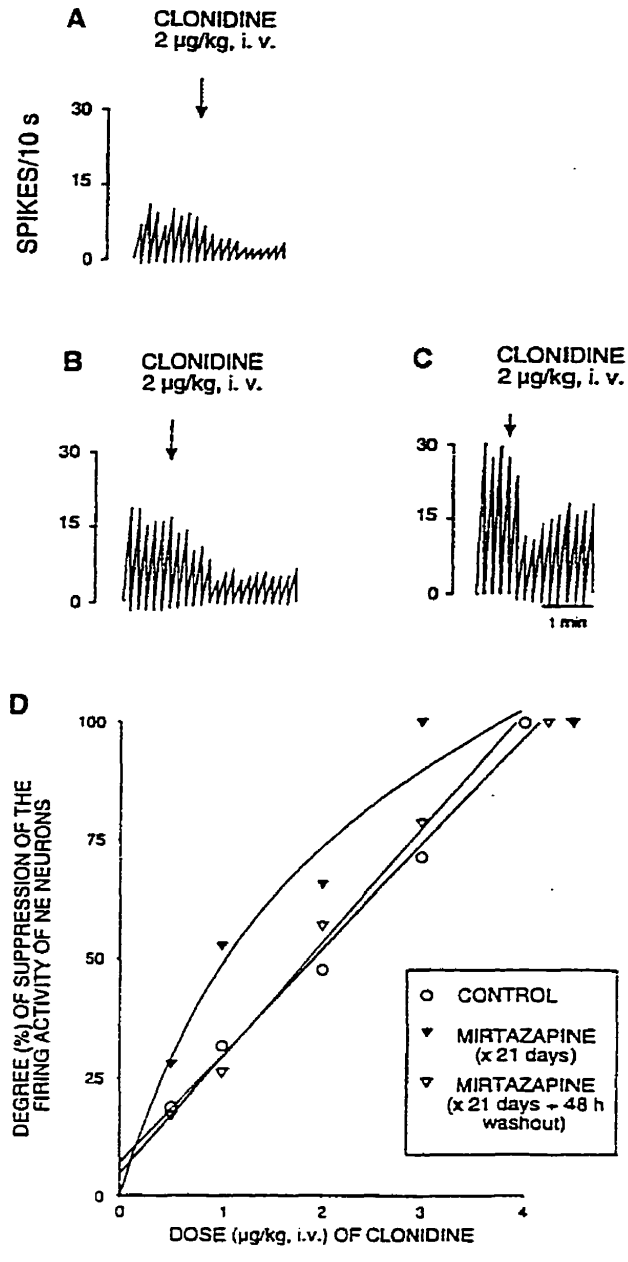

Fig. 2 Integrated firing rate histograms of LC NA neurons showing their response to clonidine in a control rat (**A**), in mirtazapine-treated rats with the minipump in place (**B**) and after a 48-h washout (**C**). **D** Dose-response curves of the effects of clonidine on the spontaneous firing activity of dorsal raphe 5-HT neurons in control rats (*black circles*), and in mirtazapine-treated rats with minipumps in place (*open triangles*) and after a 48-h washout (*black triangles*). *Each point* represents the response of one LC neuron to one dose of clonidine administered to one rat. The 95% confidence limits curves were omitted for clarity. After the two way analysis of variance, no statistical difference has been found between the three dose-response curves (using simple linear regression analysis: ED_{50} in control rats = 1.9 ± 0.2 µg/kg, i.v. with $r = 0.99$; ED_{50} in rats with minipumps in place = 1.2 ± 0.6 µg/kg, i.v. with $r = 0.91$; ED_{50} after a 48-h washout = 1.9 ± 0.1 µg/kg, i.v. with $r = 0.99$)

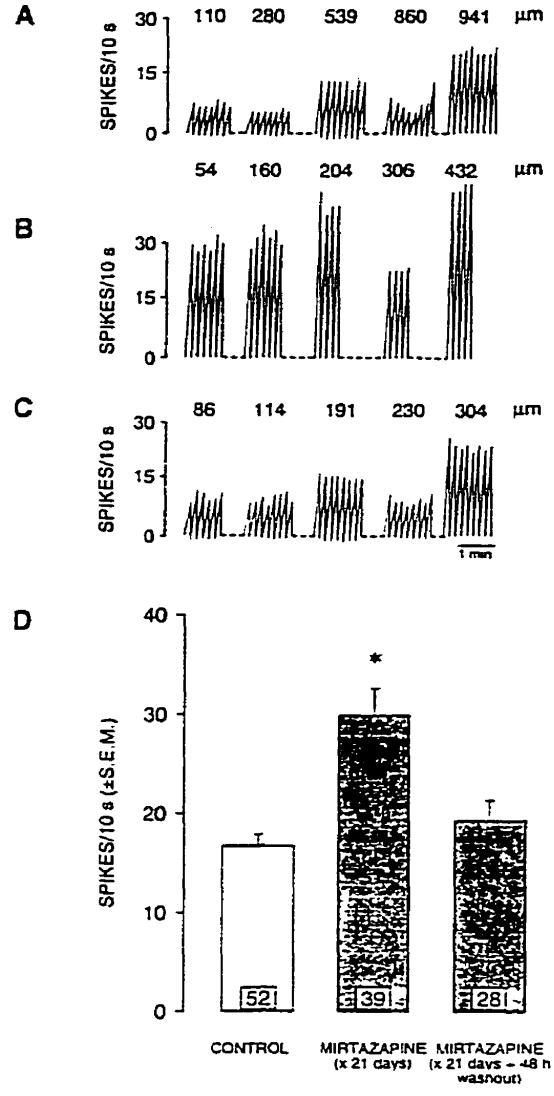

Fig. 3 Integrated firing rate histogram of dorsal raphe 5-HT neurons showing their spontaneous firing activity in a control rat (**A**), and in mirtazapine-treated rats with the minipump in place (**B**) and after a 48-h washout (**C**). The *number above each neuron* indicates the depth from the ventral border of the sylvius aqueduct at which it was recorded. Corresponding results (means ± SEM) are presented in **D**. The *numbers at the bottom of the columns* indicates the number of neurons tested in 7 rats per group. *$P<0.05$ (unpaired Student's *t*-test)

These inhibitory effects occurred in the absence of alteration of the wave form of the action potentials. As illustrated in Fig. 5B, the mirtazapine treatment (5 mg/kg/day × 21 days) did not modify the suppressant effects of NA and 5-HT. Figure 5C shows the mean suppressant effects of 5-HT and NA microiontophoretically-applied onto CA_3 pyramidal neurons and the lack of effect of the long-term mirtazapine treatment on the responsiveness of both postsynaptic α_2-adrenergic and 5-HT_{1A} receptors.

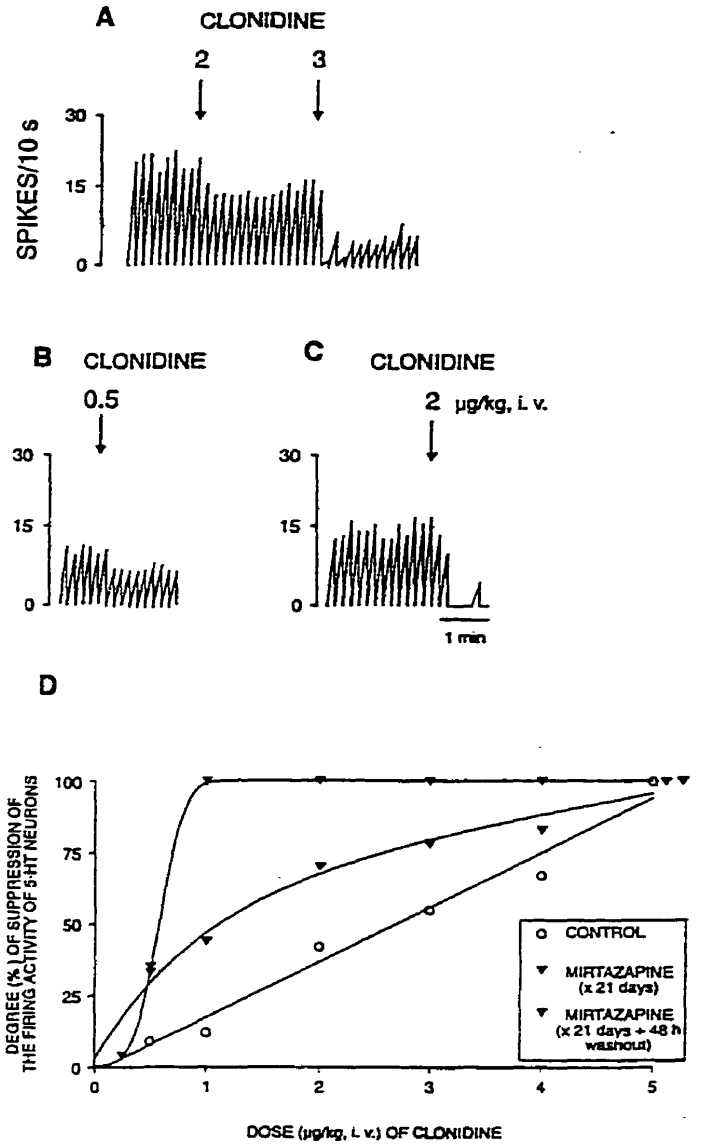

Fig. 4 Integrated firing rate histograms of dorsal raphe 5-HT neurons showing their response to clonidine in a control rat (A), in mirtazapine-treated rats with the minipump in place (B) and after a 48-h washout (C). D Dose-response curves of the effects of clonidine on the firing activity of dorsal raphe 5-HT neurons in control rats (*circles*), in rats with minipumps in place (*open triangles*) and after a 48-h washout (*black triangles*). *Each point* represents the response of one LC neuron to one dose of clonidine administered to one rat, except for curve in control rats where each point corresponds to the mean of two neurons. The shift to the left of the curve obtained in rats with minipumps in place or after a 48-h washout was statistically significant using the two way analysis of variance. $P<0.05$ (using simple linear regression analysis: ED_{50} in control rats = 2.7 ± 0.2 µg/kg, i.v. with $r = 0.97$; ED_{50} in rats with minipumps in place = 1.2 ± 0.6 µg/kg, i.v. with $r = 0.95$; ED_{50} after a 48-h washout = 0.6 ± 0.01 µg/kg, i.v. with $r = 0.99$)

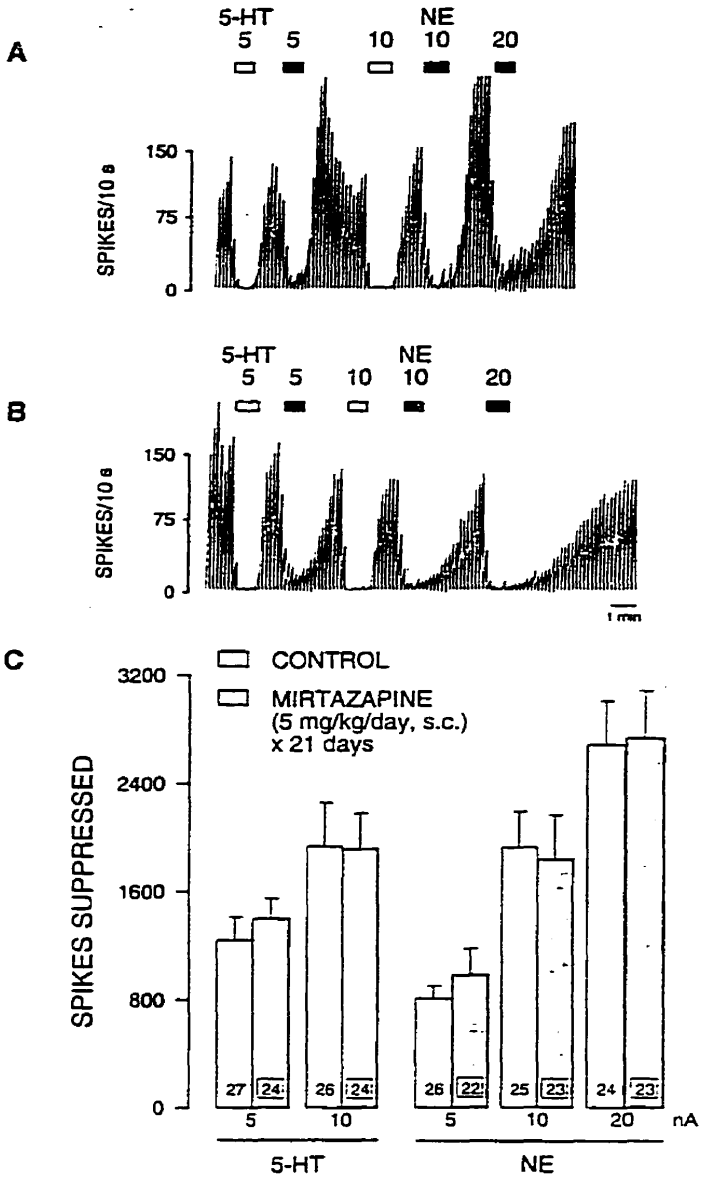

Fig. 5A,B Represent integrated firing rate histograms of dorsal hippocampus CA_3 pyramidal neurons showing their responsiveness to microiontophoretic applications of NA and 5-HT in a control rat (A) and in a mirtazapine-treated rat (B). These neurons were activated by the microiontophoretic application of quisqualate (−2 and −4 nA, respectively). *Horizontal bars* indicate the duration of the applications for which the current is given in nA. Corresponding results (means ± SEM) are presented in C. The *numbers at the bottom of the columns* indicates the number of neurons tested in 8 rats per group

Effect of long-term mirtazapine treatment on the efficacy of the electrical stimulation of the afferent 5-HT fibers to the hippocampus

Clonidine inhibits 5-HT release in vitro and in vivo through the activation of α_2-adrenergic heteroreceptors on 5-HT terminal (Starke and Montel 1973; Tao and Hjorth 1992). Previous studies from our laboratory have shown that high doses of clonidine (100 and 400 µg/kg, i.v.) also reduce the effectiveness of the stimulation of the ascend-

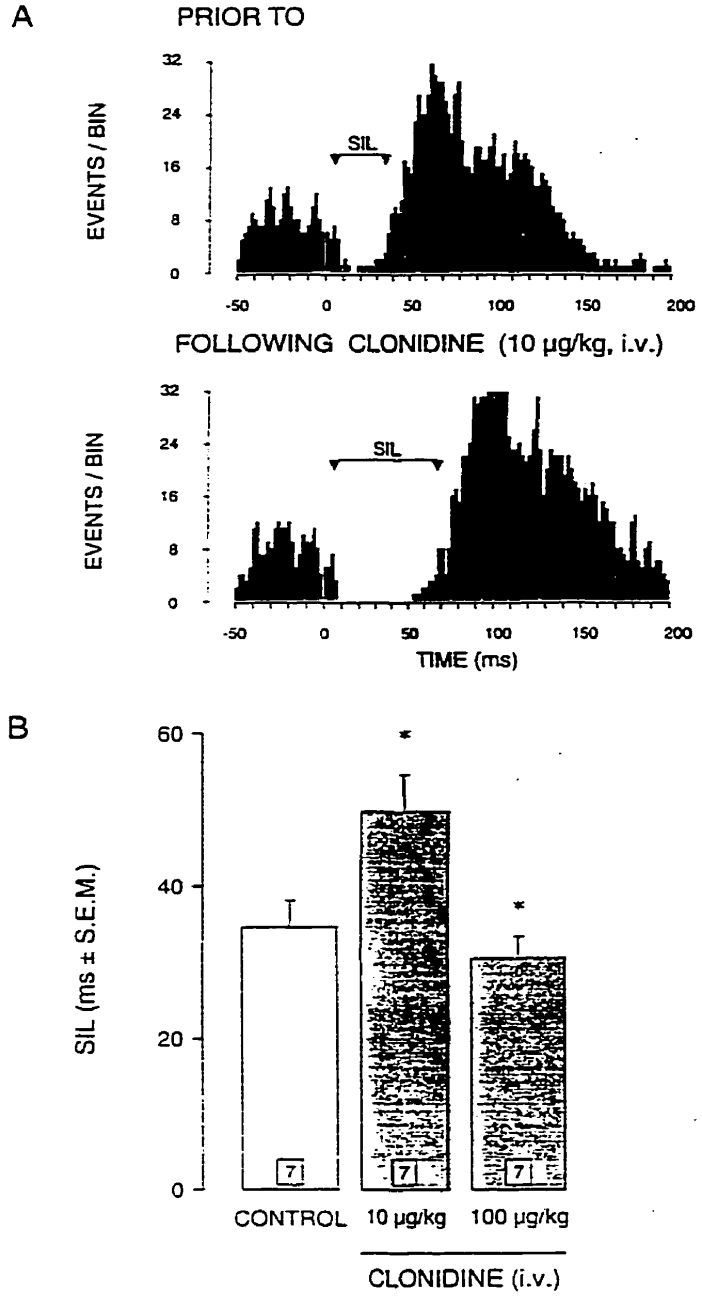

Fig. 6 A Peristimulus time histograms illustrating the effect of the electrical stimulation of the ascending 5-HT pathway at the level of the ventro-medial tegmentum on the firing activity of dorsal hippocampus CA_3 pyramidal neurons prior to and after clonidine. The SIL represents the duration of the suppression of firing (see Materials and methods). Each histogram was constructed from 200 consecutive stimulations (300 µA, 0.5 ms, 1 Hz) with a bin width of 2 ms. B Duration of suppression of firing activity (means ± SEM) of CA_3 hippocampus pyramidal neurons produced by the stimulation of the ascending 5-HT pathway, before and after the administration of clonidine (cumulative doses of 10 and 100 µg/kg, i.v.). The *number in the columns* indicates the number of neurons or rats tested. *$P<0.05$, using the paired Student *t*-test

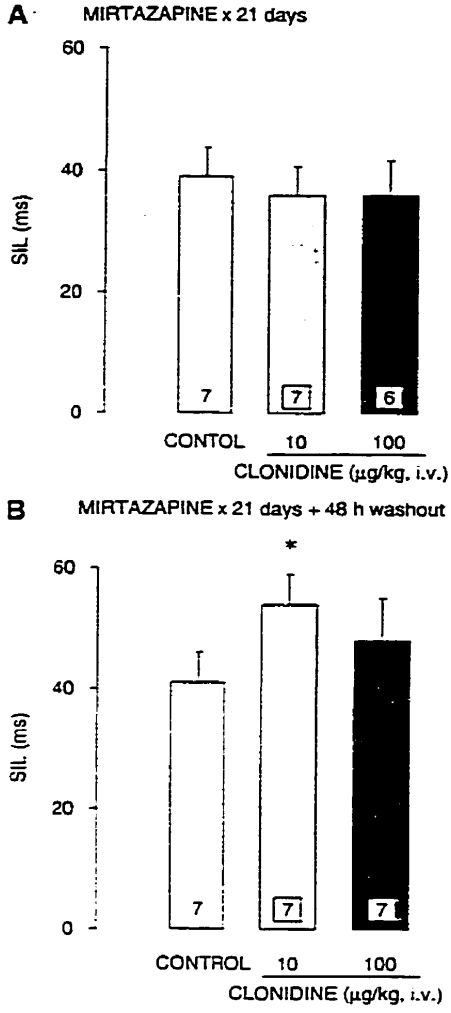

Fig. 7 Effects of the mirtazapine treatments, with minipumps on board (A) and after a 48-h washout (B), on the efficacy of the stimulation of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus pyramidal neurons and of the subsequent administrations of a low (10 µg/kg, i.v.) and a high (100 µg/kg, i.v.) dose of clonidine (means ± SEM). The *number in the columns* indicates the number of neurons or rats tested. *$P<0.05$, using the paired Student *t*-test

ing 5-HT pathway (see Introduction). In contrast, low doses of clonidine (2 and 10 µg/kg, i.v.) enhance the effectiveness of the electrical stimulation of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus CA_3 pyramidal neurons. In the present study, the low dose of clonidine (10 µg/kg, i.v.) increased by 43% the mean duration of suppression of the firing activity of CA_3 pyramidal neurons induced by the electrical stimulation of the ascending 5-HT pathway (Fig. 6A and B) and the subsequent injection of a high dose of clonidine (100 µg/kg, i.v.) significantly reduced it by 14% (Fig. 6B).

In order to determine whether mirtazapine treatment could modulate in vivo the efficacy of 5-HT synaptic transmission and antagonize the effects of the α_2-adrenoceptor agonist clonidine, its capacity to modify the duration of the suppression of the firing activity of CA_3 hippocampus pyramidal neurons produced by the electrical activation of the ascending 5-HT pathway was examined in rats with the minipump in place and after a 48-h washout.

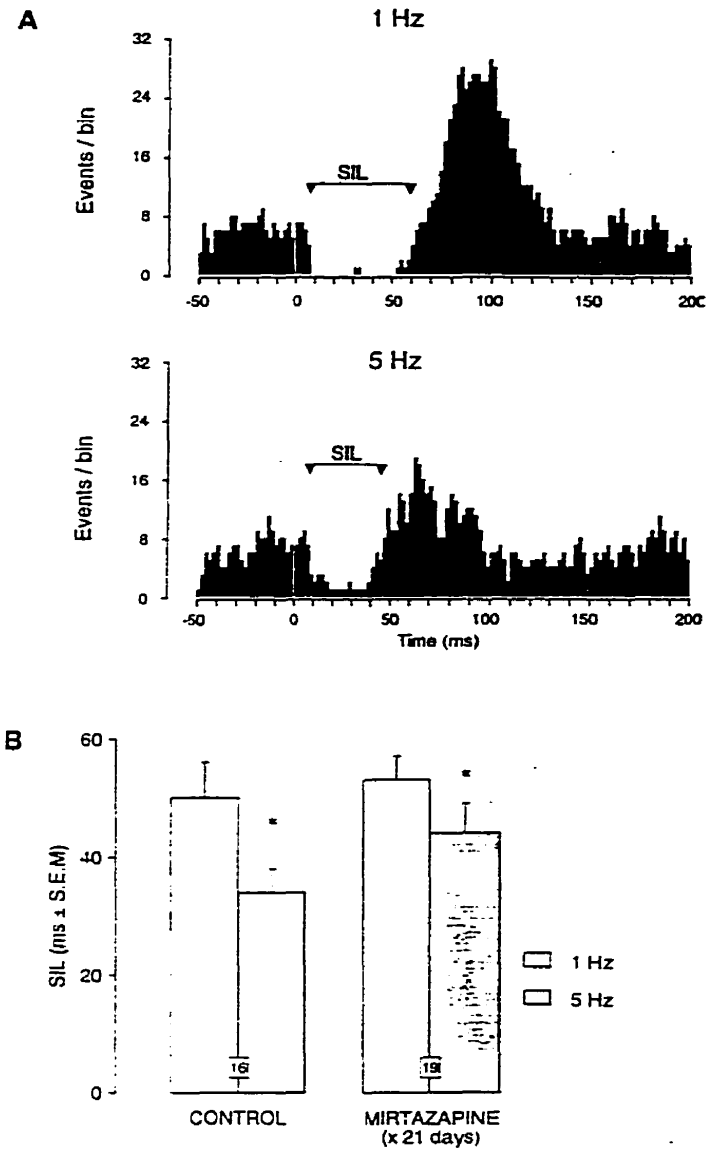

Fig. 8 A Peristimulus time histograms illustrating the effects of the electrical stimulation of the ascending 5-HT pathway at the level of the ventro-medial tegmentum on the firing activity of dorsal hippocampus CA_3 pyramidal neurons for the same neurons, when delivered at 1 and at 5 Hz in a control rat (A). Corresponding results are presented in B. The *number in the columns* indicates the number of neurons tested in 7 rats per groups. $^*P<0.05$ using the paired Student *t*-test. The decremental effect of increasing the frequency of the stimulation from 1 to 5 Hz in the mirtazapine-treated rats was not significantly different from that in control rats ($P = 0.29$, using covariance analysis)

Mirtazapine (5 mg/kg/day × 21 days) did not modify the effectiveness of the stimulation of the 5-HT pathway but prevented the effects of intravenous injections of both the low and high dose of clonidine (Fig. 7A). After the 48-h washout, only the enhancing effect of the low dose of clonidine was restored (Fig. 7B).

The responsiveness of terminal 5-HT autoreceptors was evaluated by increasing the frequency of the stimulation from 1 to 5 Hz in rats with minipumps on board. This differential effectiveness of these stimulations was not significantly different in mirtazapine-treated rats compared to control rats using an analysis of covariance (Fig. 8B).

Discussion

The present electrophysiological data show that long-term treatment with the non-selective α_2-adrenoceptor antagonist mirtazapine (5 mg/kg/day × 21 days) induced an increase of the firing activity of dorsal raphe 5-HT neurons. This indicates that mirtazapine has the capacity to produce a sustained blockade of α_2-adrenergic autoreceptors on NA neurons projecting to dorsal raphe nucleus since it has previously shown that the enhancing effect of mirtazapine, which is also present upon acute administration, is prevented by a 6-hydroxydopamine pretreatment (Haddjeri et al. 1996). This mirtazapine treatment, however, failed to antagonize the suppressant effect of clonidine on the firing activity of 5-HT neurons, but after a 48-h washout period, the responsiveness to clonidine was markedly enhanced. Long-term mirtazapine treatment also increased the spontaneous firing activity of NA LC neurons but did not antagonize the suppressant effect of clonidine. The firing rate of LC NA neurons was back to normal after a 48-h washout period without any evidence of a sensitization to clonidine. This suggests that the somatodendritic α_2-adrenergic autoreceptor was not blocked in a marked fashion by this treatment regimen. However, the mechanisms underlying the differential effect of long-term mirtazapine treatment on terminal versus somatodendritic α_2-adrenergic autoreceptors remain to be elucidated. In addition, long-term mirtazapine administration blocked both terminal α_2-adrenergic auto- and heteroreceptors on NA and 5-HT terminals, respectively, in the dorsal hippocampus, without altering the function of terminal 5-HT autoreceptors. After a 48-h washout, there was an attenuation of the responsiveness of α_2-adrenergic heteroreceptors but not that of α_2-adrenergic autoreceptors. Finally, the mirtazapine treatment did not modify the suppressant effects of microiontophoretic application of either NA or 5-HT on the firing activity of CA_3 hippocampus pyramidal neurons, indicating an unaltered responsiveness of postsynaptic α_2-adrenoceptors and 5-HT_{1A} receptors.

The presence of α_1- and α_2-adrenoceptors in the rat hippocampus has been well established (Young and Kuhar 1980; Bylund and U'Prichard 1983). Microiontophoretic application of NA onto rat dorsal hippocampus pyramidal neurons produces a suppressant effect on their firing activity mediated by postsynaptic α_2-adrenoceptors (Curet and de Montigny 1988a). On the other hand, the suppressant effect of endogenous NA released by the stimulation of LC is mediated by α_1-adrenoceptors (Curet and de Montigny 1988b). In the present study, and in contrast with the acute administration of mirtazapine (0.5 mg/kg, i.v.) which blocked the reducing effect of microiontophoretically-applied NA on CA_3 pyramidal neurons (Haddjeri et al. 1996), the mirtazapine treatment (5 mg/kg/day × 21 days) did not alter the suppressant effect of microiontophoreti-

cally-applied NA or 5-HT on the firing activity of CA_3 hippocampus pyramidal neurons, thus indicating that long-term treatment with a 5 mg/kg/day × 21 days regimen of mirtazapine does not affect the function of postsynaptic α_2-adrenoceptors and 5-HT_{1A} receptors (Fig. 5).

Using microdialysis in freely moving rats, De Boer et al. (1994, 1996) have shown that mirtazapine markedly increases 5-HT release in the ventral hippocampus at a subcutaneous dose of 2 mg/kg. The indirect α_1-adrenoceptor-mediated enhancement of 5-HT neuron firing activity and the direct blockade of inhibitory α_2-heteroreceptors located on 5-HT terminals have been proposed to account for this increase in extracellular 5-HT. Previous studies from our laboratory have shown that the in vivo activation of α_2-autoreceptors on NA terminals by a low dose of clonidine enhances the effectiveness of the electrical stimulation of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus CA_3 pyramidal neurons, whereas a high dose of clonidine activates the α_2-heteroreceptors on 5-HT terminals, thereby reducing the effectiveness of the stimulation (Mongeau et al. 1993). Using this in vivo model, we have shown that the acute injection of mirtazapine (25 μg/kg, i.v.) significantly enhanced the effectiveness of the stimulation, reflecting an enhanced release of endogenous 5-HT per impulse reaching 5-HT terminals, and prevented the effects of subsequent intravenous administration of both low and high doses of clonidine showing its capacity to block α_2-adrenergic auto- and heteroreceptors (Haddjeri et al. 1996). In the present study, long-term treatment with mirtazapine antagonized both the enhancing effect of a low dose (10 μg/kg, i.v.) and the reducing effect of a high dose (100 μg/kg, i.v.) of the α_2-adrenoceptor agonist clonidine on the effectiveness of the electrical stimulation of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus CA_3 pyramidal neurons. Furthermore, after a 48-h washout, the effect of the high dose of clonidine was still attenuated, suggesting a desensitization of α_2-adrenergic heteroreceptors on 5-HT terminals, in agreement with previous results from our laboratory showing that antidepressant treatments, including mianserin, that enhanced NA synaptic concentration induce a desensitization of these α_2-adrenergic heteroreceptors (Mongeau et al. 1994). Such a treatment did not, however, modify the function of terminal 5-HT autoreceptors as indicated by the lack of effect of long-term mirtazapine in the 1 Hz/5 Hz stimulation paradigm (Fig. 7C). This is interesting in the light of the observation that the (−)enantiomer of mirtazapine appears to be endowed with agonistic activity at this autoreceptor. Indeed, the acute administration of (−)mirtazapine (25 μg/kg, i.v.) reduces the duration of suppression of firing of CA_3 pyramidal neurons induced by the stimulation of the 5-HT pathway which is prevented by the 5-HT autoreceptor antagonist metergoline (Haddjeri et al. 1996).

Ample evidence has established that dorsal raphe 5-HT neurons receive NA projections from the LC as well as from other brainstem NA nuclei (Loizou 1969; Anderson et al. 1977; Baraban and Aghajanian 1981), and in vivo pharmacological studies have shown that the firing activity of 5-HT neurons in the dorsal raphe is dependent on a tonic activation by its NA input (Svensson et al. 1975; Baraban and Aghajanian 1980). Moreover, the α_2-adrenergic agonist clonidine suppresses the firing activity of 5-HT neurons in the dorsal raphe. This inhibitory action of clonidine was suggested to be due to the activation of α_2-adrenergic autoreceptors decreasing the endogenous NA excitatory input to α_1-adrenergic receptors located on 5-HT neurons. In support of this, the acute administration of the α_2-adrenergic antagonists idazoxan or mirtazapine increases the spontaneous firing activity of dorsal raphe 5-HT neurons and antagonizes the suppressant effect of clonidine on these neurons (Svensson et al. 1975; Freedman and Aghajanian 1984; Garrat et al. 1991; Clement et al. 1992; Haddjeri et al. 1996). Moreover, the enhancing effect of mirtazapine on the firing activity of 5-HT neurons was abolished by a 6-hydroxydopamine lesioning of NA neurons, indicating that this effect of mirtazapine was due to blockade of the α_2-adrenergic autoreceptors and not to a direct activation of α_1-adrenergic receptors located on 5-HT neurons (Haddjeri et al. 1996). In the present study, the long-term mirtazapine treatment enhanced by more than 75% the firing activity of dorsal raphe 5-HT neurons, and their activity was back to normal after a 48-h washout (Fig. 3). In contrast to the acute injection of mirtazapine, which antagonized the suppressant effect of clonidine on the firing activity of 5-HT neurons (Haddjeri et al. 1996), this effect of clonidine (0.25–5 μg/kg, i.v.) was not attenuated in rats with the minipump in place (Fig. 4D). However, after a 48-h washout, the dose-response curve of clonidine was drastically shifted to the left suggesting a sensitization of the terminal α_2-adrenergic autoreceptors in the dorsal raphe nucleus (Fig. 4). Such a sensitization phenomenon has been also reported following long-term treatments with mianserin or idazoxan (Sugrue 1980; Cerrito and Raiteri 1981; Raiteri et al. 1983; Dickinson et al. 1989). One may assume that such changes could result from an increase of the density of terminal α_2-adrenergic autoreceptors in the dorsal raphe as consequence of their sustained blockade. However, the latter needs further investigation in order to elucidate why long-term treatment with mirtazapine increased the firing activity of NA and 5-HT neurons without antagonizing the inhibitory effects of clonidine on these neurons.

Up until now, α_{2A}, α_{2B} and α_{2C} adrenoceptors have been described (see for review Bylund 1988; Bylund et al. 1994). Wamsley et al. (1992) have proposed that the oxymetazoline-sensitive receptors (α_{2A}) predominate in the rat LC. Clonidine, the challenge agent used in the present study, not only binds to α_2-adrenoreceptors, but also to non-adrenergic I_1 subtype of imidazoline sites with nanomolar affinity and with micromolar for the I_2 subtype (see for review Starke 1987; Michel and Ernsberger 1992). Data from Ernsberger and Piletz (1995) indicate that I_1 binding sites are absent in LC. On the other hand, Kovachich et al. (1993) have reported that long-term treatment with mianserin decreased the binding of [^{3}H]idazoxan in the LC without affecting its binding in the coerulean terminal fields. However, idazoxan preferentially binds to the I_2

subtype. Moreover, it has been recently revealed, using [^{3}H]rilmenidine (an oxazoline analogue of clonidine) that α_{2A}-adrenoceptors and $I_{2B\text{-like}}$ binding sites are co-localized in many rat brain areas including LC and dorsal raphe (King et al. 1995). MacKinnon et al. (1995) have shown that the new selective ligand for I_2 binding sites RS-45041-190, which has low affinity for the α_{2A}-adrenoceptors, densely labelled the LC and dorsal raphe nuclei. It is important to emphasize that, in contrast to clonidine, mirtazapine possesses hardly any affinity for these non-adrenergic imidazoline binding sites (T. De Boer, personal communication).

α_2-Adrenoceptor antagonists increase the firing activity of NA neurons whereas clonidine decreases it, both actions being presumably mediated via somatodendritic α_2-adrenergic autoreceptors (Baraban and Aghajanian 1980; Marwaha and Aghajanian 1982; Curtis and Valentino 1991). We have shown that acute administration of mirtazapine markedly increases, in a transient manner, the firing activity of NA LC neurons, presumably by blocking the somatodendritic α_2-adrenergic autoreceptors (Haddjeri et al. 1996). In keeping with this interpretation, acute mirtazapine administration caused a parallel shift to the right of the dose-response curve of clonidine on the firing activity of NA neurons. In the present study, the spontaneous firing activity of LC NA neurons was also increased in mirtazapine-treated rats with the minipump on board and was back to normal 48 h after removing the minipump (Fig. 1). In contrast with the acute administration of mirtazapine (250 μg/kg, i. v.), long-term treatment did not modify the dose-response curve of clonidine on the firing activity of NA neurons. In addition, there was no sensitization to clonidine after two days of washout suggesting that mirtazapine differentially affected the somatodendritic and terminal α_2-adrenergic autoreceptors. Since mirtazapine treatment did not modify the responsiveness of LC NA neurons to clonidine, whereas that of dorsal raphe 5-HT neurons was increased, one might assume that such differences could be due either to the presence of different subtypes of somatodendritic and terminal α_2-adrenergic autoreceptors or to the absence of I_1 binding sites in LC. Another possibility would be the presence of α_2-adrenergic heteroreceptors or I_1 binding sites on the dendrites of dorsal raphe 5-HT neurons which would become sensitized during mirtazapine treatment. Alternatively, Simson and Weiss (1989) have proposed that modulation of the responsiveness of LC neurons might be modulated via α_2-adrenoceptors distinct from those regulating the spontaneous firing activity of LC neurons.

It has been shown, using microdialysis in freely moving rats, that mirtazapine, administered acutely, increases both 5-HT and DOPAC (considered as an index of the noradrenergic presynaptic activity) release in the ventral hippocampus (De Boer et al. 1994, 1996). In keeping with these observations, we have demonstrated that the acute injection of mirtazapine increases, in a transient manner, the spontaneous firing activity of both dorsal raphe 5-HT and LC NA neurons (Haddjeri et al. 1996). Similarly, in the present study, long-term treatment with mirtazapine also produced a sustained increase of the spontaneous firing activity of both dorsal raphe 5-HT and LC NA neurons which was back to normal after the 48-h washout. The main result of long-term administration of mirtazapine is therefore the desensitization of the α_2-adrenergic heteroreceptors on 5-HT terminals in the hippocampus. Since mirtazapine, as for all antidepressant drugs, has a delayed onset of action, it is possible that the development of this adaptative change may be related to its antidepressant activity. This would allow 5-HT terminals to free themselves from the potent negative modulation by these α_2-adrenergic heteroreceptors activated by an enhanced NA release, thus leading to a greater tonic activation of postsynaptic 5-HT_{1A} receptors (unpublished observation).

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Chapter V: Fourth article

Modulation of the 5-HT system via the MAO-A inhibition:

Previous studies from our laboratory have revealed that MAO-A inhibitors could affect central NE and 5-HT neurotransmission of the rat. In fact, it has been demonstrated that repeated administration of the nonselective MAO inhibitor phenelzine and of the irreversible MAO-A inhibitor clorgyline produced an early and sustained decrease in the firing activity of rat LC NE neurons and also a reversible decrease in the firing activity of dorsal raphe 5-HT neurons. The aim of the fifth study was to assess the effect of sustained administration of befloxatone on the efficacy of the 5-HT neurotransmission and on pre- and postsynaptic 5-HT_{1A} receptors, using an *in vivo* electrophysiological paradigm.

This article entitle "*Enhancement of serotonergic neurotransmission following sustained administration of the reversible monoamine oxidase-A inhibitor befloxatone.*" by myself, Claude de Montigny and Pierre Blier has been submitted for publication to the European Journal of Pharmacology.

ENHANCEMENT OF THE SEROTONERGIC NEUROTRANSMISSION FOLLOWING SUSTAINED ADMINISTRATION OF THE REVERSIBLE TYPE A MONOAMINE OXIDASE INHIBITOR BEFLOXATONE[1]

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ABSTRACT

The aim of the present study was to assess, using *in vivo* electrophysiological paradigms, the effect of sustained adminstration of the selective and reversible monoamine oxidase-A (MAO-A) inhibitor beflotaxone on serotonin (5-HT) neurotransmission. In male Sprague-Dawley rats with the osmotic minipumps in place, a 2-day treatment with befloxatone (0.75 mg/kg/day, s.c.) decreased the spontaneous firing activity of dorsal raphe 5-HT neurons. The combination of befloxatone and the 5-$HT_{1A/1B}$ receptor antagonist (-)pindolol (15 mg/kg/day, s.c.) for 2 days increased slightly the firing activity of 5-HT neurons, whereas a 2-day treatment with (-)pindolol alone did not modify this parameter. The suppressant effects on the firing activity of 5-HT neurons of the 5-HT autoreceptor agonist LSD, injected intravenously, and of both 5-HT and the 5-HT_{1A} receptor agonist 8-OH-DPAT, applied by microiontophoresis were attenuated in 2-day treated rats with befloxatone, suggesting an early inactivation of the somatodendritic 5-HT_{1A} receptors. The firing activity of 5-HT neurons was back to normal after a 21-day treatment with befloxatone but the suppressant effects of LSD, 5-HT or 8-OH-DPAT was the same as in controls. In contrast, the suppressant effect of the α_2-adrenoceptor agonist clonidine on the firing activity of 5-HT neurons was significantly attenuated after the 21-day befloxatone treatment. At the postsynaptic level, the administration of the selective 5-HT_{1A} receptor antagonist WAY 100635 (100 μg/kg, i. v.) did not modify the firing activity of quisqualate-activated dorsal hippocampus CA_3 pyramidal neurons in control rats. In contrast, in rats treated with befloxatone in combination with (-)pindolol for 2 days as well as with befloxatone alone for 21 days, WAY 100635 significantly increased the firing of CA_3 pyramidal neurons. In conclusion, these data suggest that when the firing activity of 5-HT neurons is normal in the presence of befloxatone, either after a two-day treatment together with (-)pindolol or alone for 21 days, the tonic activation of postsynaptic 5-HT_{1A} receptors is enhanced.

INTRODUCTION

Catecholamines and serotonin (5-HT) can be metabolized by the two isoenzymes of monoamine oxidase (MAO) denoted MAO-A and MAO-B (Johnston, 1968). The oxidative deamination of 5-HT, norepinephrine (NE) and epinephrine is preferentially mediated by MAO-A whereas the MAO-B isoenzyme preferentially deaminates phenylethylamine and benzylamine, while dopamine (DA) is deaminated by both isoforms (Yang and Neff, 1973; Denney and Denney, 1985; Westlund et al., 1985; Youdim and Finberg, 1991; Saura et al., 1992). Because of the important side effects of classical MAO inhibitors (*e.g.* the hypertensive crises triggered by the ingestion of tyramine-containing foods, Murphy et al., 1987), a second-generation of reversible MAO-A inhibitors have been developed (see Thase et al., 1995 and Youdim, M.B.H., 1995, for review). Among the latter, befloxatone, an oxazolidinone derivative which is a selective and reversible MAO-A inhibitor (Curet et al., 1995), seems to be a promising and effective antidepressant in experimental models (Caille et al., 1996). The pharmacological profile of befloxatone reveals that it has a much higher affinity for MAO-A (Ki= 2.8 ± 0.4 nM) than moclobemide (Ki= 14000 ± 1250 nM) in rat brain homogenates (Curet et al., 1995). Moreover, befloxatone does not affect the binding of radioligands acting on the noradrenergic, dopaminergic, serotonergic, muscarinic, histaminic, opioid, sigma receptor subtypes nor inhibits the uptake of monoamines in rat brain synaptosomes (Curet et al., 1996a). *In vivo*, befloxatone (1-750 μg/kg, p.o.) increases rat whole brain levels of NE, DA and 5-HT, and decreases the levels of corresponding deaminated metabolites dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindolacetic acid (5-HIAA) (Curet et al., 1996a). In microdialysis studies performed in freely moving rats, befloxatone (0.75 mg/kg, i.p.) was found to increase striatal DA and decreases DOPAC, homovanillic acid (HVA) and 5-HIAA extracellular levels

(Curet et al., 1994). Befloxatone (0.75 mg/kg, i.p.) also increases the extracellular levels of NE but not those of 5-HT in the frontal cortex of freely moving rats (Curet et al., 1994). An autoradiographic study on rat brain sections showed that befloxatone binds selectively to MAO-A, very high labelling density of [^{3}H]befloxatone being found in the locus coeruleus and high level being observed in the dorsal raphe (Curet et al., 1995). It has been also shown that long-term treatment with befloxatone (0.75 mg/kg/day, s.c. x 21 days) desensitizes α_2-adrenergic heteroreceptors on 5-HT fibers in rats and in guinea-pigs (Blier and Bouchard, 1994; Mongeau et al., 1994). Previous studies from our laboratory have also revealed that MAO-A inhibitors affect central NE and 5-HT neurotransmission in the rat brain (see Blier and de Montigny, 1994). In fact, it has been demonstrated that repeated administration of nonselective MAO inhibitor phenelzine and of the irreversible MAO-A inhibitor clorgyline produces an early and sustained decrease of the firing activity of rat locus coeruleus NE neurons and also a transient decrease of the firing activity of dorsal raphe 5-HT neurons. In contrast, deprenyl (a MAO-B inhibitor ineffective in endogenous depression) did not affect the firing activity of 5-HT or NE neurons (Blier and de Montigny, 1985).

The aim of the present study was undertaken to assess the effect of sustained treatment of befloxatone on the efficacy of brain 5-HT neurotransmission and on the sensitivity of pre- and postsynaptic 5-HT$_{1A}$ receptors, using *in vivo* electrophysiological paradigms in the rat dorsal raphe and dorsal hippocampus.

MATERIALS AND METHODS

The experiments were carried out in male Sprague-Dawley rats (Charles River, St Contant, Quebec, Canada) weighing 250 to 300 g which were kept under standard laboratory conditions (12:12 light-dark cycle with free access to food and water). Four groups of rat were treated for 2 days with either befloxatone alone (0.75 mg/kg/day), (-)pindolol (15 mg/kg/day) alone, both befloxatone (0.75 mg/kg/day) and (-)pindolol (15 mg/kg/day) and vehicle (a 50% ethanol water solution) delivered by osmotic minipumps (ALZA, Palo Alto, CA) inserted subcutaneously, and one group of rat was treated for 21 days with befloxatone alone (0.75 mg/kg/day). The rats were tested with the minipumps on board. The animals were anesthetized with chloral hydrate (400 mg/kg, i.p.). Supplemental doses were given to maintain constant anesthesia and to prevent any nociceptive reaction to a tail pinch.

Recordings of dorsal raphe 5-HT neurons. Extracellular recordings were performed with single-barrelled glass micropipettes preloaded with fibreglass filaments in order to facilitate filling. The tip was broken back to 1 to 4 μm and filled with a 2 M NaCl solution saturated with Fast Green FCF. The rats (control or treated rats) were placed in a stereotaxic frame and a burr hole was drilled on midline 1 mm anterior to lambda. Dorsal raphe 5-HT neurons were encountered over a distance of 1 mm starting immediately below the ventral border of the Sylvius aqueduct. These neurons were identified using the criteria of Aghajanian (1978): a slow (0.5-2.5 Hz) and regular firing rate and long-duration (0.8-1.2 ms) positive action potentials. In order to determine the possible changes of the spontaneous firing activity of dorsal raphe 5-HT neurons, five to six electrode descents were carried out through this nucleus in controls and rats with minipumps on board. For the control and treated groups of rats, the responsiveness of somatodendritic 5-HT$_{1A}$ receptors was assessed after the intravenous injection of LSD (10 μg/kg). Microiontophoresis was performed with five-barrelled micropipettes preloaded (R & D Scientific glass CO, Spencerville, MD, USA) with fibreglass filaments in order to facilitate filling and the tip was broken back to 4 to 8 μm. The central barrel was used for recording and filled with a 2 M NaCl solution. The

side barrels contained the following solutions: 5-HT creatinine sulphate (20 mM in 200 mM NaCl, pH 4), 8-OH-DPAT (1 mM in 100 mM NaCl, pH 4) and 2 M NaCl used for automatic current balancing. Finally, the suppressant effect of the α_2-adrenoceptors agonist clonidine (10 μg/kg, i.v.) on the firing activity of 5-HT neurons was assessed in control and in rats treated with befloxatone for 21 days.

Recordings from CA_3 dorsal hippocampus pyramidal neurons. Recording and microiontophoresis were performed with five-barrelled glass micropipettes broken back to 8-12 μm under microscopic control (ASI Instruments, Warren, MI, USA). The central barrel was filled with a 2 M NaCl solution and used for extracellular unitary recordings. The pyramidal neurons were identified by their large amplitude (0.5-1.2 mV) and long-duration (0.8-1.2 ms) simple spikes alternating with complex spike discharges (Kandel and Spencer 1961). The side barrels contained the following solutions: 5-HT creatinine sulphate (20 mM in 200 mM NaCl, pH 4), quisqualate (1.5 mM in 200 mM NaCl, pH 8) and 2 M NaCl used for automatic current balancing. The rats were mounted on a stereotaxic apparatus and the microelectrodes were lowered at 4.2 mm lateral and 4.2 anterior to lambda into the CA_3 region of the dorsal hippocampus. Since most hippocampus pyramidal neurons are not spontaneously active under chloral hydrate anaesthesia, a leak or a small ejection current of quisqualate (+1 to -6 nA) was used to activate them within their physiological firing range (10-15 Hz; Ranck, 1975). Neuronal responsiveness to the microiontophoretic application of 5-HT were assessed by determining the number of spikes suppressed/nA. The duration of the microiontophoretic applications of the agonist was of 50 sec. The same current of ejection was always used before and after the i.v. injection of the selective 5-HT_{1A} receptor antagonist WAY 100635 (100 μg/kg). In order to assess the degree of activation of the postsynaptic 5-HT_{1A} receptors exerting an inhibitory influence on the firing activity of quisqualate-activated CA_3 pyramidal neurons, the firing was reduced to a frequency of about 5 Hz 3 min before the i. v. injection of WAY 100635.

Electrical activation of the afferent 5-HT fibers to the hippocampus. A bipolar electrode (NE-110; David Kopf, Tujunga, CA., USA) was implanted on the midline with a 10° backward angle in the ventromedial tegmentum, 1 mm anterior to lambda and 8.3 mm below the cortical surface. A stimulator (S8800; Grass Instruments, Quincey, MA., USA) delivered two hundred square pulses of 0.5 ms at a frequency of 1 Hz and an intensity of 300 μA. The stimulation pulses and the firing activity of the neuron recorded were fed to an IBM-PC computer equipped with a Tecmar interface. Peristimulus time histograms were generated to determine the duration of suppression of firing activity of the CA_3 pyramidal neuron, measured in absolute silence value (SIL, in ms). This value is obtained by dividing the total number of events suppressed following the stimulation by the mean frequency of firing of the neuron recorded (Chaput et al., 1986). The CA_3 region of the hippocampus receives extensive innervation from 5-HT neurons of dorsal and median raphe nuclei (Hensler et al., 1994). This brief suppressant effect ($\approx$ 50 ms) resulting from the electrical stimulation of the ascending 5-HT pathway is due to the release of 5-HT for each impulse applied to the 5-HT axons and it is mediated by postsynaptic 5-HT_{1A} receptors (Chaput et al., 1986). Thus, for the CA_3 neurons tested in control rats, the effect of the stimulation of the ascending 5-HT pathway was first determined prior to and following the intravenous injection of WAY 100635 (100 μ/kg). The effect of the stimulation was then assessed prior to and following the injection of WAY 100635 (100 μg/kg, i.v.) in treated rats with minipumps on board. In order to determine the possible changes of the responsiveness of the terminal 5-HT autoreceptors, in each groups, two series of stimulations (1 and 5 Hz) were carried out, while recording the same neuron, since it has

previously been demonstrated *in vitro* and *in vivo* that the activation of terminal 5-HT autoreceptors decreases the release of 5-HT and that this reduction was enhanced by increasing the frequency of the stimulations (Chaput et al., 1986, Göthert, 1980; Blier et al., 1989).

Determination of MAO activities and assay of monoamines in the rat brain.
These experiments were carried out in control and treated rats with befloxatone for 2 and 21 days in Synthelabo Recherche laboratories. For the determination of the MAO activity, rats were decapited and brains were dissected out and rapidly frozen. The samples were kept at -80°C until MAO-A and MAO-B assay. The different tissues were homogenised in 20 volumes of ice-cold 0.1 sodium phosphate buffer (pH 7.4). Aliquots (0.1 ml) of crude membrane suspensions were incubated with [^{14}C]-5-HT (final concentration 125 µM) for 5 min and with [^{14}C]-PEA (final concentration 8 µM) for 1 min in a total volume of 0.5 ml at 37°C and in one experiment with [^{14}C]-TYR (final concentration 100 µM) as non specific substrate for MAO, for 4 min. The reaction was stopped with 200 µl of 4 M HCl and deaminated metabolites were extracted by vigourous shaking for 10 min with 7 ml of toluene/ethyl acetate (V/V) and quantified by liquid scintillation counting.
For the assay of monoamines, whole brain (minus cerebellum) were removed, frozen, wheighed and stored at -80°C until analysis. NE, 5-HT and DA were measured by high pressure liquid chromatography (HPLC) with electrochemical detection. Frozen tissues were sonicated in 10 volumes of 0.1 M HClO4 containing 0.6 mM of EDTA and DHBA (final concentration 1 ng/60µl) as internal standard. After centrifugation, 60 µl of the supernatant were injected onto the liquid chromatographic column using a refrigirated (4°C) autoinjector Wisp 510 (Waters, Milford, MA, USA). Separation was achieved at room temperature. The HPLC system consted of a pump, a stainless steel separation column (0.45x25 cm) packed with a Ultrasphere ODS C18, 5 µm particle size (Beckman, Fullertone, CA, USA). The mobile phase contained 0.1 M NaH_2PO_4, 1 mM EDTA, 2.5 mM octane sulfonic acid, 7% CH_3CN, pH 3.4. The flow rate was 1 ml/min. Electrochemical detection was carried out by means of an amperometric detector (model 460 Waters) and achieved by setting the glassy carbon working electrode at +800 mV (with respect to a Ag/AgCl reference electrode). The chromatograms were registered on a Baseline 810 systems (Waters). The retention times for NE, DA and 5-HT were 5, 13 and 36 min respectively. Concentrations of each compound were calculated with reference to standards.

Drugs: Befloxatone (Synthélabo Recherche, Rueil-Malmaison, France); 5-HT creatinine sulphate, quisqualate and clonidine (Sigma Chemical, St. Louis, MO); WAY 100635 (Wyeth Research, Bershire, UK); 8-OH-DPAT (Research Biochemical, Natick, MA) and LSD (Ministry of Health and Welfare, Ottawa, ON). The concentrations and the doses used for these compounds were chosen on the basis of previous experiments carried out in our and other laboratories.

RESULTS

Effect of sustained treatment with befloxatone alone and in combination with (-)pindolol on the firing activity of dorsal raphe 5-HT neurons. It has been shown that the administration of befloxatone (1 mg/kg, i.p.) suppressed, with a short latency, the firing activity of rat dorsal raphe 5-HT neurons (Curet et al., 1996b). Consistent with these results, short-term treatment with befloxatone (0.75 mg/kg/day, s.c. x 2 days) decreased of the spontaneous firing activity of dorsal raphe 5-HT neurons by

27% (Fig. 1B and E). This decrease of the firing activity of 5-HT neurons observed in befloxatone-treated rats was no longer present in rats treated with both befloxatone and the 5-HT_{1A} autoreceptor antagonist (-)pindolol. In fact, the combination of the two drugs increased by 23% the firing activity of 5-HT neurons (Figs. 1C and E). In rats treated with (-)pindolol (15 mg/kg/day, s.c.) for 2 days or with befloxatone (0.75 mg/kg/day, s.c.) for 21 days, the firing activity of 5-HT was not significantly different from that measured in controls (Figs. 1D and E).

As illustrated in figure 2, the i.v. administration of LSD (10 μg/kg) suppressed the firing activity of 5-HT neurons in control rats and this effect of LSD was reversed by the administration of the selective 5-HT_{1A} receptor antagonist WAY 100635 (100 μg/kg, i.v). Interestingly, the short-term befloxatone treatment (0.75 mg/kg/day, s.c. x 2 days) attenuated the suppressant effect of LSD on the firing activity of 5-HT neurons (Fig. 2B). As illustrated in figure 2C, a 2-day treatment with befloxatone decreased by 52% the suppressant effect of LSD on the firing activity of dorsal raphe 5-HT neurons and this suppressant effect of LSD on the firing activity of 5-HT neurons was back to normal after a 21-day treatment with befloxatone (Fig. 2C). In order to confirm this apparent early inactivation of the somatodendritic 5-HT_{1A} receptors on 5-HT neurons, both 5-HT and 8-OH-DPAT were applied microiontophoretically directly into dorsal raphe 5-HT neurons. As illustrated in figures 3B and D, the suppressant effects of the two 5-HT agonists were significantly reduced after the 2-day treatment with befloxatone. It has previously been shown that a 2-day treatment with (-)pindolol (15 mg/kg/day, s.c.) reduces the suppressant effect of LSD on the firing activity of dorsal raphe 5-HT neurons (Romero et al., 1996). In the present study, the combination of befloxatone and (-)pindolol treatment also attenuated this reducing effect of LSD on the firing activity of dorsal raphe 5-HT neurons (inhibition of 10 ± 6 % with 10 μg/kg, i.v. of LSD in 3 rats). However, in rats treated with befloxatone for 21 days (0.75 mg/kg, s.c.), both the spontaneous firing activity of 5-HT neurons (Figs. 1E and F) and the suppressant effects of the i.v. injection of LSD as well as the microiontophoretic applications of 5-HT and 8-OH-DPAT (Figs. 2C, D and 3C, D) on the firing activity of these neurons were similar to controls. The i.v. injection of the α_2-adrenoceptor agonist clonidine (10 μg/kg, i.v.) suppressed the firing activity of 5-HT neurons in controls and this effect of clonidine was markedly attenuated in rats treated for 21 days with befloxatone (0.75 mg/kg/day, s.c., Fig. 4).

Effect of sustained treatment with befloxatone alone and in combination with (-)pindolol on the CA_3 dorsal hippocampus pyramidal neurons responsiveness to 5-HT. It has been previously demonstrated that the microiontophoretic application of 5-HT onto rat dorsal hippocampus pyramidal neurons produces a suppressant effect on their firing activity and that this effect is mediated by postsynaptic 5-HT_{1A} receptors (Blier and de Montigny, 1987; Chaput and de Montigny, 1988; Blier et al., 1993b). For all CA_3 hippocampus pyramidal neurons tested, 5-HT induced a reduction of firing activity, generally 100 % with a 10 nA current applied for 50 s (Fig. 5A, B and C). This suppressant effect occurred in the absence of any alteration of the shape of the action potentials. As illustrated in figures 5B, C and D, short-term treatment with befloxatone alone and in combination with (-)pindolol did not modify the suppressant effect of microiontophoretically-applied 5-HT on the firing activity of CA_3 dorsal hippocampus pyramidal neurons. Figure 5D illustrates the mean suppressant action of 5-HT microiontophoretically-applied onto CA_3 pyramidal neurons and the antagonistic effect of WAY 100635 on the responsiveness of these postsynaptic 5-HT_{1A} receptors in control and treated rats. In the present study, WAY 100635 (100 μg/kg, i.v.) significantly reduced the suppressant effect of 5-HT on CA_3 hippocampus pyramidal neurons firing by 52% in controls, by 75% in befloxatone-treated rats and by

64% in befloxatone and (-)pindolol-treated rats (Fig. 5D). The effect of WAY 100635 (100 μg/kg, i.v.) on the quisqualate-activated firing activity of CA_3 pyramidal neurons was assessed in control rats, in rats treated with befloxatone alone, and in rats treated with both befloxatone and (-)pindolol. In control rats, the i.v. administration of WAY 100635 did not modify the firing activity of dorsal hippocampus CA_3 pyramidal neurons indicating a minimal activation of postsynaptic 5-HT_{1A} receptors by endogenous 5-HT under these experimental conditions (Figs. 6A and D). In the treated groups, the firing activity of these neurons was significantly increased by the same dose of WAY 100635, thus indicating an increased tonic activation of postsynaptic 5-HT_{1A} receptors by the treatment regimens (Figs. 6B, C and D).

Electrical stimulation of the afferent 5-HT fibers to the hippocampus. In order to determine whether treatments with befloxatone alone and in combination with (-)pindolol could modulate *in vivo* the release of 5-HT per electrical impulse reaching 5-HT terminals and to assess the effect of the 5-HT_{1A} receptor antagonist WAY 100635 on the effectiveness of the stimulation of the 5-HT pathway, the capacity to modify the duration of the suppression of the firing activity of CA_3 hippocampus pyramidal neurons produced by the electrical activation of the ascending 5-HT pathway was examined in control rats and in rats with the minipump in place delivering the drugs. Neither befloxatone (0.75 mg/kg/day, s.c. x 2 days) nor befloxatone (0.75 mg/kg/day, s.c. x 2 days) plus (-)pindolol (15 mg/kg/day, s.c. x 2 days) modified the effectiveness of the stimulation of the 5-HT pathway (Fig. 7B). However, in control rats, the i.v. administration of WAY 100635 (100 μg/kg, i. v.) unexpectedly increased the efficacy of the stimulation of the 5-HT pathway (Figs. 7A and B). This enhancing effect of WAY 100635 on the effectiveness of the stimulation was also present in rats treated with befloxatone alone and in rats treated with both befloxatone and (-)pindolol for 2 days (Fig. 7B).

The responsiveness of terminal 5-HT autoreceptors was evaluated by increasing the frequency of the stimulation from 1 to 5 Hz. In control rats, the 5 Hz stimulation was 23% less effective from the 1 Hz one. However, in rats treated with befloxatone and treated with both befloxatone and (-)pindolol, the decremental effect of the 5-HT pathway stimulation was absent (Fig. 8B). In addition, in rats treated with befloxatone for 2 days, the enhancing effect of the terminal 5-HT autoreceptor antagonist metergoline on the efficacy of the stimulation of the 5-HT pathway (Haddjeri et al., 1996) was no longer present, thus suggesting an inactivation of terminal 5-HT autoreceptors (SIL value prior to: 35 ± 4 ms; SIL value after metergoline= 31 ± 5 ms, n=4). This inactivation of 5-HT autoreceptors was no longer present in rats treated with befloxatone for 21 days, as the sensitivity of these receptors was similar to controls (Fig. 8B).

Determination of MAO activities and assay of monoamines in the rat brain. The assessment of MAO-A and B activities from whole brain homogenates revealed that MAO-B activity was unchanged after 2 and 21 days of treatment with befloxatone (0.75 mg/kg/day, s.c.), whereas MAO-A activity was inhibited by 80% compared to controls both after 2 and 21 days of treatment with befloxatone. Furthermore, similarly to other MAO inhibitors (Blier and de Montigny, 1985, 1986a, 1986b; Da Prada et al., 1989), the brain concentration of NE, DA and 5-HT were significantly increased in rats treated with befloxatone for 2 and 21 days compared to controls (see Table I).

DISCUSSION

In the present study, befloxatone (0.75 mg/kg/day x 2 days) decreased the firing activity of dorsal raphe 5-HT neurons. This suggests that short-term treatment with befloxatone produces an inhibition of

MAO-A in the dorsal raphe and consequently enhances the synaptic availability of 5-HT, as previously demonstrated with other MAO inhibitors (Aghajanian et al., 1970). The release of 5-HT from the dendrites or recurrent collaterals in the dorsal raphe has been well documented (Héry et al., 1982; Piñeyro et al., 1994, 1995; Davidson and Stamford, 1995) and since the firing activity of 5-HT neurons is in part controlled by somatodendritic 5-HT_{1A} autoreceptors, one may assume that the decrease of the firing activity occurring after 2-day treatment with befloxatone was due to an increase of the availability of 5-HT with subsequent activation of somatodendritic 5-HT_{1A} autoreceptors. Indeed, an increase in the firing activity of 5-HT neurons in rats treated with both befloxatone and the 5-$HT_{1A/1B}$ autoreceptor antagonist (-)pindolol was observed as well as a marked increase of the firing activity of 5-HT neurons after i.v administration of the 5-HT_{1A} autoreceptor antagonist WAY 100635 in rats treated with befloxatone alone for 2 days (Fig. 2B). Although, the reason for the increase of the firing activity above the baseline is still unclear, preliminary data from Fornal et al. (1995) revealed that WAY 100635 blocks the action of endogenous 5-HT at 5-HT_{1A} autoreceptors in the cat dorsal raphe and also reverses the inhibition of the firing rate of 5-HT neurons produced by the 5-HT reuptake blocker fluoxetine above the baseline value.

In the present study, after a 2-day treatment with befloxatone, the decrease of the firing activity of 5-HT neurons was of only 27% which represents a small reduction in comparison with the approximate 50% of reduction of 5-HT neurons firing activity using either phenelzine (nonselective MAO inhibitor), clorgyline (irreversible MAO-A inhibitor) or amiflamine (reversible MAO-A inhibitor) (Blier and de Montigny, 1985, Blier et al., 1986a, 1986b). Moreover, a single administration of befloxatone (1 mg/kg, i.p.) has been shown to cause a total cessation of the firing activity of rat dorsal raphe 5-HT neurons (Curet et al., 1996b). Such results might be related to the fact that 5-HT_{1A} autoreceptors on 5-HT neurons of the dorsal raphe nucleus could be already inactivated only after a 2-day treatment with befloxatone. In fact, in rats treated with befloxatone for 2 days, the responsiveness of the somatodendritic 5-HT_{1A} autoreceptors to the systemic administration of LSD and to the microiontophoretic application of 5-HT and 8-OH-DPAT, was decreased by about two-fold. Similar results have also been obtained by Le Poul et al. (1995) using two selective 5-HT reuptake inhibitors (SSRIs). This group has shown that after 3 days of treatment with fluoxetine or paroxetine, followed by a one day washout period, the potency of 8-OH-DPAT to suppress the firing of dorsal raphe 5-HT neurons recorded from brain slices was already significantly reduced. Moreover, these treatments failed to modify the specific 5-HT_{1A} binding sites in the dorsal raphe or in other brain areas as measured with either [^{3}H]8-OH-DPAT or with [^{3}H]WAY 100635. The authors have suggested that, already after a 3-day treatment with SSRIs, an adaptative desensitization of the somatodendritic 5-HT_{1A} autoreceptors within the dorsal raphe nucleus had taken place without any change in the number of 5-HT_{1A} binding sites. The latter phenomenon (*i.e.* desensitization of dorsal raphe 5-HT_{1A} autoreceptors without any reduction in the number of the 5-HT_{1A} binding sites which is likely due to uncoupling of the receptor from its transduction system) has also been observed following long-term treatment with the partial 5-HT_{1A} receptor agonist ipsapirone (Schechter et al., 1990). In contrast to long-term treatment with SSRIs (see Blier and de Montigny, 1994), the early inactivation of the somatodendritic 5-HT_{1A} autoreceptors observed in the present study did not persist since the 21-day treatment with befloxatone modified neither the suppressant effect of the autoreceptor agonist LSD administered intravenously, nor those of 5-HT and 8-OH-DPAT applied by microiontophoresis. This unexpected result is not due to incomplete inhibition of MAO-A by befloxatone since the extent of MAO inhibition was similar in rats treated for 2- and 21 days with the drug (see Materials and Methods). Furthermore, an increase in brain levels of 5-HT was also present in rats treated

for 21 days with befloxatone, although the magnitude of this increase was somewhat lower than after a two-day treatment (see Table I). One possible explanation for such normalized 5-HT neuronal firing activity and for such normosensitive 5-HT_{1A} autoreceptors following sustained MAO inhibition, might be the adaptative change of locus coeruleus NA neurons which exert a major influence on the firing activity of 5-HT neurons. In fact, dorsal raphe 5-HT neurons receive NE projections from the locus coeruleus (Loizou, 1969; Anderson et al., 1977; Baraban and Aghajanian, 1981; Jones and Yang, 1985; Luppi et al., 1995). Pharmacological studies have indicated that the firing activity of 5-HT neurons in the dorsal raphe is dependent on a tonic activation by its noradrenergic input (Svensson et al., 1975; Baraban and Aghajanian, 1980). The inhibitory action of the α_2-adrenergic agonist clonidine on the firing activity of 5-HT neurons was thus suggested to be due to the activation of somatodendritic and terminal α_2-adrenergic autoreceptors decreasing the endogenous NE excitatory drive onto α_1-adrenergic receptors located on 5-HT neurons (Svensson et al., 1975, Clement et al., 1992a, 1992b). In keeping with these observations, α_2-adrenergic antagonists enhance the firing activity of dorsal raphe 5-HT neurons (Freedman and Aghajanian, 1984; Garrat et al., 1991, Haddjeri et al., 1996). In the present study, in rats treated with befloxatone for 21 days, the suppressant effect of clonidine on the firing activity of 5-HT neurons was markedly attenuated. This may reflect a desensitization of α_2-adrenergic autoreceptors. However, it was previously shown that the MAO-A inhibitors clorgyline and amiflamine do not alter the responsiveness of NE neurons in the locus coeruleus to systemic administration of clonidine (Blier et al., 1986a, 1986b). This suggests that the sustained blockade of MAO-A activity by befloxatone, as is the case with clorgyline and amiflamine, decreased the activity of NE neurons by maximally 40% (O. Curet, personnal communication), consequently leaving little modulation to be exerted by clonidine. The latter possibility is all the more likely as an attenuated NE activation of 5-HT neurons, resulting from the lesioning of NE neurons or the blockade of α_1-adrenoceptors, initially suppresses the firing activity of 5-HT neurons but this parameter returns to normal within a few days (Baraban and Aghajanian, 1980, Blier et al., 1989).

Among the 5-HT_{1A} receptor antagonists available, WAY 100635 is the most potent and selective antagonist acting at both pre- and postsynaptic 5-HT_{1A} receptors (Fletcher et al., 1996). In the CA_3 region of the dorsal hippocampus, the short- and long-term treatment with befloxatone, as well as the 2-day treatment with both befloxatone and (-)pindolol, did not modify the responsiveness of postsynaptic 5-HT_{1A} receptors to microiontophoretic application of 5-HT, indicating that the sensitivity of these receptors remains unchanged following such treatments. However, WAY 100635 (100 µg/kg, i.v.), in contrast to (-)pindolol (Romero et al., 1996), antagonized both in control and treated rats the suppressant effect of microiontophoretically-applied 5-HT onto CA_3 pyramidal neurons, thus showing its capacity to block 5-HT_{1A} receptors on the cell body of CA_3 pyramidal neurons.

It has been shown that 5-HT terminals are almost exclusively located on the dendritic trees of hippocampus pyramidal neurons (Oleskevish and Descarries, 1990) and the endogenous 5-HT, released by the electrical stimulation of the ascending 5-HT pathway, activates the intrasynaptic 5-HT_{1A} receptors located on dendrites of hippocampus pyramidal neurons (Chaput and de Montigny, 1988; Blier et al., 1993a). In the CA_3 region of the dorsal hippocampus, previous studies from our laboratory have also shown that pertussis toxin nearly abolishes the responsiveness of extrasynaptic 5-HT_{1A} receptors located on the cell body of CA_3 pyramidal neurons, but not of intrasynaptic 5-HT_{1A} receptors located on the dendritic tree of the same neurons (Blier et al., 1993b). Moreover, Hadrava et al. (1994) have demonstrated that the sustained activation of the extrasynaptic, but not of intrasynaptic 5-HT_{1A} receptors of CA_3 pyramidal neurons, achieved with treatments with 5-HT_{1A} receptors agonists flesinoxan and BMY

42568, prevents their inactivation by pertussis toxin. These data further suggest that these populations of postsynaptic 5-HT_{1A} receptors located on the same neurons are functionally distinct. In the present study, WAY 100635 (100 μg/kg, i.v.) failed to decrease the duration of suppression of firing of dorsal hippocampus CA_3 pyramidal neurons induced by the electrical stimulation of the ascending 5-HT pathway in control rats and in rats treated with befloxatone alone and in combination with (-)pindolol for both 2 days, as well as after a 21-day treatment with befloxatone. This suggests that WAY 100635 failed to block the intrasynaptic 5-HT_{1A} receptors of CA_3 pyramidal neurons, as is the case with the lack of effect of pertussis toxin on this population of postsynaptic 5-HT_{1A} receptors (Blier et al., 1993b; Hadrava et al., 1994). WAY 100635 (100 μg/kg, i.v.), in fact, even increased the efficacy of the stimulation. Another contributory factor to this unexpected effect of WAY 100635 could be due to its suppressant effect on the spontaneous firing activity of locus coeruleus NE neurons (Haddjeri et al., 1997). This suppressant effect of WAY 100635 on the firing of locus coeruleus NE neurons induces presumably a decrease of NE release in the synaptic cleft in the dorsal hippocampus. Consequently, as demonstrated in previous studies (Mongeau et al., 1993, 1994), the decrease of endogenous NE release reduces the tonic activation of α_2-adrenergic heteroreceptors, located on 5-HT fibers in the dorsal hippocampus, which in turn leads to an enhancement of the endogenous release of 5-HT induced by the electrical stimulation of the ascending 5-HT pathway.

It has previously been demonstrated *in vitro* and *in vivo* that the activation of terminal 5-HT autoreceptors decreases the release of 5-HT and that this reduction was even more evident at higher frequencies of stimulation of 5-HT axons (Göthert, 1980; Chaput et al., 1986; Blier et al., 1989). Long-term treatment with befloxatone does not affect the responsiveness of terminal 5-HT autoreceptors in the hippocampus and hypothalamus of guinea-pigs measured *in vitro* in brain slices (Blier and Bouchard, 1994). In the present study, two days of treatment with befloxatone alone did not modify the effectiveness of the stimulation of the 5-HT pathway. In fact, the decrease in the effectiveness of the stimulation upon increasing its frequency from 1 to 5 Hz (due to the activation of terminal 5-HT autoreceptors) was attenuated after a 2-day treatment with befloxatone alone. As was the case with the somatodendritic 5-HT_{1A} autoreceptors, these results suggest that befloxatone administration produced an early inactivation of terminal 5-HT_{1B} autoreceptors. This contention is further supported by the loss of the enhancing effect of the 5-HT autoreceptor antagonist metergoline on the efficacy of the stimulation in rats treated with befloxatone for 2 days. This inactivation was however transient as indicated by the return to a normal pattern to the 5 Hz and the 1 Hz stimulations after 21 days of treatment with befloxatone.

In rats treated for 2 days with befloxatone alone or in combination with (-)pindolol, as well as in rats treated with befloxatone for 21 days, the i. v. administration of WAY 100635 enhanced the firing activity of CA_3 pyramidal neurons. When WAY 100635 is injected systemically, it does not enhance the firing rate of 5-HT neurons in anesthetized rats (Fletcher et al., 1994, 1996; Haddjeri et al., unpublished observation), but it will reverse the suppressant effect of an enhanced activation of 5-HT_{1A} autoreceptors resulting from an increased synaptic availability of 5-HT itself or of an exogenous 5-HT_{1A} receptor agonist. Consequently, in rats treated with befloxatone for two days, one cannot conclude that there is an enhanced activation of postsynaptic 5-HT_{1A} receptors, which exert an inhibitory effect on the firing of CA_3 pyramidal neurons, because of the enhancing effect of WAY 100635 on the neurons, since the firing activity of 5-HT neurons is attenuated before the administration of WAY 100635 in these rats. However, in rats treated with befloxatone and (-)pindolol for two days, as well as in rats treated with befloxatone alone for 21 days, the firing activity of 5-HT neurons is not affected, and WAY 100635

markedly enhanced the firing activity of CA_3 pyramidal neurons. Therefore, it can be concluded that the latter two treatments produced an enhanced tonic activation of postsynaptic 5-HT_{1A} and possibly of other 5-HT receptors.

In conclusion, the present results show that the short-term administration of befloxatone reduces the firing activity of 5-HT neurons which is followed by a recovery after 21 days of sustained administration. This sequence of events correlates well with the delayed onset of MAO inhibitors in major depression. Given that the firing activity of 5-HT neurons is even above the normal value following a two-day treatment with befloxatone and (-)pindolol, this combined treatment could be expected to produce the same enhancing effect on 5-HT neurotransmission as after a long-term treatment with befloxatone alone.

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FIGURE LEGENDS

Figure 1: Integrated firing rate histograms of dorsal raphe 5-HT neurons showing their spontaneous firing activity in control (**A**), rat with a 2-day treatment with befloxatone (0.75 mg/kg/day, s.c.) (**B**), rat with a 2-day treatment with both befloxatone and (-)pindolol (15 mg/kg/day, s.c.) (**C**) and rat with a 21-day treatment with befloxatone (0.75 mg/kg/day, s.c.) (**D**). The number above each neuron indicates the depth from the floor of the sylvius aqueduct at which it was recorded. Corresponding results (means ± S.E.M.) are presented on **E**. Note the unchanged firing activity of 5-HT neurons in the group treated with

(-)pindolol alone. The numbers at the bottom of the columns indicates the number of neurons tested corresponding to 6-8 rats per group. $^{*}P < 0.05$ (unpaired Student's *t* test).

Figure 2: Integrated firing rate histograms of dorsal raphe 5-HT neurons showing their response to LSD and WAY 100635 in control (**A**) and treated rat with befloxatone for 2 days (**B**). Corresponding results (means ± S.E.M.) of the suppressant effect of LSD (10 µg/kg, i.v.) of the firing activity of 5-HT neurons in controls and treated rats with befloxatone for 2 and 21 days are presented on **C**. The numbers at the bottom of the columns indicates the number of neurons or rats tested. $^{*}P < 0.05$ (unpaired Student's *t* test).

Figure 3: **A**, **B** and **C** represent integrated firing rate histograms of three dorsal raphe 5-HT neurons showing their responsiveness to microiontophoretic application of 5-HT and 8-OH-DPAT in control (**A**), treated rat with befloxatone for 2 days (**B**) and treated rat with befloxatone for 21 days (**C**). Horizontal bars indicate the duration of the applications for which the current is given in nanoampers. In **D**, the responsiveness to 5-HT and 8-OH-DPAT is expressed as the number of spikes per nanoampers (means ± S.E.M.). The numbers at the bottom of the columns indicates the number of neurons tested. $^{*}P < 0.05$ (unpaired Student's *t* test).

Figure 4: Integrated firing rate histograms of dorsal raphe 5-HT neurons showing their response to clonidine in control (**A**) and rat treated with befloxatone for 21 days (**B**). Corresponding results (means ± S.E.M.) of the suppressant effect of clonidine (10 µg/kg, i.v.) of the firing activity of 5-HT neurons in controls and treated rats with befloxatone for 21 days are presented on **C**. The numbers at the bottom of the columns indicates the number of neurons or rats tested. $^{*}P < 0.01$ (unpaired Student's *t* test).

Figure 5 : **A**, **B** and **C** represent integrated firing rate histograms of three dorsal hippocampus CA_3 pyramidal neurons showing their responsiveness to microiontophoretic application of 5-HT prior to and after the i.v. administration of WAY 100635 in control (**A**) and treated rat for 2 days with befloxatone alone (0.75 mg/kg/day, s.c.) (**B**) and in combination with (-)pindolol (0.75 mg/kg/day, s.c.) (**C**). These neurons were activated with quisqualate ejection current. Horizontal bars indicate the duration of the applications for which the current is given in nanoampers. In **D**, the responsiveness to 5-HT is expressed as the number of spikes suppressed per nanoampers (means ± S.E.M.). The numbers at the bottom of the columns indicates the number of neurons tested. Note the altered effectiveness of 5-HT to suppress firing activity after WAY 100635 administration in each group. $^{*}P < 0.05$ (unpaired Student's *t* test).

Figure 6 : **A**, **B** and **C** represent integrated firing rate histograms of three dorsal hippocampus CA_3 pyramidal neurons showing their responsiveness to the i.v. administration of WAY 100635 (100 µg/kg, i.v.) in control (**A**) and treated rat for 2 days with befloxatone alone (0.75 mg/kg/day, s.c.) (**B**) and in combination with (-)pindolol (0.75 mg/kg/day, s.c.) (**C**). These neurons were activated with quisqualate ejection current. In **D**, the responsiveness to WAY 100635 is expressed as degree (%) of increase of the firing of CA_3 pyramidal neurons (means ± S.E.M.). The numbers at the bottom of the columns indicates the number of neurons tested. $^{*}P < 0.05$ (unpaired Student's *t* test).

Figure 7: **A**: Peristimulus time histograms illustrating the effect of the electrical stimulation of the ascending 5-HT pathway at the level of the ventro-medial tegmentum on the firing activity of dorsal hippocampus CA_3 pyramidal neurons in a control rat prior to and after WAY 100635 (100 µg/kg, i.v.). The SIL represents the duration of suppression of firing (see Materials and Methods). Each histogram was constructed from 200 consecutive stimulations (300 µA, 0.5 ms, 1 Hz) with a bin width of 2 ms. **B**: Duration of suppression of firing activity (means ± S.E.M) of CA_3 hippocampus pyramidal neurons produced by the stimulation of the ascending 5-HT pathway, before and after the administration of WAY 100635 (100 µg/kg, i.v.) in controls and treated rats for 2 days with befloxatone alone (0.75 mg/kg/day, s.c.) and in combination with (-)pindolol (0.75 mg/kg/day, s.c.). The number in the columns indicates the number of neurons tested. $^{*}P < 0.05$, using the paired Student *t* test.

Figure 8: **A**: Peristimulus time histograms illustrating the effect of the electrical stimulation of the ascending 5-HT pathway at the level of the ventro-medial tegmentum on the firing activity of dorsal hippocampus CA_3 pyramidal neurons for the same neurons at 1 and 5 Hz in a control rat. Note the reduction of the SIL which represents the duration of suppression of firing

(see Materials and Methods) by increasing frequency. **B**: Effects of treatments on the efficacy of the stimulation of the ascending 5-HT pathway of subsequent increase of the frequency from 1 to 5 Hz (means ± S.E.M.). The number in the columns indicate the number of neurons tested. $^{*}P < 0.05$ using the paired Student *t* test. Only the responsiveness of terminals 5-HT autoreceptors in rats treated with befloxatone for 21 days was not significantly modified by comparison the decrease of the effectiveness in control versus treated rats.

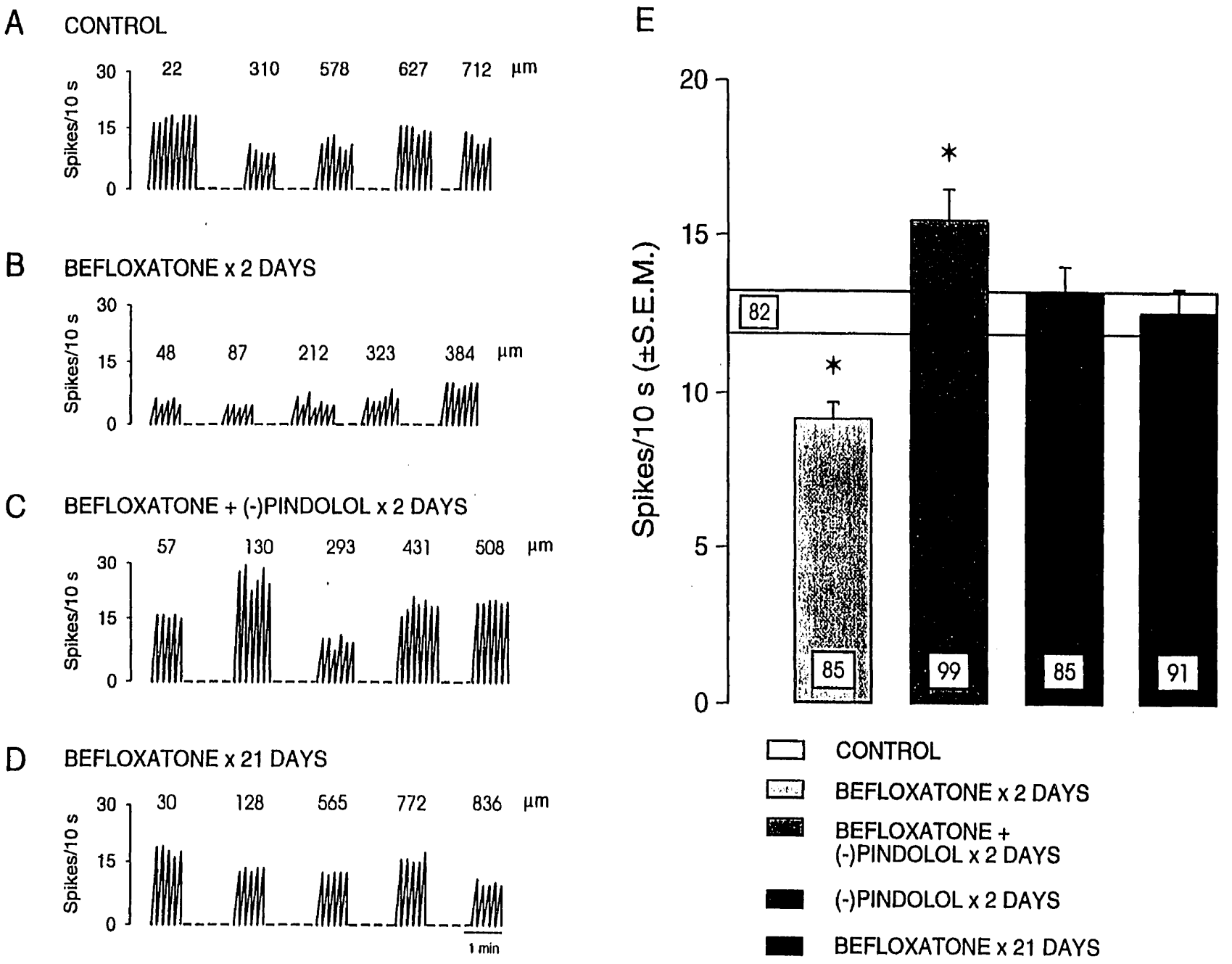
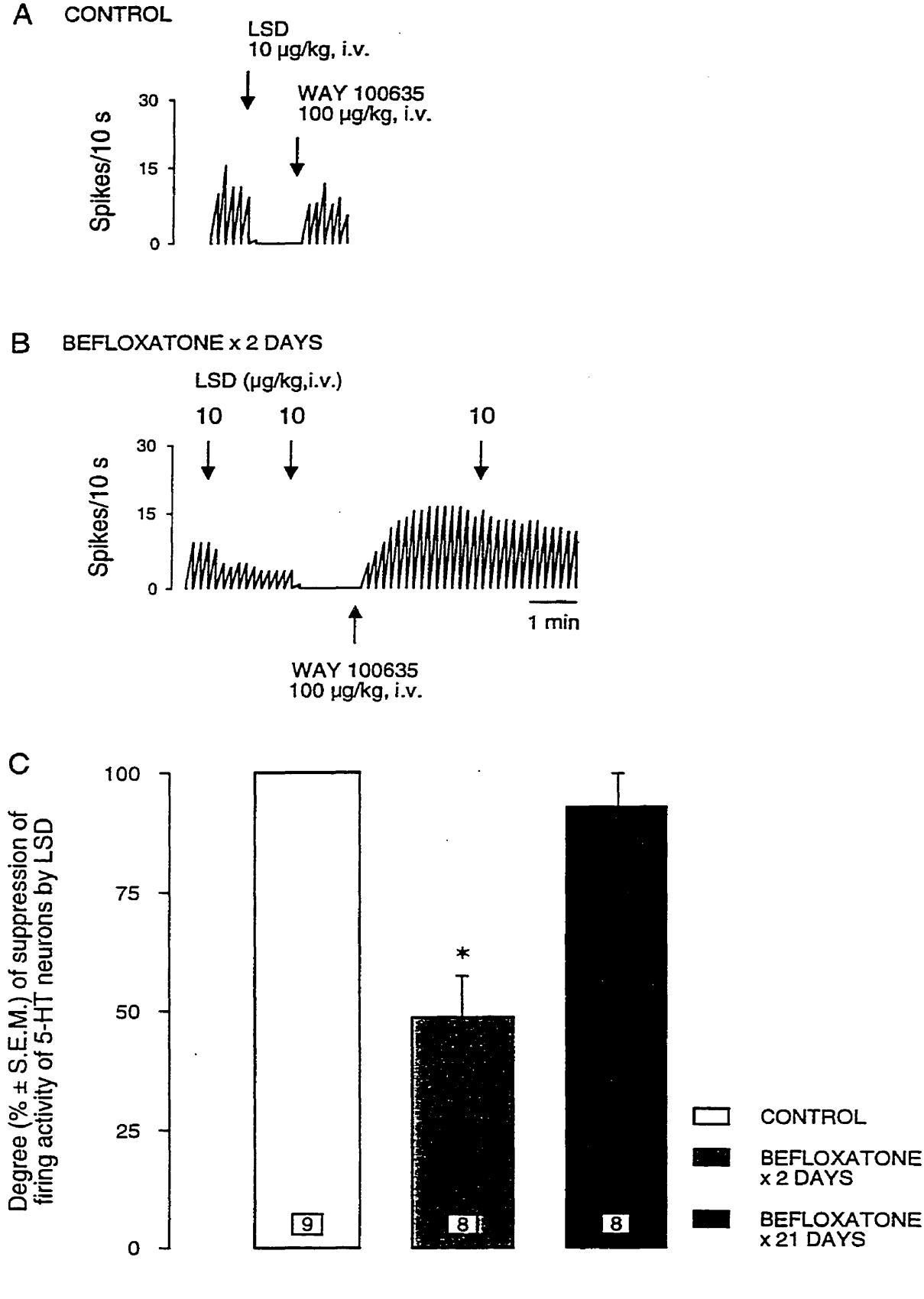
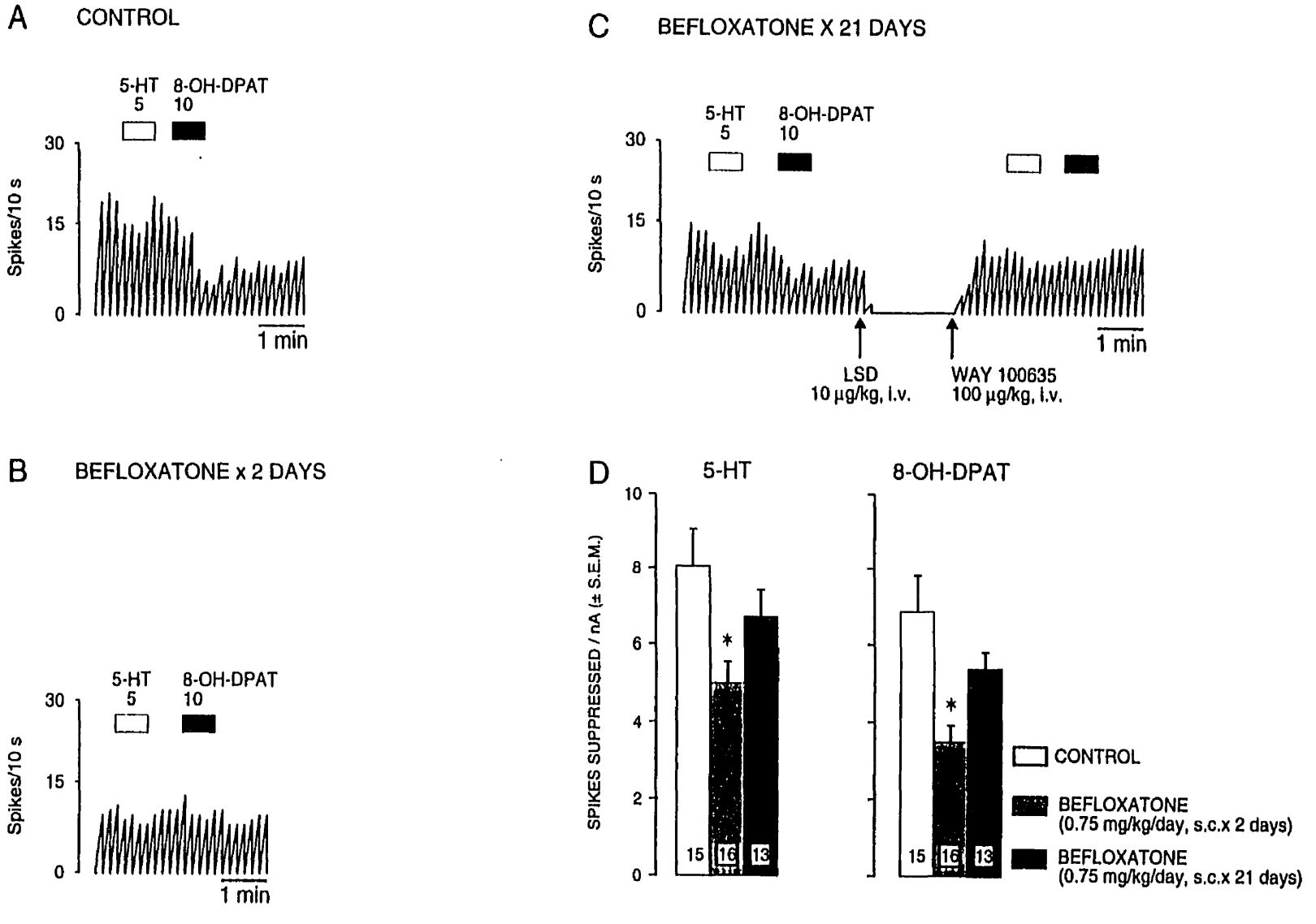
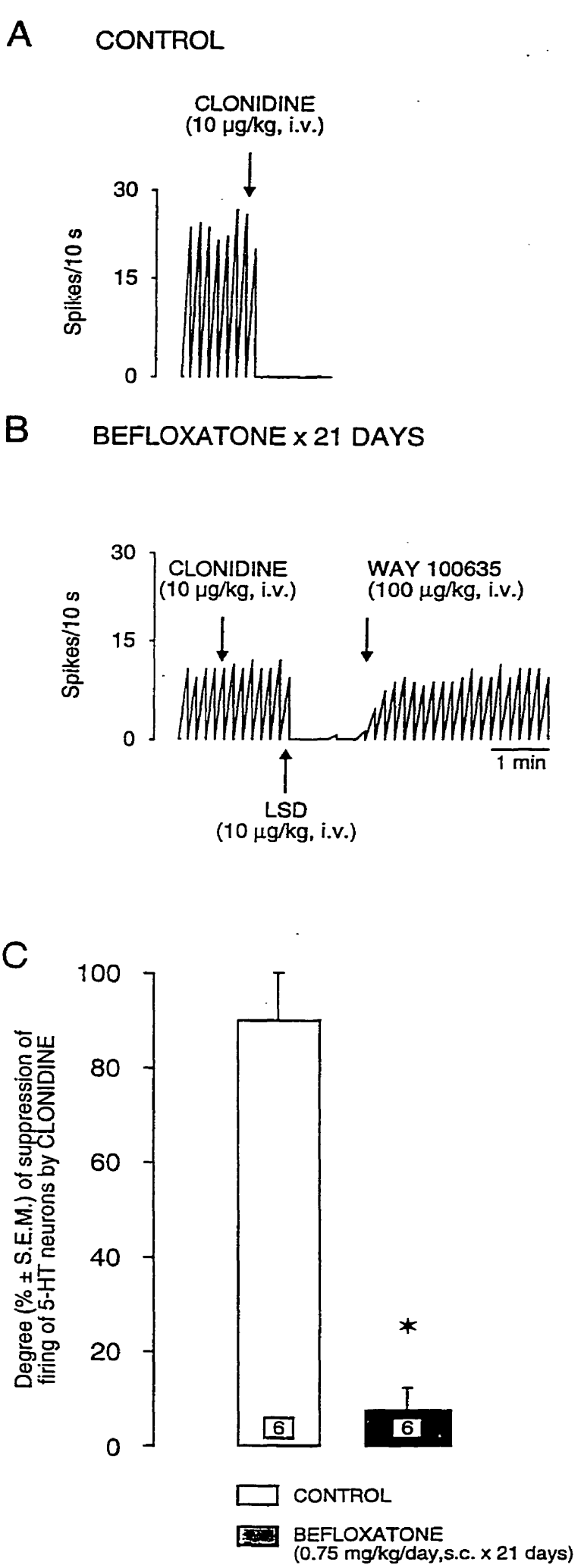

A
CONTROL
Spikes/10 s
30
15
0
22
310
578
627
712
µm
B
BEFLOXATONE x 2 DAYS
48
87
212
323
384
C
BEFLOXATONE + (-)PINDOLOL x 2 DAYS
57
130
293
431
508
D
BEFLOXATONE x 21 DAYS
30
128
565
772
836
1 min
E
Spikes/10 s (±S.E.M.)
20
10
5
82
85
99
85
91
CONTROL
BEFLOXATONE x 2 DAYS
BEFLOXATONE + (-)PINDOLOL x 2 DAYS
(-)PINDOLOL x 2 DAYS
BEFLOXATONE x 21 DAYS

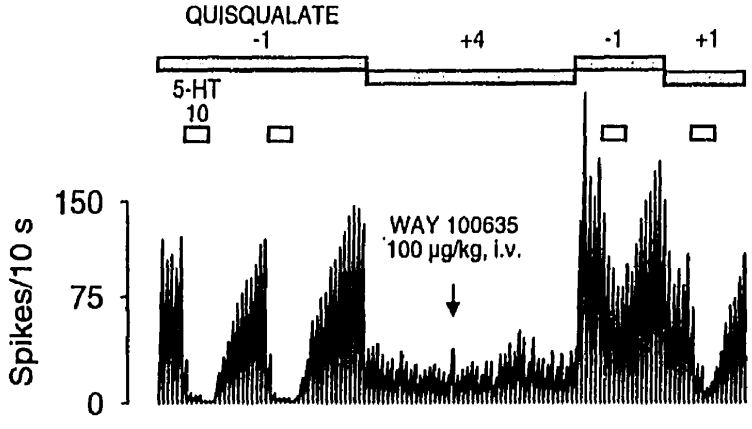
Fig. 4


A CONTROL
LSD
10 µg/kg, i.v.
WAY 100635
100 µg/kg, i.v.
Spikes/10 s
30
15
0
B BEFLOXATONE x 2 DAYS
LSD (µg/kg,i.v.)
10
10
10
Spikes/10 s
30
15
0
1 min
WAY 100635
100 µg/kg, i.v.
C
Degree (% ± S.E.M.) of suppression of firing activity of 5-HT neurons by LSD
100
75
50
25
0
*
9
8
8
CONTROL
BEFLOXATONE x 2 DAYS
BEFLOXATONE x 21 DAYS

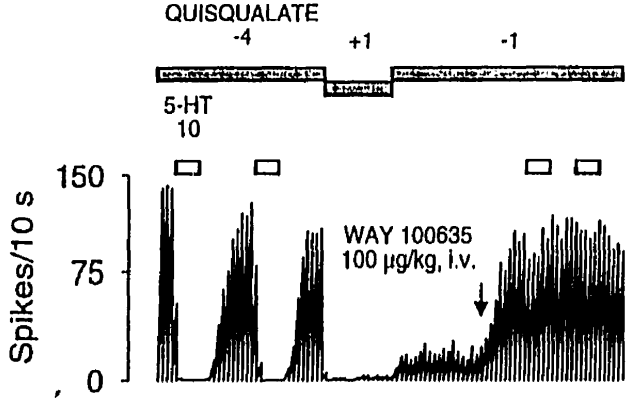

A
CONTROL
5-HT
5
8-OH-DPAT
10
Spikes/10 s
30
15
0
1 min
B
BEFLOXATONE x 2 DAYS
5-HT
5
8-OH-DPAT
10
Spikes/10 s
30
15
0
1 min
C
BEFLOXATONE X 21 DAYS
5-HT
5
8-OH-DPAT
10
Spikes/10 s
30
15
0
1 min
LSD
10 µg/kg, i.v.
WAY 100635
100 µg/kg, i.v.
D
5-HT
8-OH-DPAT
SPIKES SUPPRESSED / nA (± S.E.M.)
10
8
6
4
2
0
15
16
13
*
CONTROL
BEFLOXATONE
(0.75 mg/kg/day, s.c.x 2 days)
BEFLOXATONE
(0.75 mg/kg/day, s.c.x 21 days)


A
CONTROL
CLONIDINE
(10 µg/kg, i.v.)
30
15
0
Spikes/10 s
B
BEFLOXATONE x 21 DAYS
30
15
0
Spikes/10 s
CLONIDINE
(10 µg/kg, i.v.)
WAY 100635
(100 µg/kg, i.v.)
1 min
LSD
(10 µg/kg, i.v.)
C
100
80
60
40
20
0
Degree (% ± S.E.M.) of suppression of firing of 5-HT neurons by CLONIDINE
6
6
*
CONTROL
BEFLOXATONE
(0.75 mg/kg/day,s.c. x 21 days)

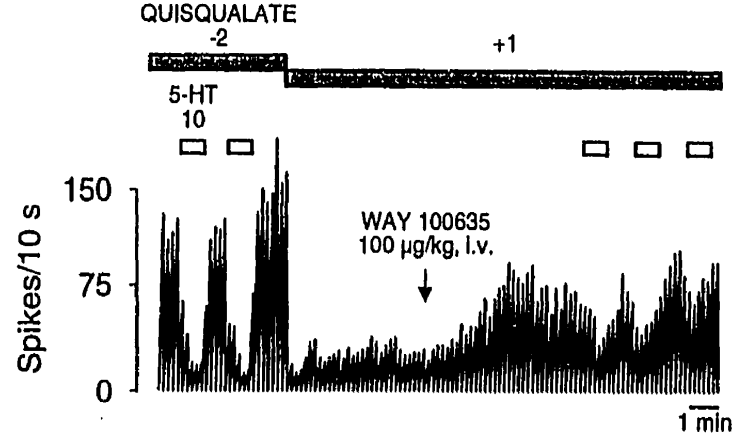
A CONTROL



B BEFLOXATONE x 2 DAYS



C BEFLOXATONE + (-)PINDOLOL x 2 DAYS



D

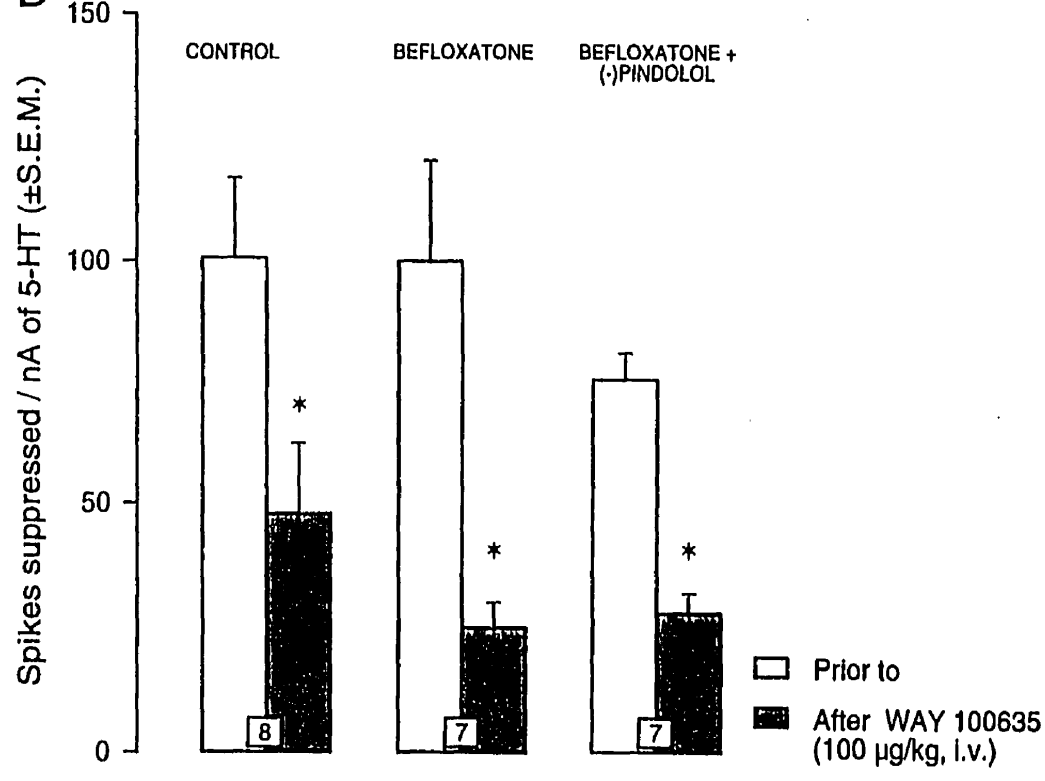


Fig. 5

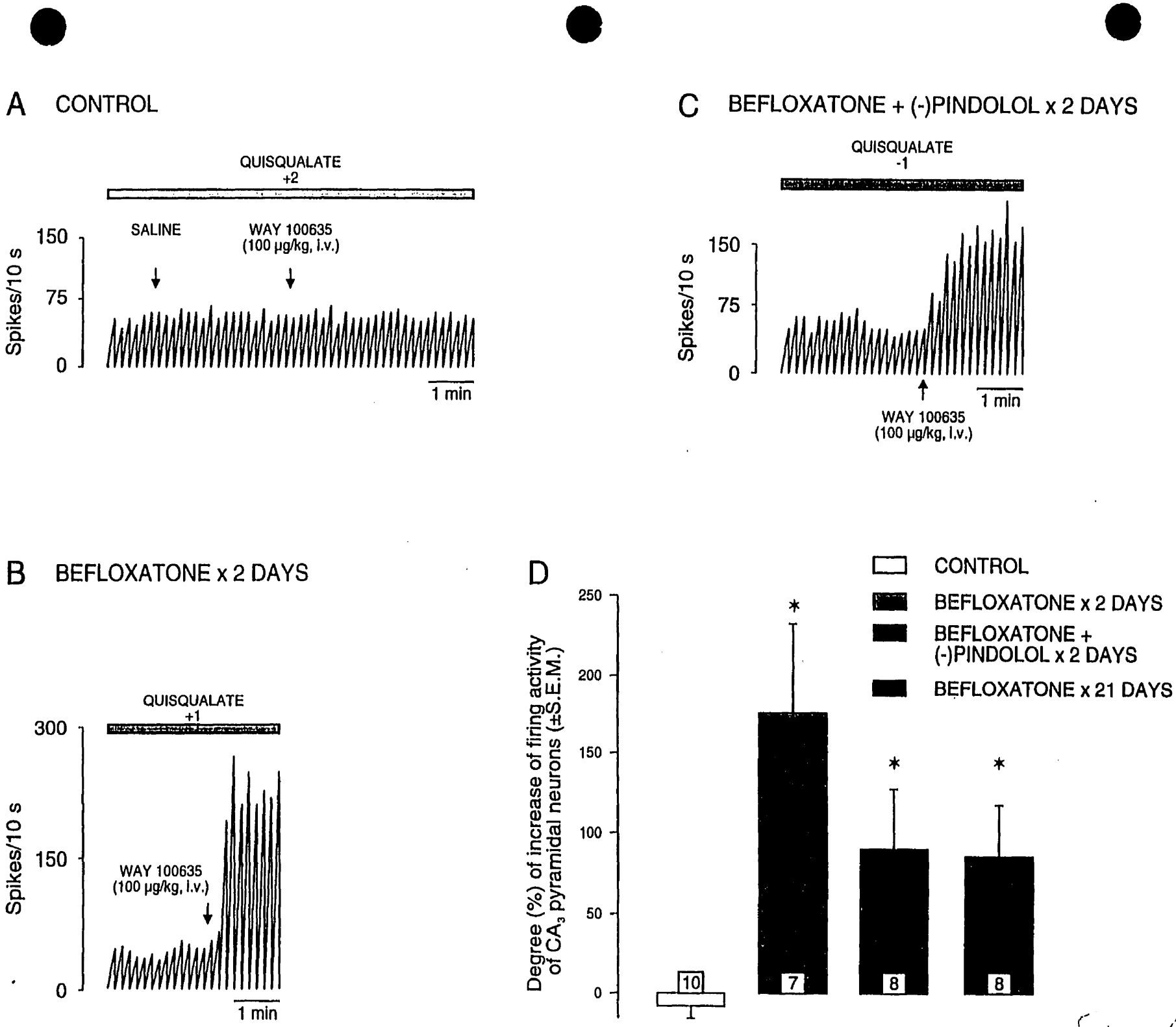
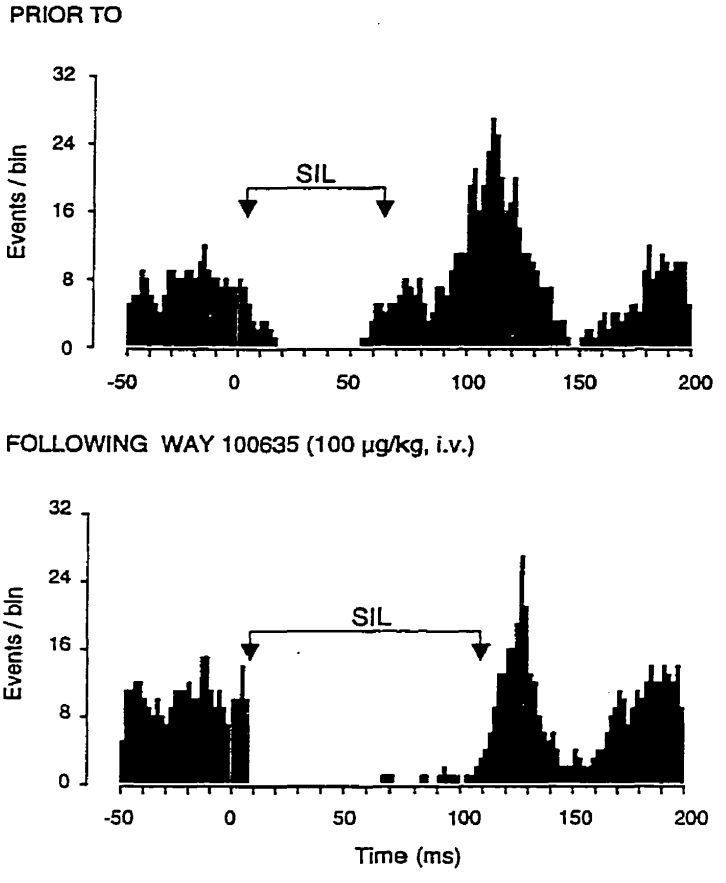
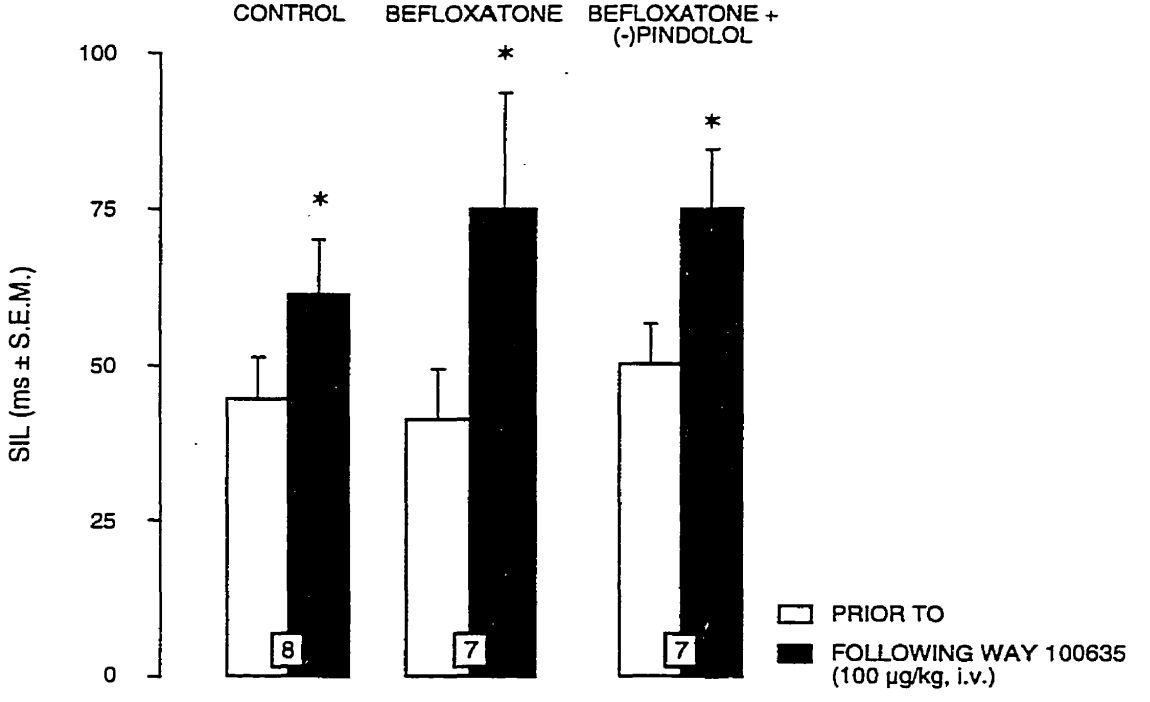

A CONTROL
QUISQUALATE
+2
SALINE
WAY 100635
(100 µg/kg, i.v.)
Spikes/10 s
150
75
0
1 min
B BEFLOXATONE x 2 DAYS
QUISQUALATE
+1
300
150
0
WAY 100635
(100 µg/kg, i.v.)
Spikes/10 s
1 min
C BEFLOXATONE + (-)PINDOLOL x 2 DAYS
QUISQUALATE
-1
150
75
0
Spikes/10 s
1 min
WAY 100635
(100 µg/kg, i.v.)
D
CONTROL
BEFLOXATONE x 2 DAYS
BEFLOXATONE +
(-)PINDOLOL x 2 DAYS
BEFLOXATONE x 21 DAYS
Degree (%) of increase of firing activity
of CA3 pyramidal neurons (±S.E.M.)
250
200
150
100
50
0
10
7
8
8
*
*
*

Fig. 6

A


PRIOR TO
Events / bin
32
24
16
8
0
SIL
-50
0
50
100
150
200
FOLLOWING WAY 100635 (100 µg/kg, i.v.)
Events / bin
32
24
16
8
0
SIL
-50
0
50
100
150
200
Time (ms)

B


CONTROL
BEFLOXATONE
BEFLOXATONE + (-)PINDOLOL
SIL (ms ± S.E.M.)
100
75
50
25
0
*
*
*
8
7
7
PRIOR TO
FOLLOWING WAY 100635 (100 µg/kg, i.v.)

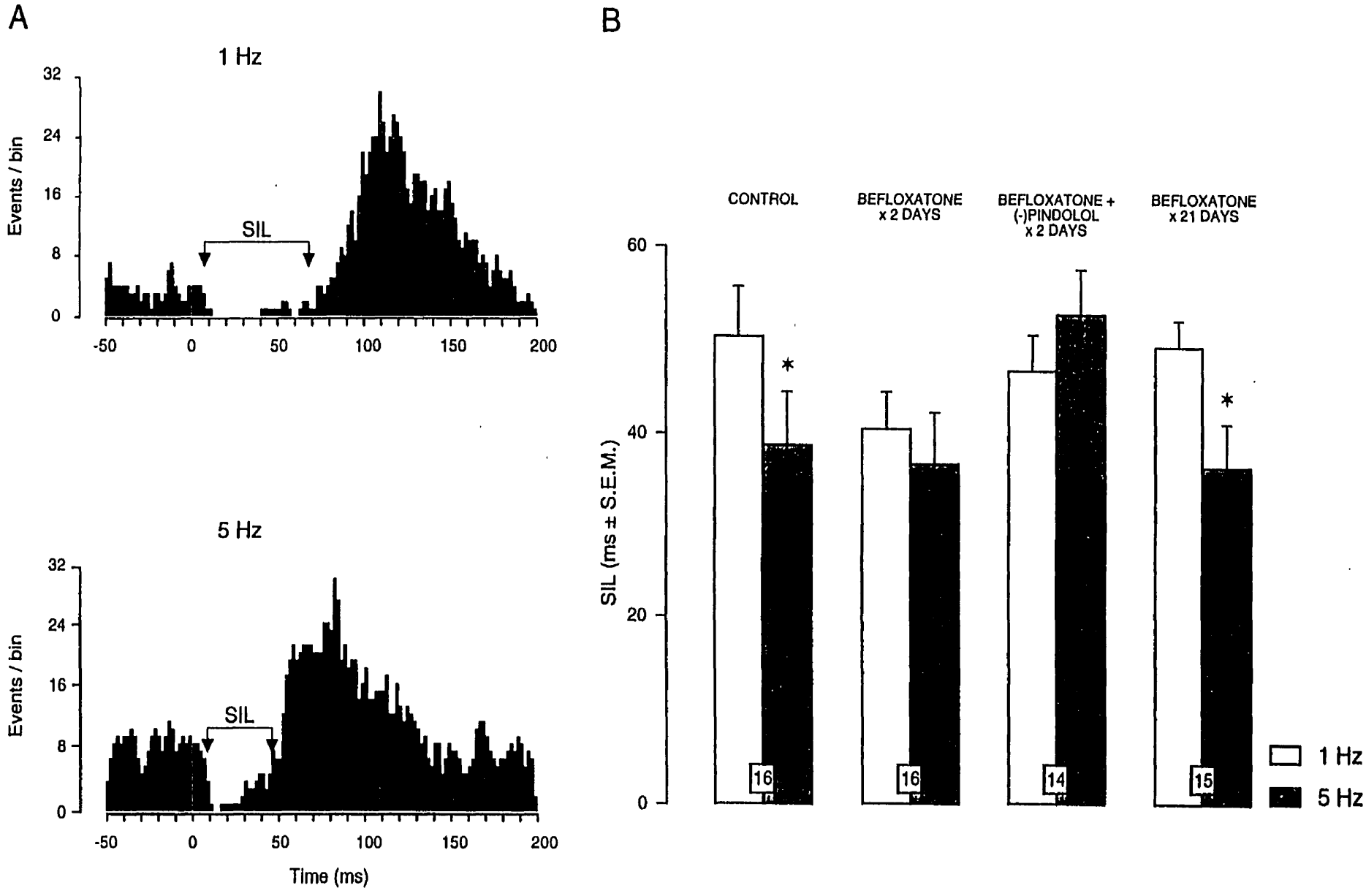

A
1 Hz
Events / bin
32
24
16
8
0
SIL
-50
0
50
100
150
200
5 Hz
Time (ms)
B
CONTROL
BEFLOXATONE x 2 DAYS
BEFLOXATONE + (-)PINDOLOL x 2 DAYS
BEFLOXATONE x 21 DAYS
SIL (ms ± S.E.M.)
60
40
20
0
16
16
14
15
1 Hz
5 Hz

Fig 8

Table I: Brain concentrations of monoamines in control and befloxatone (0.75 mg/kg/day, s.c.) treated rats
Results represent the means ± S.E.M. obtained from group of 6-7 animals.
% of variation vs control values were give in parenthese. * $p<0.05$, ** $p<0.01$, using Dunett's test:

	NE	DA	5-HT
	ng/g of tissue		
Control	328 ± 17	1003 ± 34	810 ± 45
Befloxatone 2 Days	472 ± 33 ** (+ 43 %)	1384 ± 58 ** (+ 38 %)	1236 ± 56 ** (+ 52 %)
21 Days	436 ± 25 * (+ 33 %)	1373 ± 58 ** (+ 37 %)	1119 ± 77 ** (+ 38 %)

Chapter VI: Fifth article

Intermodulations of NA and 5-HT systems:

This study was undertaken in an attempt to further characterize the interactions between 5-HT and NE systems. An important drawback of the results obtained with various pharmacological agents to study the 5-HT-NE interactions is the lack of selectivity of the drugs used. WAY 100635 (*N*-{2-[4(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydroxychloride), on the other hand, has been shown to be a potent and selective antagonist of both pre- and postsynaptic 5-HT_{1A} receptors. This study was undertaken to characterize the effects of WAY 100635, using *in vivo* electrophysiological paradigms. The effects of WAY 100635, administered locally or intravenously, on the suppressant effect of microiontophoretically-applied 5-HT on the firing activity of CA_3 dorsal hippocampus pyramidal neurons and on the spontaneous firing activity of LC NE neurons were assessed in an attempt to further characterize the 5-HT modulation of the NE system.

This article entitle "*Modulation of the firing activity of rat locus coeruleus norepinephrine neurons by the serotonin system.*" by myself, Claude de Montigny and Pierre Blier was published in British Journal of Pharmacology (1997, vol. 120, pp. 865-875).

Modulation of the firing activity of noradrenergic neurones in the rat locus coeruleus by the 5-hydroxtryptamine system

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1 The aim of the present study was to investigate the putative modulation of locus coeruleus (LC) noradrenergic (NA) neurones by the 5-hydroxytryptaminergic (5-HT) system by use of *in vivo* extracellular unitary recordings and microiontophoresis in anaesthetized rats. To this end, the potent and selective 5-HT_{1A} receptor antagonist WAY 100635 (*N*-{2-[4(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydroxychloride) was used.

2 In the dorsal hippocampus, both local (by microiontophoresis, 20 nA) and systemic (100 $\mu g\ kg^{-1}$, i.v.) administration of WAY 100635 antagonized the suppressant effect of microiontophorectically-applied 5-HT on the firing activity of CA_3 pyramidal neurones, indicating its antagonistic effect on postsynaptic 5-HT_{1A} receptors.

3 WAY 100635 and 5-HT failed to modify the spontaneous firing activity of LC NA neurones when applied by microiontophoresis. However, the intravenous injection of WAY 100635 (100 $\mu g\ kg^{-1}$) readily suppressed the spontaneous firing activity of LC NA neurones.

4 The lesion of 5-HT neurones with the neurotoxin 5,7-dihydroxytryptamine increased the spontaneous firing activity of LC NA neurones and abolished the suppressant effect of WAY 100635 on the firing activity of LC NA neurones.

5 In order to determine the nature of the 5-HT receptor subtypes mediating the suppressant effect of WAY 100635 on NA neurone firing activity, several 5-HT receptor antagonists were used. The selective 5-HT_3 receptor antagonist BRL 46470A (10 and 100 $\mu g\ kg^{-1}$, i.v.), the 5-HT_{1D} receptor antagonist GR 127935 (100 $\mu g\ kg^{-1}$, i.v.) and the 5-$HT_{1A/1B}$ receptor antagonist (−)-pindolol (15 $mg\ kg^{-1}$, i.p.) did not prevent the suppressant effect of WAY 100635 on the firing activity of LC NA neurones. However, the suppressant effect of WAY 100635 was prevented by the non-selective 5-HT receptor antagonists spiperone (1 $mg\ kg^{-1}$, i.v.) and metergoline (1 $mg\ kg^{-1}$, i.v.), by the 5-HT_2 receptor antagonist ritanserin (500 $\mu g\ kg^{-1}$, i.v.). It was also prevented by the 5-HT_{1A} receptor/α_{1D}-adrenoceptor antagonist BMY 7378 (1 $mg\ kg^{-1}$, i.v.) and by the α_1-adrenoceptor antagonist prazosin (100 $\mu g\ kg^{-1}$, i.v.).

6 These data support the notion that the 5-HT system tonically modulates NA neurotransmission since the lesion of 5-HT neurones enhanced the LC NA neurones firing activity and the suppressant effect of WAY 100635 on the firing activity of NA neurones was abolished by this lesion. However, the location of the 5-HT_{1A} receptors involved in this complex circuitry remains to be elucidated. It is concluded that the suppressant effect of WAY 100635 on the firing activity of LC NA neurones is due to an enhancement of the function of 5-HT neurones via a presynaptic 5-HT_{1A} receptor. In contrast, the postsynaptic 5-HT receptor mediating this effect of WAY 100635 on NA neurones appears to be of the 5-HT_{2A} subtype.

Keywords: Extracellular unitary recordings; microiontophoresis; presynaptic modulation; 5-HT receptors; dorsal hippocampus; locus coeruleus; WAY 100635

Introduction

It is well established that noradrenergic (NA) neurones modulate the 5-hydroxytryptaminergic (5-HT) system. Dorsal raphe 5-HT neurones receive adrenergic projections from the locus coeruleus (LC) (Loizou, 1969; Anderson *et al.*, 1977; Baraban & Aghajanian, 1981; Jones & Yang, 1985; Luppi *et al.*, 1995), and pharmacological studies have suggested that the firing activity of 5-HT neurones in the dorsal raphe is dependent on a tonic activation by a noradrenergic input mediated via α_1-adrenoceptors (Svensson *et al.*, 1975; Baraban & Aghajanian, 1980; Clement *et al.*, 1992). Yoshioka *et al.* (1992) have shown that α_2-adrenoceptor activation reduces 5-HT synthesis in both hippocampus and dorsal raphe nucleus. On the other hand, several lines of evidence support the notion that the 5-HT system also influences brain NA neurones. NA neurones of the LC receive dense 5-HT projections (Pickel *et al.*, 1977; Cedarbaum & Aghajanian, 1978; Léger & Descarries, 1978; Segal, 1979; Maeda *et al.*, 1991; Vertes & Kocsis, 1994), which do not originate from the dorsal raphe nucleus (Pieribone *et al.*, 1989; Aston-Jones *et al.*, 1991a). Electrophysiological and biochemical studies have revealed an inhibitory role of 5-HT on the function of LC NA neurones. In particular, although microiontophoretic application of 5-HT agonists does not modify the spontaneous firing activity of LC NA neurones, the activation of 5-HT_1 receptors reduces both glutamate-induced activation and glutamatergic synaptic potentials of these NA neurones (Bobker & Williams, 1989; Aston-Jones *et al.*, 1991b). However, in contrast to 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), partial 5-HT_{1A} agonists, such as buspirone, gepirone, ipsapirone and their common metabolite 1-(2-pyrimidinyl)-piperazine, which is an α_2-adrenoceptor antagonist, have been shown to increase LC neurone firing activity (Sanghera *et al.*, 1983; 1990; Engberg, 1992). Systemic but not local administration of selective 5-HT_2 receptor antagonists increase (Rasmussen & Aghajanian, 1986; Gorea & Adrien, 1988; Aghajanian *et al.*, 1990) whereas the 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane (DOI), administered systemically but not locally, de-

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creases the firing activity of LC NA neurones. The latter effect has been proposed to be due to an increased activation of the GABA inhibitory input to the LC (Chiang & Aston-Jones, 1993). Taken together, these data suggest an indirect effect of 5-HT_2 receptor ligands. Raphe nuclei lesions induce an increase of the tyrosine hydroxylase activity in LC (McRae-Degueurce *et al.*, 1982) and pretreatment with the 5-HT synthesis inhibitor parachlorophenylalanine increases both tyrosine hydroxylase and the firing activities of LC NA neurones (Crespi 1980; Reader 1986). Furthermore, several studies have demonstrated, *in vivo* and *in vitro*, 5-HT modulations of the release of NA (Blandina *et al.*, 1991; Done & Sharp, 1992; Mongeau *et al.*, 1994; Matsumoto *et al.*, 1995).

An important drawback of the results obtained with various pharmacological agents to study the 5-HT-NA interactions is the lack of selectivity of the drugs used. WAY 100635 (*N*-{2-[4(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl) cyclohexanecarboxamide trihydroxychloride), has been shown to be a potent and selective antagonist of both pre- and postsynaptic 5-HT_{1A} receptors (Fletcher *et al.*, 1994; 1996; Fornal *et al.*, 1994; Khawaja *et al.*, 1994; Gurling *et al.*, 1994; Golzan *et al.*, 1995; Mundey *et al.*, 1994; 1995). The present studies were undertaken to characterize the effects of WAY 100635 by use of *in vivo* electrophysiological paradigms, i.e. extracellular unitary recording and microiontophoresis in anaesthetized male Sprague-Dawley rats on the firing activity of LC NA neurones. The effects of WAY 100635, administered locally or intravenously, on the spontaneous firing activity of LC NA neurones were assessed in an attempt to characterize further the 5-HT modulation of the noradrenergic system.

Methods

The experiments were carried out in male Sprague-Dawley rats weighing 250 to 300 g which were kept under standard laboratory conditions (12:12 light-dark cycle with free access to food and water). They were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.) and additional doses were given to maintain constant anaesthesia, monitored by the absence of nociceptive reaction to a tail pinch.

Extracellular unitary recording from CA_3 dorsal hippocampus pyramidal neurones

Recording and microiontophoresis were performed with five-barrelled glass micropipettes broken back to 8–12 μm under microscope control (ASI Instruments, Warren, MI, U.S.A.). The central barrel was filled with a 2 M NaCl solution and used for extracellular unitary recordings. The pyramidal neurones were identified by their large amplitude (0.5–1.2 mV) and long-duration (0.8–1.2 ms) simple spikes alternating with complex spike discharges (Kandel & Spencer, 1961). The side barrels contained the following solutions: 5-HT creatinine sulphate (20 mM in 200 mM NaCl, pH 4), WAY 100635 (10 mM in 200 mM NaCl, pH 3.8), quisqualate (1.5 mM in 200 mM NaCl, pH 8) and 2 M NaCl used for automatic current balancing. The rats were mounted in a stereotaxic apparatus and the micropipettes were lowered at 4.2 mm lateral and 4.2 anterior to lambda into the CA_3 region of the dorsal hippocampus. Since most hippocampus pyramidal neurones are not spontaneously active under chloral hydrate anaesthesia, a leak or a small ejection current of quisqualate (+1 to −6 nA) was used to activate them within their physiological firing range (10–15 Hz; Ranck, 1975). Neuronal responsiveness to the microinotophoretic application of 5-HT was assessed by determining the number of spikes suppressed per nA. The duration of the microiontophoretic applications of the agonist was 50 s. The same ejection current of 5-HT was always used before and during the microiontophoretic application of WAY 100635 for a duration of about 5 min. In order to assess the effectiveness of the lesion of 5-HT neurones with the neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), the recovery time 50 (RT_{50}) method was used. The RT_{50} value represents the time (in s.) required by the neurone to recover 50% of its initial firing rate from the end of the microiontophoretic application of 5-HT. The RT_{50} value has been shown to be a reliable index of the *in vivo* activity of the 5-HT reuptake process in the rat hippocampus (Piñeyro *et al.*, 1994). For instance, in 5,7-DHT-lesioned rats, according to the current of 5-HT used, the RT_{50} value is 2 to 3 times greater than in control rats (Piñeyro *et al.*, 1994).

Microiontophoresis and unitary extracellular recordings from LC noradrenergic neurones

The microiontophoresis were performed with five-barrelled micropipettes (R & D Scientific glass CO, Spencerville, MD, U.S.A.) preloaded with fibreglass filaments in order to facilitate filling and the tip was broken back to 4 to 8 μm. The central barrel was used for recording and filled with a 2 M NaCl solution. The side barrels contained the following solutions: 5-HT creatinine sulphate (20 mM in 200 mM NaCl, pH 4), noradrenaline bitartrate (20 mM in 100 mM NaCl, pH 4), WAY 100635 (10 mM in 200 mM NaCl, pH 3.8) and 2 M NaCl used for automatic current balancing. The duration of the microiontophoretic applications of the drugs were 40 s. Locus coeruleus NA neurones were recorded with micropipettes lowered at −0.7 mm interaural and 1.1. to 1.4 mm lateral (Paxinos & Watson, 1982). The NA neurones were identified by their regular firing rate (1–5 Hz), long-duration (0.8–1.2 ms) positive action potentials and their characteristic burst discharge in response to nociceptive pinch of the contralateral hind paw (Aghajanian, 1978).

By unitary extracellular recording with single-barrelled glass micropipettes, the responsiveness of LC NA neurones to the intravenous injection of WAY 100635 (100 μg kg^{-1}) was assessed alone and after the following drugs: the selective 5-HT_3 antagonist BRL 46470A (10 and 100 μg kg^{-1}, i.v.; Newberry *et al.*, 1993), the 5-$HT_{1B/1D}$ antagonist GR 127935 (100 μg kg^{-1}, i.v.; Skingle *et al.*, 1993), the 5-HT_2 antagonist ritanserin (500 μg kg^{-1}, i.v.; Leysen *et al.*, 1985), the 5-HT_{1A} receptor α_{1D}-adrenoceptor antagonist BMY 7378 (1 mg kg^{-1}, i.v.; Chaput & de Montigny, 1988) and the 5-$HT_{1A/1B}$ antagonist (−)-pindolol (15 mg kg^{-1}, i.p.; Romero *et al.*, 1996), the non-selective 5-HT antagonists spiperone (1 mg kg^{-1}, i.v.; Griebel, 1995) and metergoline (1 mg kg^{-1}, i.v.; Fuxe *et al.*, 1975), and the α_1-adrenoceptor antagonist prazosin (0.1 mg kg^{-1}, i.v.; Marwaha & Aghajanian, 1982). The effect of WAY 100635 (100 μg kg^{-1}, i.v.) on the spontaneous firing rate of NA neurones was also assessed after a pretreatment with 5,7-DHT to lesion 5-HT neurones. The latter was performed under chloral hydrate anaesthesia by injecting 5,7-DHT intracerebroventricularly (200 μg free base in 20 μl of 0.9% NaCl and 0.1% ascorbic acid) 1 h after the injection of desipramine (25 mg kg^{-1}, i.p.) to protect NA neurones from the neurotoxic action of 5,7-DHT. The rats were tested 10 days later. In order to determine possible changes of the spontaneous firing activity of LC NA neurones, four to five electrode descents were carried out through this nucleus in control and 5,7-DHT-pretreated rats.

Drugs

WAY 100635 (Wyeth Research, Berkshire, U.K.); clonidine, NA bitartrate, quisqualic acid, 5,7-DHT creatinine sulphate (Sigma Chemical, St. Louis, MO, U.S.A.); BMY 7378 (8-[2-[4-(2-methyoxyphenyl)-1-piperazinyl]ethyl-8-azapirol[4.5]-decane-7.9-dione dihydrochloride; Bristol-Myers Squibb, Wallingford, CT, U.S.A.); metergoline (Farmitalia, Milano, Italia); ritanserin, spiperone, (−)-pindolol and desipramine HCl (Research Biochemicals, Natick, MA, U.S.A.); GR 127935 (*N*-[methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1,-biphenyl]-4-carboxamide; Glaxo Research, Greenford, U.K.); BRL 46470A ((endo-N-methyl-8-azabicyclo[3.2.1]oct-3yl)-2, 3-dihy-

dro-3, 3-dimethyl-indole-1-carboxamide; Smith Kline Beecham, Harlow, U.K.); (±)-mirtazapine (Organon, Oss, The Netherlands); prazosin HCl (Pfizer, Kirkland, Canada). The concentrations and the doses used for these compounds were chosen on the basis of previous successful experiments carried out in our and other laboratories.

Results

Effect of WAY 100635 on the responsiveness of CA_3 dorsal hippocampus pyramidal neurones to 5-HT

In an initial series of experiments, the capacity of WAY 100635 to block postsynaptic 5-HT_{1A} receptors was verified. It has been previously demonstrated *in vivo* that the microiontophoretic application of 5-HT onto rat dorsal hippocampus pyramidal neurones produces a suppressant effect on their firing activity and that this effect is mediated by postsynaptic 5-HT_{1A} receptors (Blier & de Montigny, 1987; Chaput & Montigny, 1988). When applied on dorsal hippocampus CA_3 pyramidal neurones, 5-HT induced a current-dependent (5–10 nA) reduction of firing activity, generally from 30 to 100% (Figure 1a and b). This suppressant effect occurred in the absence of alteration of the shape of the action potential. In the present study, the systemic administration of WAY 100635 (100 $\mu g\ kg^{-1}$, i.v.) significantly reduced the suppressant effect of 5-HT, applied microiontophoretically, on the firing activity of CA_3 hippocampus pyramidal neurones (spikes suppressed/nA of 5-HT before WAY 100635 = 101 ± 16; after WAY 100635 injection = 48 ± 14; $P<0.05$ by paired Student's t test, $n=8$). As illustrated in Figure 1a, the microiontophoretic application of WAY 100635 (20 nA) did not reduce by itself the firing activity of these neurones, but it attenuated the suppressant effect of 5-HT co-applied through the same micropipette. Figure 1b shows the mean reduction (31%) of the suppressant effect of 5-HT by the concomitant microiontophoretic application of WAY 100635 (20 nA). This confirms the 5-HT_{1A} antagonistic activity of WAY 100635 at postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus.

Effect of WAY 100635 on the firing activity of LC noradrenergic neurones

The effect of WAY 100635 administered locally and systemically was then investigated on electrophysiologically-identified LC NA neurones. As illustrated in Figures 2a and 7a, WAY 100635 (100 $\mu g\ kg^{-1}$, i.v.) reduced the firing activity of LC NA neurones. This suppressant effect of WAY 100635 on the firing activity of LC NA neurones always appeared with a short latency (<60 s).

In order to determine whether the suppressant effect of WAY 100635 on the spontaneous firing rate of the LC NA neurones was due to direct action of this compound on postsynaptic 5-HT receptors in the immediate vicinity of the cell body of NA neurones, this 5-HT_{1A} antagonist was applied by microiontophoresis onto these neurones. As illustrated in Fig-

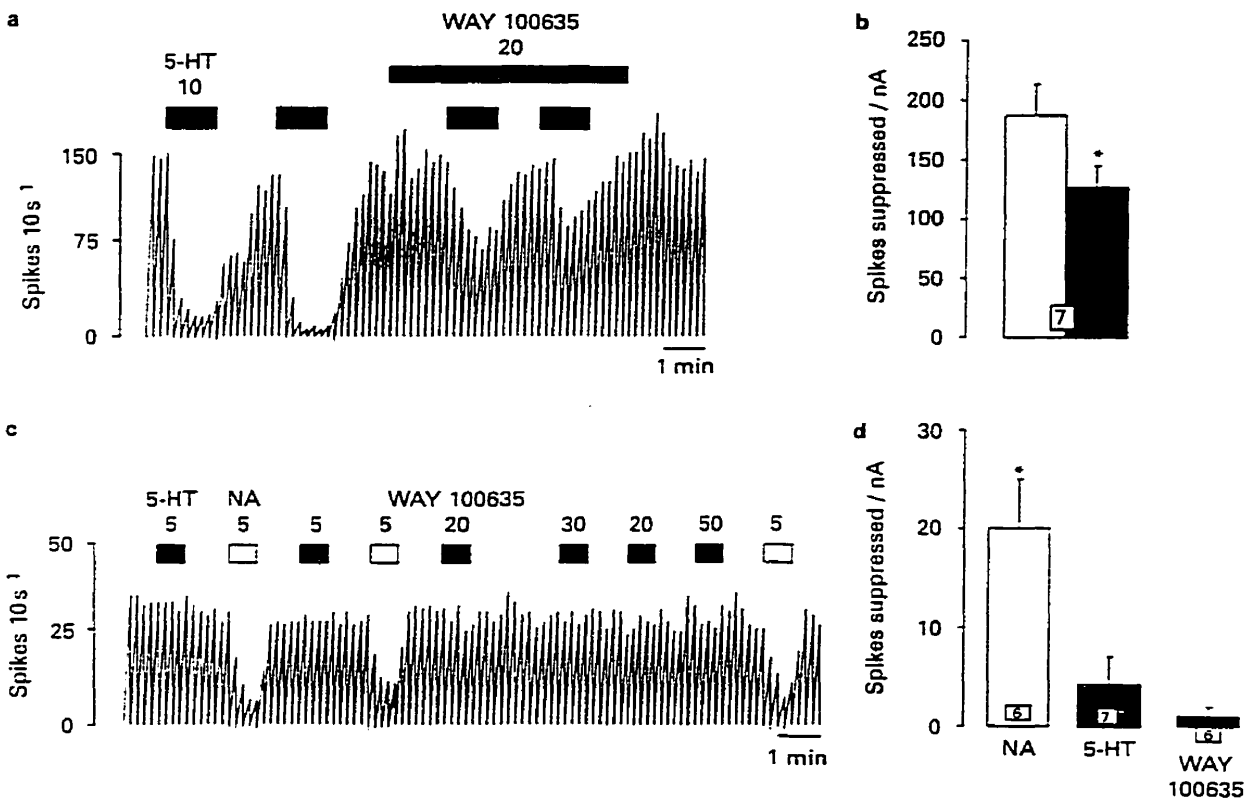

Figure 1 (a) Integrated firing rate histograms of the dorsal hippocampus CA_3 pyramidal neurone showing its responsiveness to microiontophoretic application of 5-HT before and during the local administration of WAY 100635. This neurone was activated with quisqualate ejection current of −2 nA. Horizontal bars indicate the duration of the applications for which the current is given in nA. Corresponding results (mean ± s.e.mean) are presented in (b); responses to 5-HT (open column) before and (stippled column) after the application of WAY 100635. The numbers at the bottom of the columns indicate the number of neurones tested. *$P<0.05$, by paired Student's t test. (c) Integrated firing rate histograms of LC noradrenergic neurone showing its responsiveness to microiontophoretic application of NA, 5-HT and WAY 100635. The firing activity of this neurone was spontaneous. Horizontal bars indicate the duration of the applications for which the current is given in nA. Corresponding results (means ± s.e.mean) are presented in (d). Note that only the microiontophoretic application of NA significantly modified the firing activity of LC neurones. *$P<0.05$, by unpaired Student's t test (Comparing the number of spikes in the 40 s period immediately preceding the microiontophoretic application and the number of spikes during the application).

ures 1c and d), the spontaneous firing activity of LC NA neurones was not altered by the microiontophoretic application of WAY 100635. Furthermore, 5-HT applied microiontophoretically did not modify the firing activity of LC NA neurones whereas NA reduced it (Figure 1c and d).

In order to determine a possible involvement of 5-HT neurones in the suppressant effect of WAY 100635 on the firing activity of LC NA neurones, the lesioning of 5-HT neurones was performed with the neurotoxin 5,7-DHT. As illustrated in Figure 2, the suppressant effect of WAY 100635 was markedly attenuated by the 5,7-DHT pretreatment. In 5,7-DHT-lesioned rats, the suppressant effect of WAY 100635 on the spontaneous firing activity of LC NA neurones was reduced by 63% (Figure 2c). Moreover, the increased firing activity observed after 5,7-DHT pretreatment could not account for the reduced suppressant effect of WAY 100635 ($P>0.05$, by ANCOVA).

In each 5,7-DHT-lesioned rat, the effectiveness of the 5,7-DHT pretreatment was verified by use of the RT_{50} method in the dorsal hippocampus. As illustrated in Figure 3, 5-HT denervation markedly prolonged the effect of 5-HT microiontophorectically-applied onto dorsal hippocampus CA_3 pyramidal neurones: the RT_{50} value was increased by 488% in 5,7-DHT-lesioned rats, thus confirming a thorough destruction of 5-HT neurones.

In order to determine the spontaneous firing activity of LC NA neurones, four to five electrode descents through the LC were carried out in each of the control and 5,7-DHT-pretreated rats. As illustrated in Figure 4, the lesioning of 5-HT neurones produced a 67% increase in the firing activity of LC NA neurones.

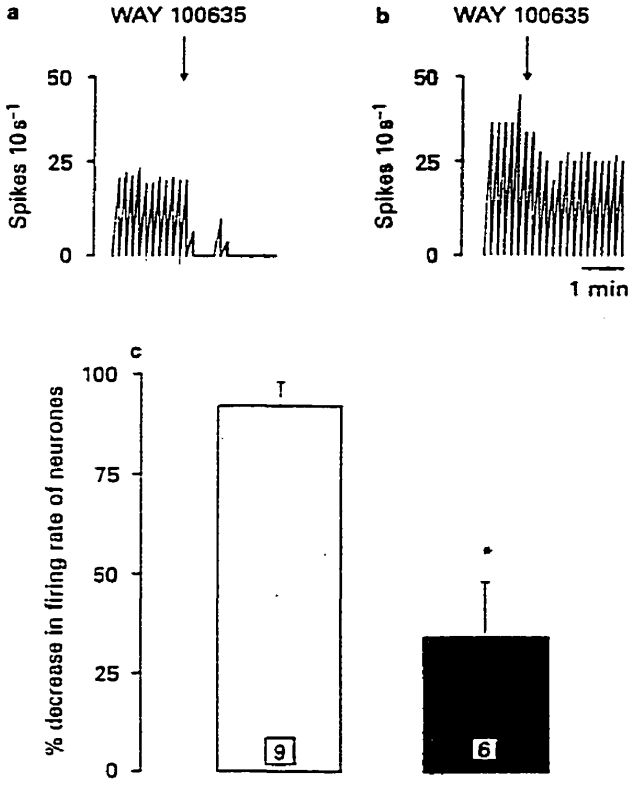

Figure 2 Integrated firing rate histogram of noradrenergic neurones recorded in the LC showing their responses to WAY 100635 (100 $\mu g\ kg^{-1}$, i.v.) in a sham-operated rat (a) and in a 5,7-DHT pretreated rat (b). (c) Responsiveness of noradrenergic neurones to WAY 100635 (100 $\mu g\ kg^{-1}$, i.v.) in control (open columns) and 5,7-DHT-pretreated (stippled column) rats (means±s.e.mean). The numbers at the bottom of the columns indicate the number of neurones tested. *$P<0.05$, by unpaired Student's t test.

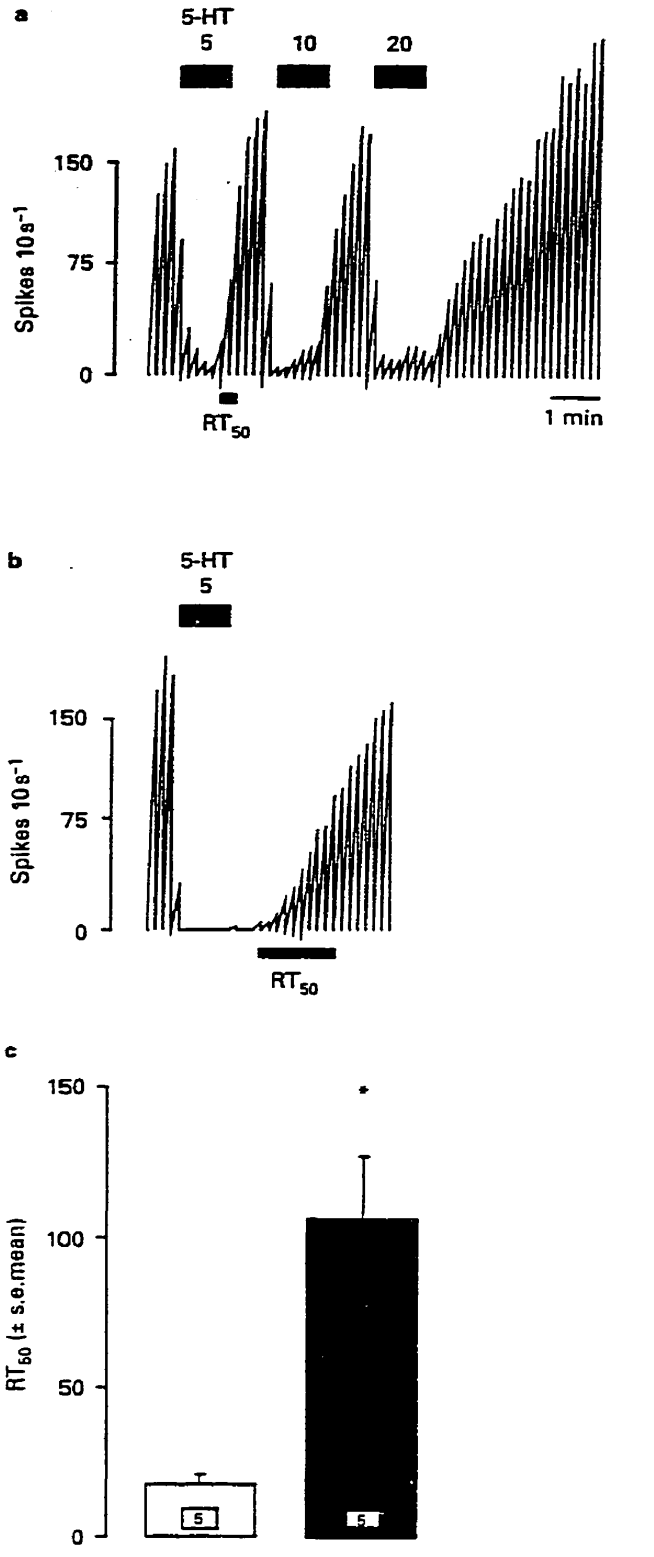

Figure 3 Integrated firing rate histograms of two dorsal hippocampus CA_3 pyramidal neurones in sham operated (a) and 5,7-DHT pretreated (b) rats showing their responsiveness to microiontophoretic application of 5-HT. These neurones were activated with quisqualate ejection current of −1 and −3 nA, respectively. The solid horizontal bars indicate the duration of the applications for which the current is given in nA and the hatched bars indicated RT_{50} (see Methods section). For 5 nA applications of 5-HT, corresponding results (mean±s.e.mean) are presented (c); (open column) sham operated rats, (stippled column) 5,7-DHT pretreated rats. The number at the bottom of the columns indicate the number of neurones tested, one neurone in each rat. *$P<0.05$, by unpaired Student's t test.

Effects of 5-HT antagonists on the suppression of the firing activity of LC noradrenergic neurones by WAY 100635

Given that the suppressant effect of WAY 100635 on the firing activity of LC NA neurones was prevented by lesioning 5-HT neurones, the nature of the presynaptic 5-HT receptor involved was then investigated with 5-HT antagonists. As illustrated in Figure 5(a, b and c), the selective 5-HT_3 receptor antagonist BRL 46470A (10 and 100 $\mu g\ kg^{-1}$, i.v.) and the 5-$HT_{1B/1D}$ receptor antagonist GR 127935 (100 $\mu g\ kg^{-1}$, i.v.) did not modify by themselves the spontaneous firing activity of LC NA neurones and failed to alter the suppressant effect of WAY 100635 (100 $\mu g\ kg^{-1}$, i.v.) on the firing activity of LC NA neurones (Figure 5d). Figure 6 illustrates the effects of pretreatment, with the 5-HT receptor antagonists ritanserin, metergoline and spiperone. As shown by Rasmussen and Aghajanian (1986), the 5-HT_2 receptor antagonist ritanserin (500 $\mu g\ kg^{-1}$, i.v.) increased the firing activity of LC NA neurones by 20±6%, ($P<0.05$, $n=7$), although not all neurones were affected (see Figure 6a). Ritanserin blocked the suppressant effect of WAY 100635 on the firing activity of these neurones (Figure 6a and d). The 5-$HT_{1/2}$ antagonist metergoline (1 mg kg^{-1}, i.v.) by itself, but not the 5-$HT_{1A/2A}$ antagonist spiperone (1 mg kg^{-1}, i.v.), increased the firing activity of LC NA neurones, by 26±8% ($P<0.05$, $n=7$, data not shown), and both drugs reduced the suppressant effect of WAY 100635 on the firing activity of these neurones. The mean antagonistic effects of ritanserin, metergoline and spiperone are presented in Figure 6d. The 5-HT_{1A} antagonist BMY 7378 increased the firing activity of LC NA neurones and prevents the suppressant effect of (−)-mirtazapine, an α_2-heteroreceptor antagonist, on these neurones (Haddjeri *et al.*, 1996). In addition, BMY 7378 inhibits the firing activity of dorsal raphe 5-HT neurones with an ED_{50} of about 20 $\mu g\ kg^{-1}$, i.v. (Chaput & de Montigny, 1988; Cox *et al.*, 1993) and reduces the release of 5-HT in the ventral hippocampus (Hjorth *et al.*, 1995). In the present study, the suppressant effect of WAY 100635 was both prevented (Figure 7c and d) and reversed (Figure 5b) by BMY 7378 (1 mg kg^{-1}, i.v.). Pretreatment with either WAY 100635 or BMY 7378 did not modify the suppressant effect of the α_2-adrenoceptor agonist clonidine, showing that these two 5-HT antagonists did not block the somatodendritic α_2-autoreceptor (Figure 7c). In order to determine whether the suppressant effect of WAY 100635 on the firing activity of LC NA neurones was due to an antagonism of the cell body 5-HT_{1A} autoreceptors of 5-HT neurones, (−)-pindolol, an effective antagonist of these receptors (Romero *et al.*, 1996), was used. (−)-Pindolol (15 mg kg^{-1}, i.p., administered 20 min before the recording from LC NA neurones) appeared to reduce by itself the firing activity of LC NA neurones; 4 of the 6 neurones recorded had a firing activity below 1 Hz (1.2±0.2 Hz, $n=6$) (Figure 7b). However, (−)-pindolol failed to alter the suppressant effect of WAY 100635 on the firing activity of these neurones (Figure 7b and d). WAY 100635 has also moderate (0.23 μM) affinity for α_1-adrenoceptor binding sites (Fletcher *et al.*, 1996). In order to assess the possible involvement of α_1-adrenoceptors in the suppressant effect of WAY 100635, the α_1-adrenoceptor antagonist prazosin was used. For 7 rats, prazosin (0.1 mg kg^{-1}, i.v.) by itself did not modify significantly the spontaneous firing activity of LC NA neurones (data not shown). However, this dose of prazosin prevented the suppressant effect of WAY 100635 on the firing activity of LC NA neurones (decrease of firing in control: 92±6%; decrease in firing after prazosin: 11±7%, $P<0.01$).

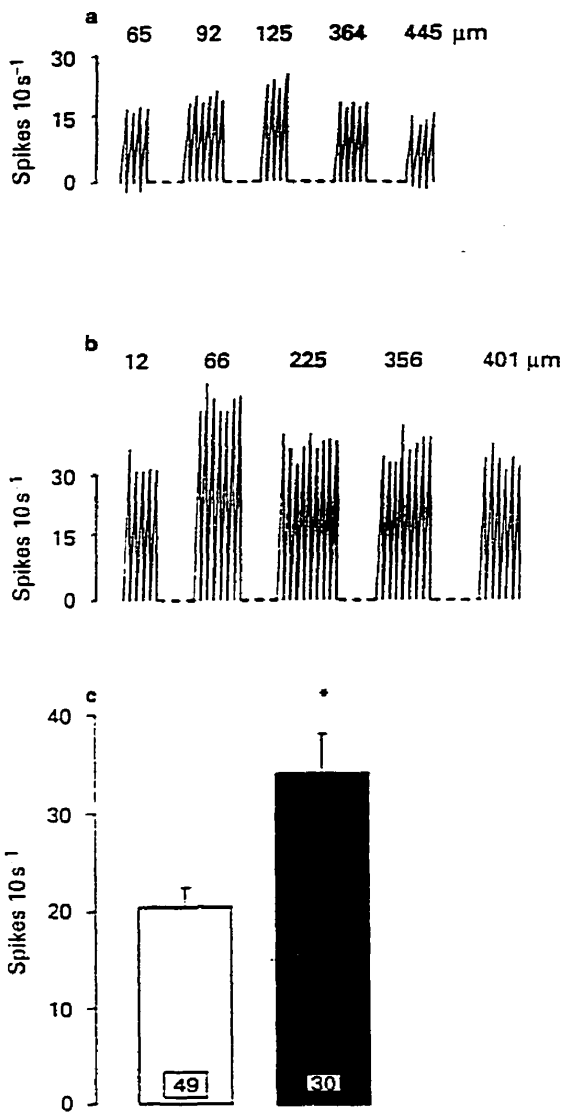

Figure 4 Integrated firing rate histograms of LC noradrenergic neurones, recorded in one electrode descent in the LC, showing their spontaneous firing activity in control (a) and 5,7-DHT-pretreated rats (b). The number above each neurone indicates the depth from the floor of the fourth ventricle at which it was recorded. Corresponding results (means±s.e.mean) are presented on (c): (open column) control and (stippled column) 5,7-DHT pretreated rats. The numbers at the bottom of the columns indicate the number of neurones tested. *$P<0.05$ (unpaired Student's t test).

Discussion

The microiontophoretic application of WAY 100635, as well as its intravenous administration, significantly attenuated the suppressant effect of microiontophoretically-applied 5-HT on the firing activity of CA_3 dorsal hippocampus pyramidal neurones, thus demonstrating its antagonistic action at postsynaptic 5-HT_{1A} receptors (Figure 1a and b). In contrast to NA, WAY 100635 and 5-HT, applied directly by microiontophoresis onto NA neurones, failed to modify their spontaneous firing activity (Figure 1b and d). However, the systemic administration of WAY 100635 (100 $\mu g\ kg^{-1}$, i.v.) suppressed the spontaneous firing activity of LC NA neurones (Figure 2a). This suppressant effect of WAY 100635 on the firing activity of LC NA neurones was prevented by a 5,7-DHT pretreatment (Figure 2b and c). Furthermore, after the 5-HT system had been lesioned, the firing activity of LC NA neurones was increased, suggesting the existence of a tonic inhibition of LC NA neurones by their 5-HT afferents (Figure 4). In order to determine the nature of the presynaptic 5-HT receptor involved in this suppressant effect of WAY 100635, several 5-HT receptor antagonists were used. The 5-HT_3 receptor antagonist BRL 46470A, the 5-$HT_{1B/1D}$ receptor antagonist GR 127935 and the 5-$HT_{1A,1B}$ receptor antagonist (−)-pindolol failed to prevent the effect of WAY 100635 (Figures 5; 7b and d). However, the suppressant effect of WAY

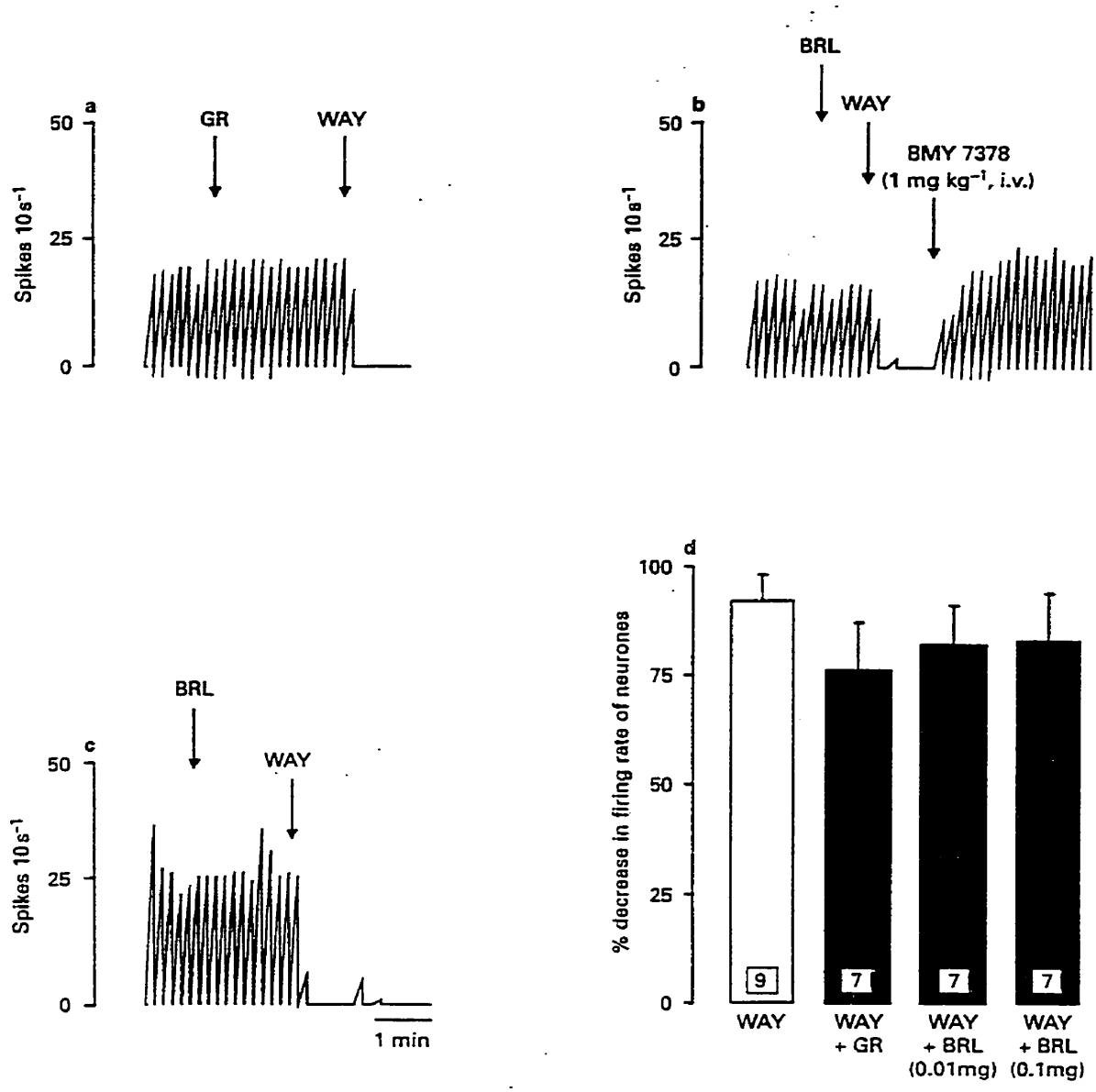

Figure 5 Integrated firing rate histograms of noradrenergic neurones recorded in the LC showing their responses to WAY 100635 (WAY, 100 μg kg^{-1}, i.v.) after the administration of GR 127935 (GR, 100 μg kg^{-1}, i.v.) (a), in another rat after the injection of BRL 46470A, (BRL, 10 μg kg^{-1}, i.v.) in (b) and 100 μg kg^{-1}, i.v., in (c). (d) Responsiveness of NA neurones to WAY 100635 (100 μg kg^{-1}, i.v.) in control, in GR 127935 and BRL 46470A-pretreated rats (means ± s.e.mean).

100635 was prevented by the 5-HT$_{1A/2A}$ receptor antagonist spiperone, by the non-selective 5-HT receptor antagonist metergoline, by the 5-HT$_2$ receptor antagonist ritanserin (Figure 6 and 7), by the 5-HT$_{1A}$ receptor α_{1D}-adrenoceptor antagonist BMY 7378 and by the α_1-adrenoceptor antagonist prazosin.

WAY 100635 is a potent and selective antagonist at both pre- and postsynaptic 5-HT$_{1A}$ receptors (Fletcher *et al.*, 1996). Khawaja *et al.* (1994) have shown, by use of [^{3}H]-WAY 100635, that this ligand has an affinity of 0.37 nM and a maximal binding capacity of 312 fmol mg^{-1} protein at 5-HT$_{1A}$ binding sites in rat hippocampal membranes. Moreover, the maximal number of binding sites labelled with [^{3}H]-WAY 100635 was 36% higher than those labelled by [^{3}H]-8-OH-DPAT. This is probably due to the fact that WAY 100635 is a high affinity ligand of both G-protein-coupled and free 5-HT$_{1A}$ receptors binding subunits, whereas 8-OH-DPAT only binds to G-protein-coupled 5-HT$_{1A}$ receptors (Golzan *et al.*, 1995). In rat cortical membranes, WAY 100635 can also bind to α_1-adrenoceptors labelled with [^{3}H]-prazosin, but with an affinity 300 times lower than that for 5-HT$_{1A}$ receptors (Fletcher *et al.*, 1996). It is unlikely that the low α_1-adrenoceptor affinity of WAY 100635 could contribute to the suppressant effect on LC NA neurones firing activity since Marwaha & Aghajanian (1982) have previously shown that α_1-adrenoceptor antagonists, including prazosin, do not affect the spontaneous firing activity of LC NA neurones. Mundey *et al.* (1994) in the guinea-pig and Gartside *et al.* (1995) in the rat have observed that intravenous administration of WAY 100635 increases the firing activity of dorsal raphe 5-HT neurones, whereas Fletcher *et al.* (1994) found no change in the rat. WAY 100635, applied microiontophoretically, prevented the suppressant effect of the 5-HT$_{1A}$ agonist 8-OH-DPAT on the firing activity of dorsal raphe 5-HT neurones in guinea-pigs (Mundey *et al.*, 1995) and WAY 100635 (applied by microiontophoresis and administered intravenously) antagonized the suppressant effect of microiontophoretically-applied 5-HT on the rat dorsal raphe 5-HT neurones (Author's unpublished observations). Furthermore, several studies have shown that systemic administration of WAY 100635 can antagonize the suppressant effects of 5-HT and of 8-OH-DPAT (Fletcher *et al.*, 1994; Mundey *et al.*, 1994; Craven *et al.*, 1994), and that of the selective 5-HT reuptake inhibitor (SSRI), paroxetine (Gartside *et al.*, 1995) on 5-HT neurone firing activity. Furthermore, the increase of extracellular 5-HT levels in the rat ventral hippocampus induced by the SSRI, citalopram, was enhanced by WAY 100635 (Hjorth & Milano, 1995) and WAY 100635 prevented the

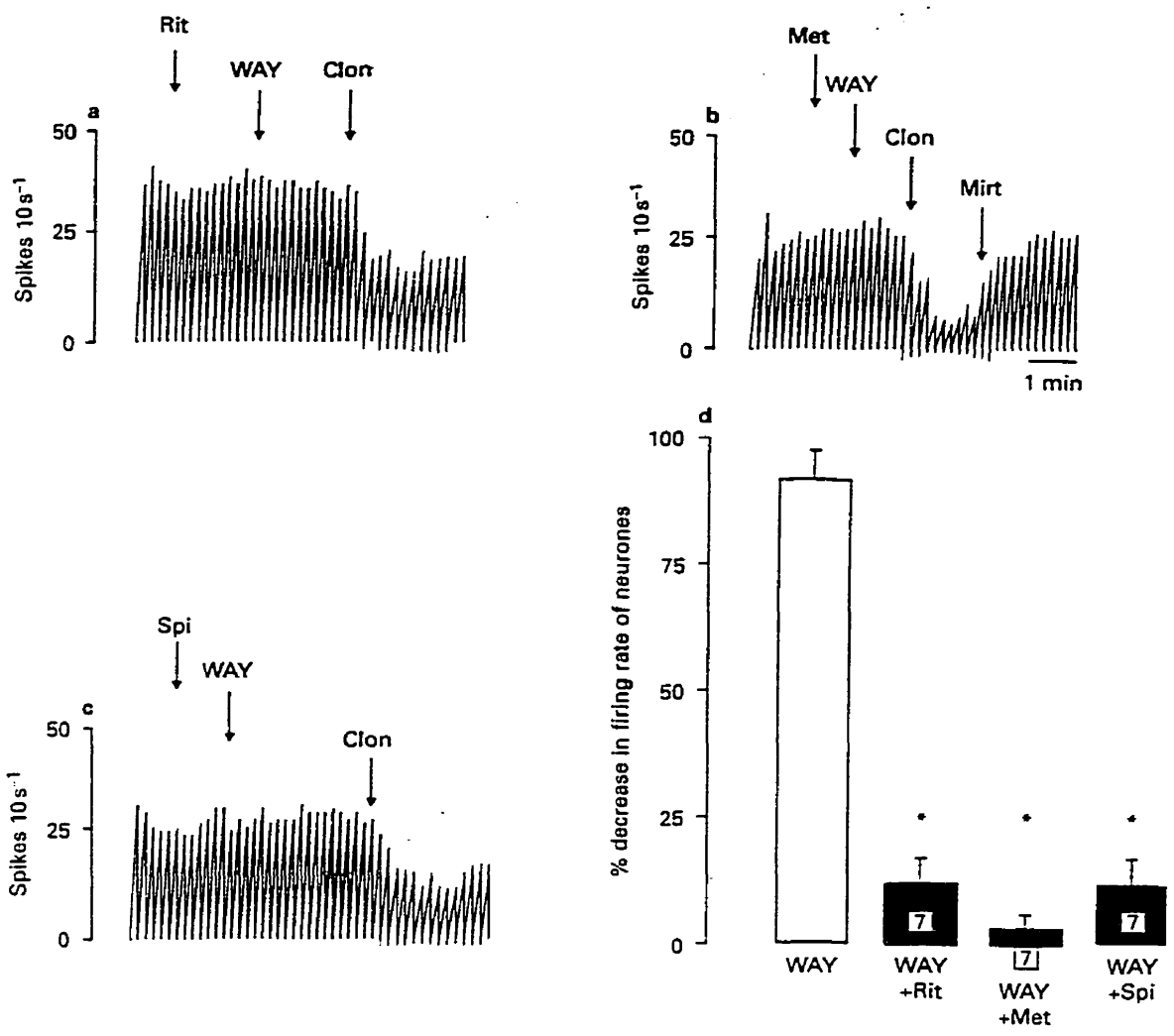

Figure 6 Integrated firing rate histogram of noradrenergic neurones recorded in the LC showing their responses to WAY 100635 (WAY, 100 $\mu g\ kg^{-1}$, i.v.) after the administration of ritanserin (Rit, 0.5 mg kg^{-1}, i.v.) (a), after the injection of metergoline (Met, 1 mg kg^{-1}, i.v.) (b) and spiperone (Spi, 1 mg kg^{-1}, i.v.) in (c). Responses to clonidine (Clon, 3 $\mu g\ kg^{-1}$, i.v.) are also shown (in (b) 5 $\mu g\ kg^{-1}$, i.v.) and in (b) to (±)-mirtazapine (Mirt, 250 $\mu g\ kg^{-1}$, i.v.). (d) Responsiveness of NA neurones to WAY 100635 (100 $\mu g\ kg^{-1}$, i.v.) in control, ritanserin, metergoline and spiperone-pretreated rats (means±s.e.mean). $^*P<0.05$ (unpaired Student's t test).

decrease of 5-HT release induced by systemic administration of 8-OH-DPAT in the rat ventral hippocampus (Gurling *et al*., 1994) and in the dorsal raphe (Davidson & Stamford, 1995). Taken together, these results indicate that WAY 100635 is indeed an effective antagonist of both pre- and postsynaptic 5-HT_{1A} receptors.

In the present study, the intravenous administration, but not the microiontophoretic application, of WAY 100635 suppressed the firing activity of LC NA neurones. It is important to emphasize that the lack of effect observed with local application of WAY 100635 on the soma of LC NA neurones does not exclude a possible involvement of receptors on the dendritic tree, as is the case with the suppressant effect of apomorphine on the firing activity of nigral dopamine neurones (Akaoka *et al*., 1992). However, the suppressant effect of WAY 100635 was prevented by 5,7-DHT pretreatment, implying the involvement of a presynaptic action of WAY 100635 on the 5-HT system. The LC receives a dense 5-HT innervation and electrophysiological and biochemical studies have suggested that the 5-HT system exerts a tonic inhibition of LC NA neurones (see Introduction). Consistent with this notion, the lesioning 5-HT neurones with 5,7-DHT increased the spontaneous firing activity of LC NA neurones (Figure 4b and c).

In order to determine the nature of the 5-HT receptor mediating the suppressant effect of WAY 100635 on the firing activity of LC NA neurones, 5-HT receptor antagonists were used. The selective 5-HT_3 receptor antagonist BRL 46470A, the 5-$HT_{1B/1D}$ receptor antagonist GR 127935, and the 5-$HT_{1A/1B}$ receptor antagonist (−)-pindolol did not modify the suppressant effect of WAY 100635. On the basis of the latter results, one may assume that the [redacted], 5-HT_{1B}, 5-HT_{1D} and 5-HT_3 receptor subtypes are not involved in the mediation of the effect of WAY 100635 on LC NA neurones. Hence, it then appears paradoxical that the 5-HT_{1A} receptor antagonist BMY 7378 completely prevented the suppressant effect of WAY 100635 (Figure 7c). However, it has previously been shown that the systemic administration of BMY 7378 suppresses the activity of dorsal raphe 5-HT neurones (Chaput & de Montigny, 1988; Cox *et al*., 1993). It is therefore possible that BMY 7378 prevented the suppressant effect of WAY 100635 on the firing activity of LC NA neurones by shutting off the firing of 5-HT neurones as a result of either its α_{1D}-adrenoceptor antagonistic activity (Goetz *et al*., 1995) or of its partial 5-HT_{1A} agonistic activity (Chaput & de Montigny, 1988; Cox *et al*., 1993). It has been shown that the $\alpha_{1A/1B/1D}$-adrenoceptor antagonist prazosin suppresses the spontaneous firing activity of dorsal raphe 5-HT neurones ($ED_{50}=50\ \mu g\ kg^{-1}$, i.v.) without modifying that of LC NA neurones (Marwaha & Aghajanian, 1982). In the present study, the suppressant effect of WAY 100635 on the firing activity of LC NA neurones was significantly prevented by prazosin (100 $\mu g\ kg^{-1}$, i.v.), providing further evidence for the crucial role of the 5-HT input in this effect of WAY 100635.

It has been shown that 5-HT_3 receptors are located postsynaptically (Leyson *et al*., 1982; Stockmeier & Kellar, 1986;

Fischette *et al.*, 1987) and an *in situ* hybridization study has revealed the presence of 5-HT_{2C} mRNA in the LC (Pompeiano *et al.*, 1994), whereas Wright *et al.* (1995) did not observe the presence of 5-$HT_{1A/2}$ mRNAs in this nucleus. In the present study, the non-selective 5-HT receptor antagonist metergoline, but not spiperone, increased by itself the firing activity of LC NA neurones and yet, both drugs prevented the suppressant effect of WAY 100635 on the firing activity of LC NA neurones. Binding studies have shown that metergoline has high affinity for 5-$HT_{1A/B/D}$, 5-$HT_{2A/C}$ and 5-HT_7 sites, whereas spiperone has high affinity for 5-HT_{1A}, 5-HT_{2A} and 5-HT_7 sites, and (−)-pindolol has only high affinity for 5-$HT_{1A/B}$ re-

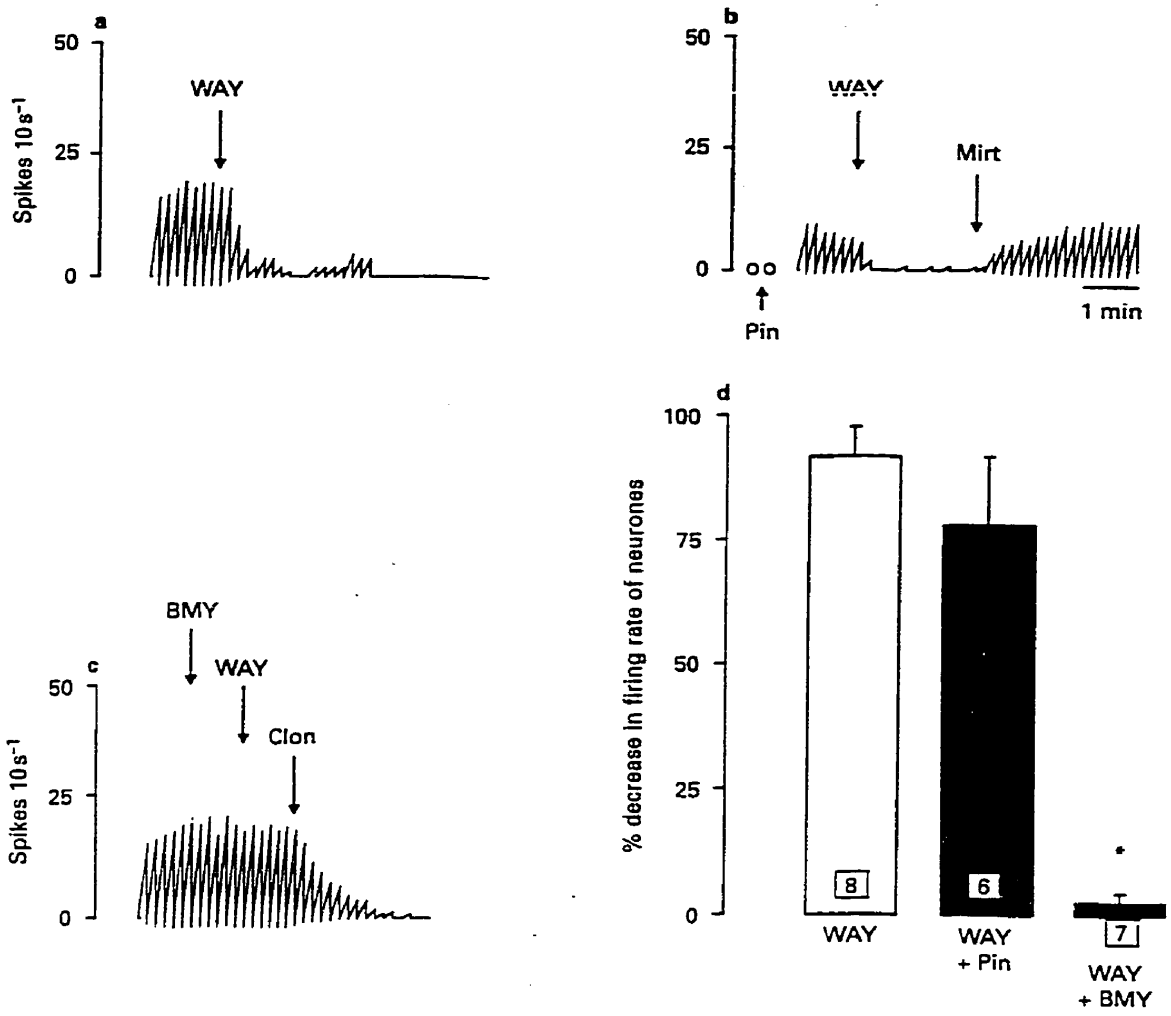

Figure 7 Integrated firing rate histogram of noradrenergic neurones recorded in the LC showing their responses to WAY 100635 (WAY, 100 $\mu g\ kg^{-1}$, i.v.) in control rat (a), after the administration of (−) pindolol (Pin, 15 $mg\ kg^{-1}$, i.p.) (b), and in another rat after the injection of BMY 7378 (BMY, 1 $mg\ kg^{-1}$, i.v.) (c). Responses to mirtazapine (Mirt, 250 $\mu g\ kg^{-1}$, i.v.) and clonidine (Clon, 5 $\mu g\ kg^{-1}$, i.v.) are also shown in (b) and (c), respectively, (d) Responsiveness of NA neurones to WAY 100635 (100 $\mu g\ kg^{-1}$, i.v.) in control, BMY 7378 and (−)-pindolol-pretreated rats (means ± s.e.mean). The numbers at the bottom of the columns indicate the number of neurones tested. $^*P<0.05$ (unpaired Student's t test).

Table 1 Affinities of 5-HT receptor antagonists and their effects on the spontaneous firing rate and on the inhibitory action of WAY 100635 on locus coeruleus noradrenergic neurones

Affinities	*5-HT_{1A}*	*5-HT_{2A}*	*5-HT_{2C}*	*5-HT_7*	*Effect on firing rate*[1]	*Inhibitory action of WAY 100635*[1]
Drugs						
Ritanserin	−	+	−	+	↗	↓
Spiperone	+	+	−	+	0	↓
(−)-Pindolol	+	−	−	−	↘	0
Metergoline	+	+	−	+	↗	↓
BMY 7378	+	−	−	−	↗	↓
GR 127935	−	−	−	NA	0	0
BRL 4640A	−	−	−	NA	0	0

[1]All drugs were injected i.v. (except pindolol, i.p.) before WAY 100635 (0.1 $mg\ kg^{-1}$ i.v.), 0 = no change, ↗ = increase, ↓ = decrease. [2](−) = $pK_i > 7$ and (−) = $pK_i < 6$; NA = not avaliable. Data from Baxter *et al.* (1995); Fiorella *et al.* (1995); Hoyer *et al.* (1994); Ruat *et al.* (1993); Shen *et al.* (1993); Zgombick *et al.* (1995).

ceptor subtypes (see Table 1). Ritanserin, which has high affinity for both 5-$HT_{2A/B/C}$ and 5-HT_7 receptors prevented the effect of WAY 100635 on the firing activity of LC NA neurones. However, in the LC, it has been suggested that the effects 5-HT_2 receptor ligands are indirect (Chiang & Aston-Jones, 1993).

High levels of 5-HT_7 receptor mRNA were observed in rat thalamus, hypothalamus, brainstem (including dorsal raphe) and hippocampus (Lovenberg *et al.*, 1993; Ruat 1993). Using [^{3}H]-lysergic acid diethylamide and transfected cell lines, these groups have shown that metergoline, ritanserin and spiperone have high affinities for the recombinant 5-HT_7 receptors. Using whole-cell voltage-clamp recordings from rat suprachiasmatic neurones Kawahara *et al.* (1994) have shown that 5-HT inhibits a GABA-activated current, presumably via 5-HT_7 receptors. This latter effect of 5-HT was antagonized by ritanserin, but not by pindolol nor by the 5-$HT_{2A,2C}$ receptors antagonist ketanserin. In the present study, metergoline, ritanserin and spiperone, which also have a high affinity for the 5-HT$_7$ receptors (Zgombick *et al.*, 1995), prevented the suppressant effect of WAY 100635 on the firing activity of LC NA neurones. On the other hand, BMY 7378 has very low affinity (≈ 2 μM) for the 5-HT_7 binding sites (F.D. Yocca, personal communication) and since WAY 100635 has no significant affinity for these receptors (Fletcher *et al.*, 1996), their involvement can be ruled out. Taking into account the affinities for the different 5-HT receptor subtypes of the drugs used in the present study (see Table 1) and the fact that prazosin prevented the effect of WAY 100635, the present results suggest that, the suppressant effect of WAY 100635 on the firing activity of LC NA neurones is mediated via 5-HT_{1A} receptors located presynaptically on the NA neurones. Given that spiperone, ritanserin and metergoline are 5-HT_{2A} receptor antagonists which, unlike BMY 7378, do not alter the firing activity of dorsal raphe 5-HT neurones in anaesthetized rats, it is possible that postsynaptic 5-HT_{2A} receptors might also be involved in the suppressant effect of WAY 100635 on the firing activity of LC NA neurones. Using microdialysis in freely moving rats Bosker *et al.* (1996) have recently shown that WAY 100635 (>50 μg kg^{-1}, s.c.) tended to increase extracellular 5-HT in the median raphe and the dorsal hippocampus and we have shown that WAY 100635 (100 μg kg^{-1}, i.v.) increased the duration of suppression of firing (corresponding to the endogenous 5-HT release) of dorsal hippocampus CA_3 pyramidal neurones induced by the electrical stimulation of the ascending 5-HT pathway (Author's unpublished observations). It is conceivable that the effect of WAY 100635 on the firing activity of LC NA neurones could be due to a direct blockade of presynaptic 5-HT_{1A} receptors, leading to an increase of 5-HT in the LC which results ultimately in the activation of postsynaptic 5-HT_{2A} receptors. It is also possible, on the other hand, that this suppressant effect of WAY 100635 on the LC NA neurones firing could be mediated through the 5-HT-mediated suppression of the excitatory glutamatergic input from the paragigantocellularis nucleus to LC neurones, as has been previously described (Aston-Jones *et al.*, 1991). Other experiments are needed to confirm the nature of the pre- and postsynaptic 5-HT receptors involved in the effect of WAY 100635 on LC NA neurones firing activity and to elucidate further this complex 5-HT neuromodulation of the LC noradrenergic system.

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Chapter VII: Sixth article

Tonic activation of postsynaptic 5-HT receptors:

In this study, several classes of antidepressant drugs have been used to assess whether their long-term administration could modify the tonic activation of postsynaptic 5-HT_{1A} receptors on CA_3 pyramidal neurons. In rats treated for 3 weeks with either MIR, the SSRI paroxetine, the selective MAO-A inhibitor befloxatone and the partial 5-HT_{1A} receptors agonist gepirone, the effects of WAY 100635, administered intravenously, were assessed on the suppressant effect of 5-HT microiontophoretically-applied on dorsal hippocampus CA_3 pyramidal neurons and on the firing activity of quisquilate-activated CA_3 pyramidal neurons.

This article entitle "*Antidepressant treatments tonically activate forebrain 5-HT_{1A} receptors.*" by myself, Pierre Blier and Claude de Montigny has been submitted for publication to the Journal of Neuroscience.

Systems Neuroscience
Dr. Stephen G. Lisberger

ANTIDEPRESSANT TREATMENTS TONICALLY ACTIVATE FOREBRAIN 5-HT_{1A} RECEPTORS.[1]

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Abbreviated title: Tonic activation of postsynaptic 5-HT_{1A} receptors.

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ABSTRACT

For over two decades, considerable evidence has accumulated to suggest that antidepressant treatments enhance the serotonin (5-HT) neurotransmission through different adaptative changes. We report here the first direct functional evidence of an increase activation of postsynaptic 5-HT_{1A} receptors by antidepressant treatments. As the activation of 5-HT_{1A} receptors of dorsal hippocampus CA_3 pyramidal neurons produces an hyperpolarization, an *in vivo* electrophysiological paradigm was used whereby the degree of disinhibition produced by the selective 5-HT_{1A} receptor antagonist WAY 100635 (100 μg/kg, i.v.) was determined. Unexpectedly, no disinhibition could be detected in control rats, suggesting that, under the experimental conditions used, 5-HT_{1A} receptors are not tonically activated. However, in rats treated for 3 weeks with the selective 5-HT reuptake inhibitor paroxetine, the selective and reversible monoamine oxidase-A inhibitor befloxatone, the α_2-adrenergic antagonist mirtazapine or for 2 weeks with the 5-HT_{1A} receptor agonist gepirone, WAY 100635 induced a marked increase (60-200%) in the firing activity of dorsal hippocampus pyramidal neurons. These data indicate that these antidepressant treatments, which act on entirely different primary targets, share the property of enhancing the tonic activation of postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus and provide the first direct *in vivo* functional evidence of an increased 5-HT neurotransmission induced by antidepressant treatments. In keeping with the clinical data suggesting an enhanced 5-HT neurotransmission in healthy subjects and depressed patients treated with antidepressants, the present data suggest that an enhanced activation of postsynaptic 5-HT_{1A} receptors might contribute to the alleviation of depression.

INTRODUCTION

Although the physiopathology of depressive illness is not fully understood, there is a growing body of evidence that implicate the serotonin (5-HT) system in the therapeutic effect of antidepressant treatments (Price et al., 1990; Blier and de Montigny, 1994). Hence, several lines of clinical and preclinical evidence have revealed that various classes of antidepressant treatments lead to an enhanced 5-HT neurotransmission with a time course that is consistent with their delayed therapeutic effect (Heninger and Charney, 1987; Van Praag et al., 1990; Delgado et al., 1990; Cummings, 1993). This enhanced 5-HT neurotransmission occurs through different adaptative changes (see Blier and de Montigny, 1994 for review). Previous studies from our laboratory have shown that tricyclic antidepressant drugs and electroconvulsive shock therapy lead to an enhanced 5-HT neurotransmission through a progressive sensitization of the postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus (de Montigny and Aghajanian, 1978; de Montigny, 1984). However, although still controversial, few antidepressant treatments have been shown to increase the density of 5-HT_{1A} binding sites (Welner et al., 1989; Nowak and Dulinski, 1991;

Stockmeier et al., 1992; Burnet et al., 1994). Long-term treatments with monoamine oxidase inhibitors (MAOIs) and selective 5-HT reuptake inhibitors (SSRIs) treatments desensitize the somatodendritic 5-HT_{1A} autoreceptors of 5-HT neurons in the dorsal raphe nucleus, thereby allowing their firing rate to recover in the presence of the drugs (Blier et al., 1986; Chaput et al., 1988). In addition, long-term SSRI treatment desensitizes terminal 5-$HT_{1B/D}$ autoreceptors, whereas long-term MAOI treatment desensitizes terminal α_2-adrenoceptors located on 5-HT fibers (Mongeau et al. 1994a; Blier and Bouchard, 1994). Both of the two latter subtypes of receptor modulate 5-HT release in the terminal field. Chronic treatment with α_2-adrenoceptors antagonists, such as mirtazapine, probably increase 5-HT neurotransmission, as a result of a sustained increase in 5-HT neuron firing activity in the presence of decreased function of α_2-adrenergic heteroreceptors located on 5-HT terminals in the dorsal hippocampus (Mongeau et al., 1994b; Haddjeri et al., 1996b). Finally, long-term treatment with 5-HT_{1A} receptors agonists, such as gepirone, desensitize the presynaptic 5-HT_{1A} receptors on 5-HT neurons but not the postsynaptic 5-HT_{1A} receptors located on CA_3 pyramidal neurons (Blier and de Montigny, 1987). Consequently, in the presence of the exogenous 5-HT_{1A} receptors agonist in the brain and a normalized endogenous 5-HT release, it was hypothesized that 5-HT_{1A} receptor agonists lead to an enhanced tonic activation of postsynaptic 5-HT_{1A} receptors. Taken together, these data indicate that changes in 5-HT system are important in the therapeutic effect of antidepressant treatments.

In the present study, several classes of antidepressant drugs were used to verify whether their long-term administration could indeed modify the tonic activation of postsynaptic 5-HT_{1A} receptors on CA_3 pyramidal neurons. Rats were treated for 2 or 3 weeks with either the SSRI paroxetine, with the selective and reversible MAO-A inhibitor befloxatone, with the α_2-adrenergic antagonist mirtazapine or with the 5-HT_{1A} receptor agonist gepirone. The effect of the potent and selective 5-HT_{1A} receptor antagonist WAY 100635, administered intravenously, was assessed on the firing activity of CA_3 pyramidal neurons in the dorsal hippocampus in order to unveil the degree of activation of postsynaptic 5-HT_{1A} receptors, which exert an inhibitory effect on that parameter in that brain region.

MATERIALS AND METHODS

The experiments were carried out in male Sprague-Dawley rats weighing 250 to 300 g which were kept under standard laboratory conditions (12:12 light-dark cycle with free access to food and water). Four groups of rats were treated for 21 days with either befloxatone (Synthelabo Recherche, Rueil-Malmaison, France; 0.75 mg/kg/day), mirtazapine (Organon, Oss, The Netherlands; 5 mg/kg/day), paroxetine (SmithKline Beecham, Harlow, England; 10 mg/kg/day) or vehicle (50% ethanol water solution) delivered by osmotic minipumps (ALZA, Palo Alto, CA)

inserted subcutaneously (s.c.). One group of rat was treated with gepirone (Bristol-Myers Squib, Wallingford, CT, U.S.A; 15 mg/kg/day, solubilized in water) for 2 weeks. The rats were tested after the long-term treatments with the minipumps on board. The animals were anesthetized with chloral hydrate (400 mg/kg, i.p.). Supplemental doses were given to maintain constant anaesthesia and to prevent any nociceptive reaction to a tail pinch.

Recordings from CA_3 dorsal hippocampus pyramidal neurons. Recording and microiontophoresis were performed with five-barrelled glass micropipettes broken back to 8-12 μm under microscopic control (ASI Instruments, Warren, MI, USA). The central barrel was filled with a 2 M NaCl solution and used for extracellular unitary recordings. The pyramidal neurons were identified by their large amplitude (0.5-1.2 mV) and long-duration (0.8-1.2 ms) simple spikes alternating with complex spike discharges (Kandel and Spencer 1961). The side barrels contained the following solutions: 5-HT creatinine sulphate (Sigma Chemical, St. Louis, MO, USA; 20 mM in 200 mM NaCl, pH 4), quisqualate (Sigma Chemical, St. Louis, MO,USA; 1.5 mM in 200 mM NaCl, pH 8) and 2 M NaCl used for automatic current balancing. The rats, control or treated with the minipumps on board, were mounted in a stereotaxis apparatus and the microelectrodes were lowered at 4.2 mm lateral and 4.2 anterior to lambda into the CA_3 region of the dorsal hippocampus. Since most hippocampus pyramidal neurons are not spontaneously active under chloral hydrate anaesthesia, a leak or a small ejection current of quisqualate (+1 to -6 nA) was used to activate them within their physiological firing range (Ranck, 1975). Neuronal responsiveness to the microiontophoretic application of 5-HT were assessed by determining the number of spikes suppressed for 10 nA applications of 5-HT. The duration of the microiontophoretic applications of 5-HT was of 50 s. The same current of ejection was always used before and after the i.v. injection of the selective $5\text{-}HT_{1A}$ receptors antagonist WAY 100635 (Wyeth Research, Bershire, UK; 100 μg/kg). Two minutes prior the i.v. administration of WAY 100635, the firing activity of quisquilate-activated CA_3 pyramidal neurons tested was decreased down to about 5 Hz in order to allow possible changes of the firing activity to be readily detectable following WAY 100635 administration in control and treated rats. To assess the effectiveness of the long-term treatment with paroxetine, the recovery time 50 (RT_{50}) method was used. The RT_{50} value has been shown to be a reliable index of the *in vivo* activity of the 5-HT reuptake process in the rat hippocampus (Piñeyro et al., 1994). This value is obtained by calculating the time in seconds required by the neuron to recover 50% of its initial firing rate from the end of the microiontophoretic application of 5-HT onto CA_3 pyramidal neurons.

RESULTS

Effects of long-term antidepressant treatments on the responsiveness of CA_3 dorsal hippocampus pyramidal neurons to 5-HT. It has previously been demonstrated that the microiontophoretic application of 5-HT onto rat dorsal hippocampus pyramidal neurons produces a suppressant effect on their firing activity which is mediated by 5-HT_{1A} receptors (Blier and de Montigny, 1987; Chaput and de Montigny, 1988). For all CA_3 hippocampus pyramidal neurons tested, 5-HT (10 nA) induced a reduction of firing activity (Figs. 1 and 2). This inhibitory effect occurred in the absence of alteration of the shape of the action potentials. Long-term treatment with either befloxatone (Figs. 2A and 3B), with mirtazapine (Figs. 2B and 3A) or with gepirone (Figs. 2C and 3B) did not modify the suppressant effect of microiontophoretically-applied 5-HT on the firing activity of CA_3 dorsal hippocampus pyramidal neurons. On the other hand, the long-term treatment with paroxetine markedly prolonged the effect of 5-HT microiontophoretically-applied onto dorsal hippocampus CA_3 pyramidal neurons: the RT_{50} value was increased by 344% in paroxetine-treated rats, thus confirming the blockade of the 5-HT uptake process by paroxetine (Fig. 1B). In control and treated rats, the i.v. administration of WAY 100635 (100 μg/kg) attenuated the suppressant effect of 5-HT. Figure 3 illustrates the mean suppressant action of 5-HT microiontophoretically-applied onto CA_3 pyramidal neurons and the antagonistic effect of WAY 100635 on the responsiveness of these postsynaptic 5-HT_{1A} receptors in control rats. In the present study, WAY 100635 (100 μg/kg, i.v.) significantly reduced the suppressant effect of 5-HT on CA_3 hippocampus pyramidal neurons by 52% in controls (Fig. 3A), by 59% in mirtazapine-treated rats (Fig. 3A), by 62% in gepirone-treated rats (Fig. 3B) and by 57% in befloxatone-treated rats (Fig. 3B).

The effect of WAY 100635 (100 μg/kg, i.v.) on the quisqualate-activated firing activity of CA_3 pyramidal neurons was assessed in control and in treated rats. In controls, the i.v. administration of WAY 100635 did not modify the firing activity of dorsal hippocampus CA_3 pyramidal neurons (firing activity prior to: 5.1 ± 0.4 Hz; following WAY 100635 injection: 4.7 ± 0.5 Hz, n=12; Figs. 4A and E) whereas in paroxetine-treated group (Figs. 1B and 4E), the firing activity of these neurons was markedly and significantly increased (firing activity prior to: 4.8 ± 0.6 Hz; following WAY 100635 injection: 11.1 ± 1.4 Hz, n=7). The firing activity of dorsal hippocampus CA_3 pyramidal neurons in befloxatone (firing activity prior to: 4.2 ± 0.5 Hz; following WAY 100635 injection: 8.1 ± 0.8 Hz, n=8; Figs. 4B and E), mirtazapine (firing activity prior to: 5.1 ± 0.5 Hz; following WAY 100635 injection: 8.2 ± 0.8 Hz, n=12; Figs. 4C and E) and gepirone (firing activity prior to: 5 ± 0.8 Hz; following WAY 100635 injection: 15.2 ± 2.5 Hz, n=7; Figs. 4D and E) treated group, was also significantly increased after the i.v. administration of WAY 100635.

DISCUSSION

The present electrophysiological studies show that chronic treatments with either the SSRI paroxetine (10 mg/kg/day, s.c. x 21 days), the selective and reversible MAO-A inhibitor befloxatone (0.75 mg/kg/day, s.c. x 21 days), the α_2-adrenergic antagonist mirtazapine (5 mg/kg/day, s.c. x 21 days) or with the 5-HT_{1A} receptor agonist gepirone (15 mg/kg/day, s.c. x 14 days) enhanced the tonic activation of postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus, as put into evidence by the enhancing effect of the administration of the 5-HT_{1A} receptor antagonist WAY 100635.

Among the 5-HT_{1A} receptors antagonists available, WAY 100635 is thus far the most potent and selective antagonist at both pre- and postsynaptic 5-HT_{1A} receptors (Fletcher et al., 1996). Khawaja et al. (1994) have reported, using tritiated WAY 100635, that this 5-HT_{1A} receptor ligand has an affinity of 0.37 nM in rat hippocampal membranes. Mundey et al. (1994) in guinea-pigs and Gartside et al. (1995) in rats have observed that i.v. administration of WAY 100635 increased the firing activity of dorsal raphe 5-HT neurons, probably by removing the tonic inhibitory effect exerted by endogenous 5-HT on cell body 5-HT_{1A} autoreceptors controlling their firing activity, whereas Fletcher et al. (1994) found no such change in rats. Furthermore, several studies have shown that systemic administration of WAY 100635 antagonizes the suppressant effect of 5-HT and of the prototypical 5-HT_{1A} receptor agonist 8-OH-DPAT (Fletcher et al., 1994; Mundey et al., 1994; Craven et al., 1994), and that of the SSRI paroxetine (Gartside et al., 1995) on 5-HT neuron firing activity. The increase of extracellular 5-HT levels in the rat ventral hippocampus induced by the SSRI citalopram was enhanced by WAY 100635 (Hjorth & Milano, 1995) and WAY 100635 prevented the decrease of 5-HT level induced by systemic administration of 8-OH-DPAT in the rat ventral hippocampus (Gurling et al., 1994) and in the dorsal raphe (Davidson & Stamford, 1995), presumably as a result of 5-HT_{1A} autoreceptors blockade. Taken together, these results indicate that WAY 100635 is indeed an effective antagonist of both pre- and postsynaptic 5-HT_{1A} receptors. In the present experiments, the capacity of WAY 100635 to block the 5-HT_{1A} autoreceptors is not expected to alter its effectiveness to increase the firing activity of postsynaptic neurons in the treated rats. If any interference exists, it would tend to lead to an underestimation of the effect of WAY 100635 as it was reported that in the presence of an SSRI in freely moving cats (Fornal et al., 1994) and of befloxatone in anesthetized rats (Haddjeri et al., unpublished observation), WAY 100635 actually increases 5-HT neuronal firing rate above the control range.

Paroxetine, a phenylpiperidine compound, is a potent and selective SSRI (Tuloch and Johnson, 1992) which is currently used in the treatment of depressive illness (Claghorn et al., 1993, Nemeroff, 1994). Previous study from our laboratory have shown that chronic paroxetine treatment enhances 5-HT release induced by the electrical stimulation of the ascending 5-HT pathway by reducing the function of terminal 5-HT autoreceptors in the dorsal hippocampus

(Chaput et al., 1991). This treatment has also been shown to desensitize somatodendritic 5-HT_{1A} autoreceptors in the dorsal raphe (Le Poul et al., 1995). Furthermore, Piñeyro et al. (1994) have recently shown *in vivo* that long-term treatment with paroxetine desensitizes the neuronal 5-HT carrier in the dorsal hippocampus. As previously reported (Piñeyro et al., 1994), chronic treatment with paroxetine increases the RT_{50} value, thus confirming a thorough blockade of the terminal 5-HT uptake process in the dorsal hippocampus by such a treatment (Fig. 1B). In rats treated with paroxetine, WAY 100635 significantly increased the firing activity of quisqualate-activated CA_3 pyramidal neurons, revealing an enhancement of the tonic activation postsynaptic 5-HT_{1A} receptors (Figs. 1B and 4E). This could be due to an increase of synaptic 5-HT concentration in the dorsal hippocampus resulting from the desensitization of somatodendritic and terminal 5-HT autoreceptors, as well as the desensitization of the neuronal 5-HT carrier (Piñeyro et al., 1994).

Because of the important side-effects of classical MAO-A inhibitors, especially the possible hypertensive crises secondary to the cheese-effect (Murphy et al., 1987), a second-generation of reversible MAO-A inhibitors have recently been developed which are devoid of such pressor effects (see Thase et al., 1995 for review). Among the latter, befloxatone, an oxazolidinone derivative which is a selective and reversible MAO-A inhibitor, seems to be a promising effective antidepressant (Caille et al., 1996). The pharmacological profile of befloxatone reveals that it has a higher affinity for MAO-A (Ki= 2.8 ± 0.4 nM) than the older MAO-A inhibitors like moclobemide (Ki= 14000 ± 1250 nM) using rat brain membranes. *In vitro*, befloxatone (1-750 µg/kg, p.o.) increases whole rat brain levels of norepinephrine, dopamine and 5-HT, and decreases the levels of the metabolites dihydroxyphenylacetic acid and 5-hydroxyindolacetic acid. Using microdialysis in the striatum of freely moving rats, befloxatone (0.75 mg/kg, i.p.) increases the dopamine and decreases dihydroxyphenylacetic acid, homavalenic acid and 5-hydroxyindolacetic acid extracellular levels. In the frontal cortex of freely moving rats, acute befloxatone (0.75 mg/kg, i.p.) increases the norepinephrine level but not that of 5-HT (Curet et al., 1994, 1995, 1996). Recently, it has been shown that long-term treatment with befloxatone (0.75 mg/kg/day, s.c. x 21 days) desensitizes, *in vivo* in rats and *in vitro* in guinea-pigs, α_2-adrenergic heteroreceptors on 5-HT fibers. In the present study, in rats treated with befloxatone for 21 days, WAY 100635 significantly increased the firing of quisqualate-activated CA_3 pyramidal neurons, revealing an enhancement of the tonic activation of normosensitive postsynaptic 5-HT_{1A} receptors by such a treatment (Figs. 4B and E). This could be due to an increase in the synaptic availability of 5-HT in the dorsal hippocampus induced by the inhibition of the MAO-A and the release of 5-HT not being dampened as a result of the desensitization of terminal α_2-adrenoceptors located on 5-HT fibers, leading to an increased 5-HT neurotransmission (Mongeau et al., 1994a; Blier and Bouchard, 1994).

Mirtazapine (ORG 3770 or Remeron®), is a tetracyclic compound with antidepressant activity in humans (Smith et al., 1990; Claghorn and Lesem, 1995; vanMoffaert et al., 1995). Its

pharmacological profile is characterized by α_2-adrenergic, 5-HT_2, 5-HT_3 and histaminergic$_1$ antagonistic activities. It is devoid of anticholinergic activity and has no effect on the reuptake of 5-HT or catecholamines (Nickolson et al., 1982; De Boer et al., 1988). Among these neurochemical effects, the blockade of presynaptic α_2-adrenoceptors has been proposed to be a possible substratum for its antidepressant activity (see: Pinder and Wieringa, 1993 for review). Using microdialysis in freely moving rats, De Boer et al. (1994, 1996) have shown that mirtazapine increases extracellular 5-HT and dihydroxyphenylacetic acid (used as an index of presynaptic noradrenergic activity) in the ventral hippocampus. The indirect α_1-adrenoceptor-mediated enhancement of 5-HT neuron firing and the direct blockade of inhibitory α_2-adrenergic heteroreceptors located on 5-HT terminals may be responsible for this increase in extracellular 5-HT (Haddjeri et al., 1996a). Indeed, it was previously demonstrated that acute administration of mirtazapine increases in a transient manner both the spontaneous firing activity of locus coeruleus NE and dorsal raphe 5-HT neurons whereas a 21-day treatment with mirtazapine (5 mg/kg/day, s.c.) increases it in a sustained manner. It has been suggested that chronic treatment with mirtazapine increases the 5-HT neurotransmission, as a result of a sustained increase in 5-HT neuron firing activity in the presence of decreased function of α_2-adrenergic heteroreceptors located on 5-HT terminals in the dorsal hippocampus (Haddjeri et al., 1996b). In the present study, the sensitivity of postsynaptic 5-HT_{1A} receptors on CA_3 pyramidal neurons, assessed with the microiontophoretic application of 5-HT, remained unchanged in rats treated with mirtazapine for 21 days (Fig. 2B and 3A). However, in this group, the administration of WAY 100635 significantly increased the firing of quisqualate-activated CA_3 pyramidal neurons, showing that the blockade of postsynaptic 5-HT_{1A} receptors by WAY 100635 revealed an enhancement of their tonic activation (Figs. 4C and E). This could be the consequence of an increased 5-HT neurotransmission due to the sustained increase of the firing activity of 5-HT neurons leading to an increase of the endogenous 5-HT release in the dorsal hippocampus and permitted by the desensitization of terminal α_2-adrenoceptors located on 5-HT fibers, receptors which modulate 5-HT release in the terminal field (Haddjeri et al., 1996b).

Gepirone is an azapirone derivative endowed with anxiolytic/antidepressant activity (Rausch et al., 1990; Jenkins et al., 1990). Previous studies have shown that acute administration of gepirone dose-dependently decreases the firing activity of dorsal raphe 5-HT neurons and microiontophoretic application of gepirone also decreases the firing activity of dorsal hippocampus CA_3 pyramidal neurons (Blier and de Montigny, 1987). The sustained administration of gepirone produces an initial decrease of the firing activity of dorsal raphe 5-HT neurons, which is followed by a progressive recovery to normal after two weeks of treatment. It has been also shown that gepirone treatment desensitized the presynaptic 5-HT_{1A} receptors on 5-HT neurons but not the postsynaptic 5-HT_{1A} receptors located on CA_3 pyramidal neurons (Blier and de Montigny, 1987). Such a treatment, however, fails to modify the effectiveness of the electrical stimulation

of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus CA_3 pyramidal neurons. Therefore, it could be suggested that such a treatment results in an augmented tonic activation of the postsynaptic 5-HT_{1A} receptors, but at that time selective 5-HT_{1A} receptors antagonists were not available to verify this hypothesis. In the present study, the i.v. administration of WAY 100635 significantly increased the firing activity of CA_3 pyramidal neurons at a time when 5-HT neurons have regained their normal firing activity, thus, showing that the blockade of the postsynaptic 5-HT_{1A} receptors by WAY 100635 revealed an enhancement of their tonic activation (Figs. 4D and E). This now constitutes direct evidence that 5-HT neurotransmission is in fact enhanced at 5-HT_{1A} receptors in the dorsal hippocampus following long-term administration of the 5-HT_{1A} receptor agonist gepirone.

The results obtained with paroxetine, befloxatone and mirtazapine indicate that synaptic 5-HT levels are increased following long-term administration. Therefore, the degree of tonic activation of 5-HT receptors, but not all, other than those of 5-HT_{1A} subtypes may also be increased. However, it has been demonstrated that some postsynaptic 5-HT receptors other than those of the 5-HT_{1A} subtypes become sensitized following long-term antidepressant treatments. For instance, repeated tricyclic antidepressant administration sensitizes postsynaptic 5-HT_2 receptors in the facial motor nucleus and yet uncharacterized 5-HT receptor subtypes in the amygdala (Menkes et al., 1980; Wang and Aghajanian, 1980). Consequently, the present data by no means rule out that the tonic activation of 5-HT receptors other than those of the 5-HT_{1A} subtype may be modified by certain antidepressant drugs.

In conclusion, the present electrophysiological studies show that chronic treatment with either the selective and reversible MAO-A befloxatone, the SSRI paroxetine, the α_2-adrenergic antagonist mirtazapine or with the 5-HT_{1A} receptor agonist gepirone enhanced the tonic activation of postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus, as measured with the administration of the selective 5-HT_{1A} receptors antagonist of WAY 100635. This results constitute further direct evidence that an enhanced 5-HT neurotransmission may underlie the antidepressant response in humans.

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FIGURE LEGENDS

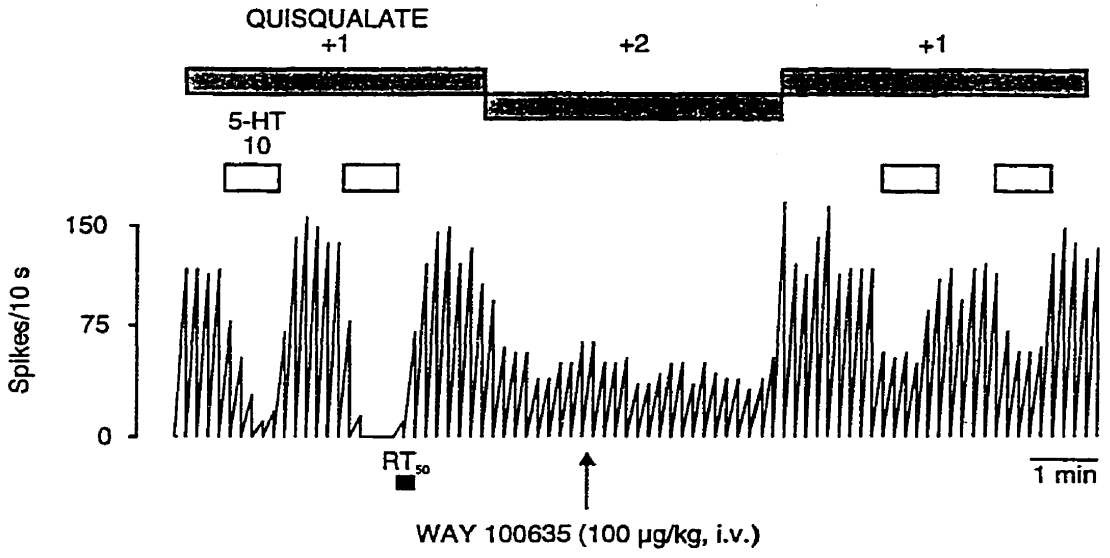
Figure 1: Integrated firing rate histogram of a dorsal hippocampus CA_3 pyramidal neuron showing its responsiveness to microiontophoretic application of 5-HT in control (**A**) and treated rat with paroxetine (**B**). These neurons were activated with a quisquilate ejection current of +1 and -3 nA. Horizontal bars indicate the duration of the applications for which the current is given in nanoampers. Note the altered effectiveness of 5-HT to suppress firing activity after administration of WAY 100635 (0.1 mg/kg) in control (**A**). The black horizontal bars indicated RT_{50} values (see Materials and Methods section).

Figure 2: Integrated firing rate histogram of a dorsal hippocampus CA_3 pyramidal neuron showing its responsiveness to microiontophoretic application of 5-HT before and after the i.v. injection of WAY 100635 (0.1 mg/kg) in rat treated with befloxatone (**A**), treated with mirtazapine (**B**) and with gepirone (**C**). These neurons were activated with a quisquilate ejection current of -1 to -3 nA. Horizontal bars indicate the duration of the applications for which the current is given in nanoampers. Note the altered effectiveness of 5-HT to suppress firing activity after administration of WAY 100635 in all treated rats.

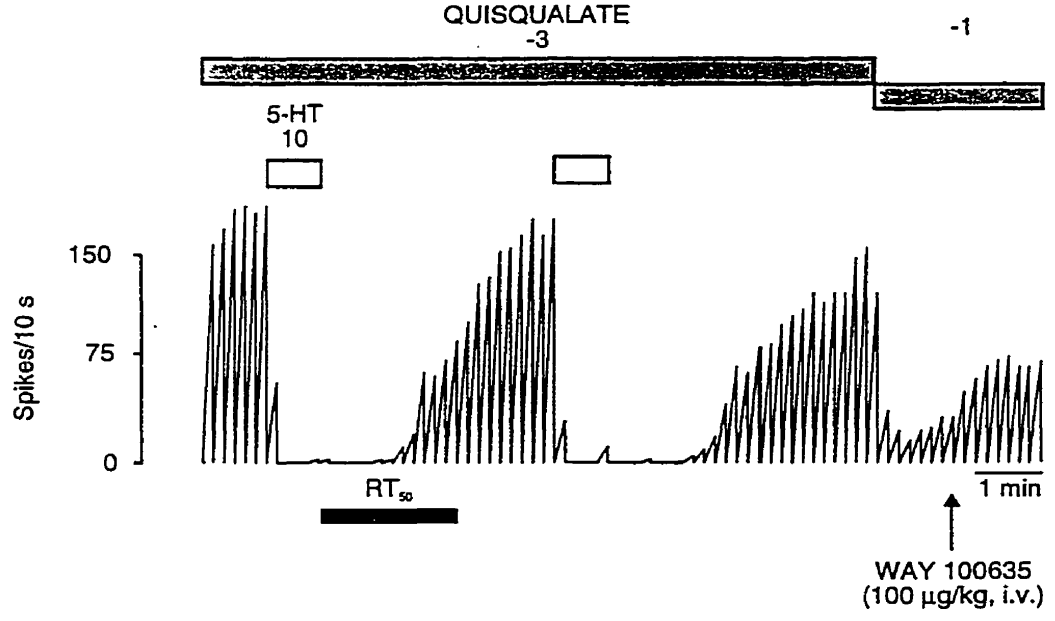
Figure 3: In panel **A**, the responsiveness to 5-HT in controls and treated rats with mirtazapine is expressed as the number of spikes suppressed per 10 nanoamps, the number in the columns indicate the number of neurons and rat tested. $P < .05$ (paired Student's *t* test). In panel **B**, the responsiveness to 5-HT in treated rats with befolxatone and gepirone is expressed as the number of spikes suppressed per 10 nanoamps, the number in the columns indicate the number of neurons and rat tested. $P < .05$ (paired Student's *t* test). Note the altered effectiveness of 5-HT to suppress firing activity after administration of WAY 100635 in all groups.

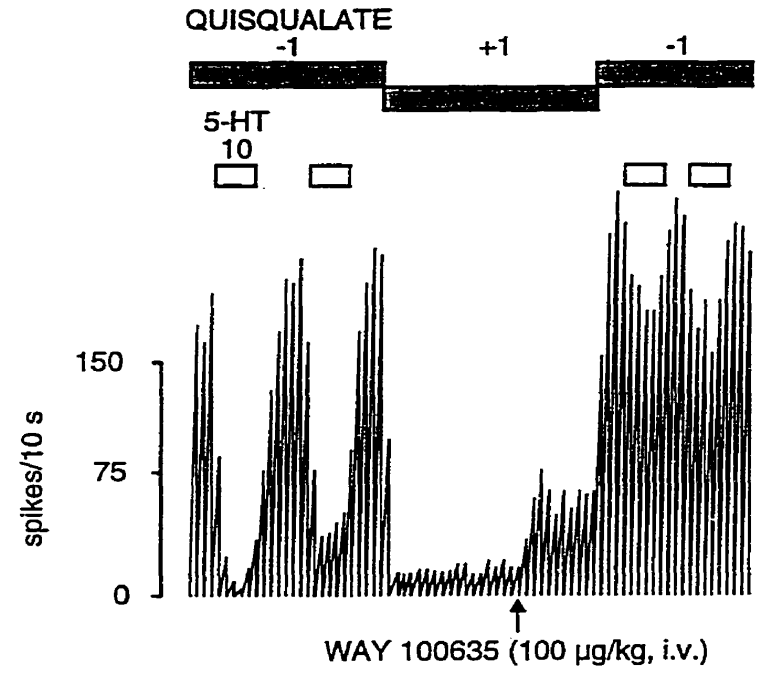
Figure 4: Integrated firing rate histogram of a dorsal hippocampus CA_3 pyramidal neuron showing its responsiveness to the i.v. injection of WAY 100635 (0.1 mg/kg) in control rat (**A**), rat treated with befloxatone (**B**), rat treated with mirtazapine (**C**) and rat treated with gepirone (**D**). These neurons were activated with a quisquilate ejection current of -1 to -3 nA. Note the altered firing activity after administration of WAY 100635 rat treated with either befloxatone (**B**) with mirtazapine (**C**) or with gepirone (**D**). In panel **E**, the responsiveness to WAY 100635 is expressed as changes in firing activity. For each group, 7-12 neurons or rats have been tested, $P < 0.05$ (unpaired Student's *t* test).

A CONTROL

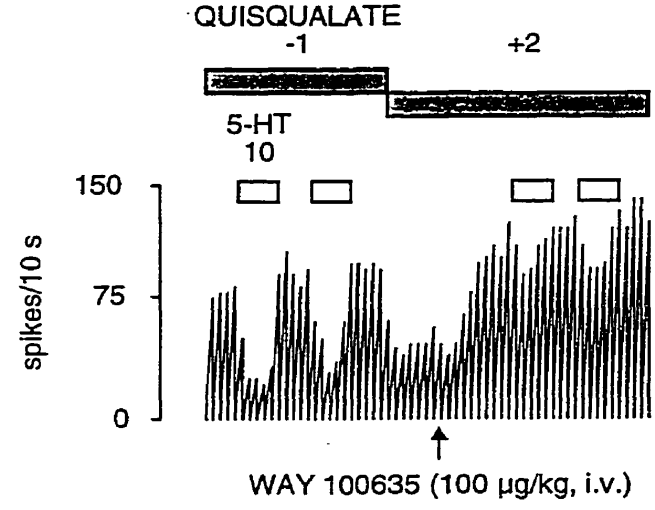


B PAROXETINE (10 mg/kg/day x 21 days)

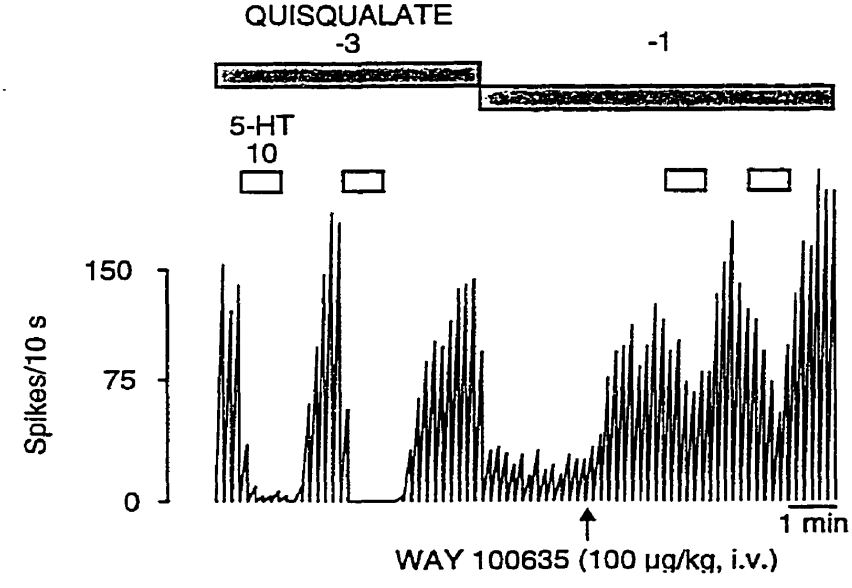




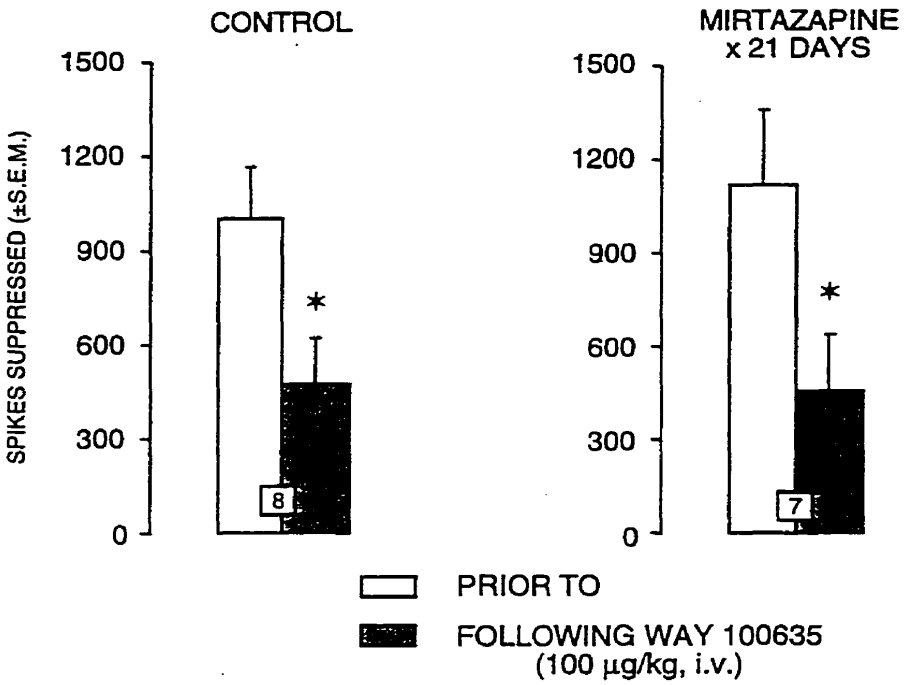
B MIRTAZAPINE (5 mg/kg/day x 21 days)



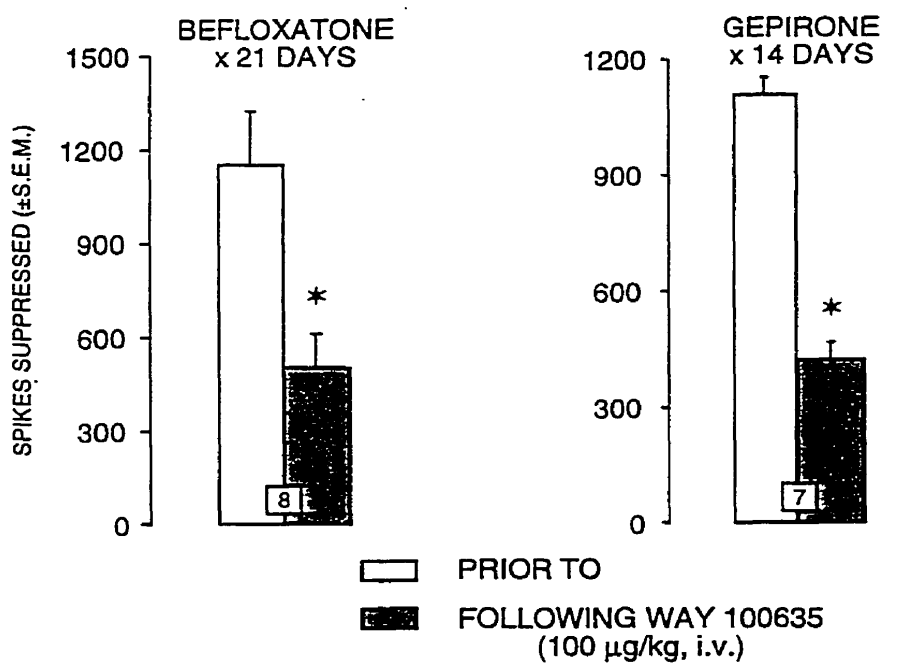
C GEPIRONE (15 mg/kg/day x14 days)

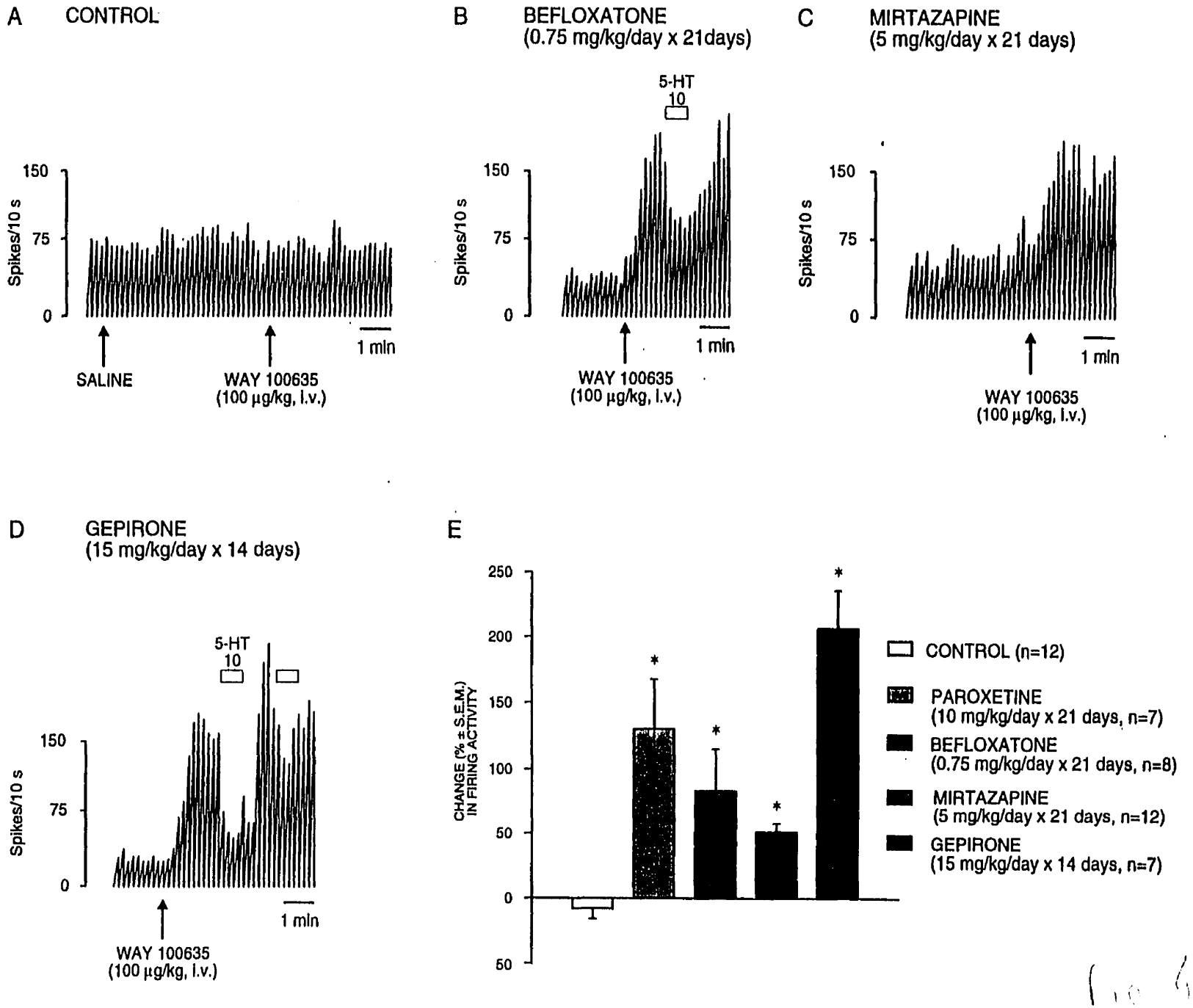


A


CONTROL
MIRTAZAPINE
x 21 DAYS
SPIKES SUPPRESSED (±S.E.M.)
1500
1200
900
600
300
0
*
8
7
PRIOR TO
FOLLOWING WAY 100635
(100 µg/kg, i.v.)

B


BEFLOXATONE
x 21 DAYS
GEPIRONE
x 14 DAYS
SPIKES SUPPRESSED (±S.E.M.)
1500
1200
900
600
300
0
*
8
7
PRIOR TO
FOLLOWING WAY 100635
(100 µg/kg, i.v.)


A
CONTROL
150
75
0
Spikes/10 s
1 min
SALINE
WAY 100635
(100 µg/kg, i.v.)
B
BEFLOXATONE
(0.75 mg/kg/day x 21days)
5-HT
10
150
75
0
Spikes/10 s
1 min
WAY 100635
(100 µg/kg, i.v.)
C
MIRTAZAPINE
(5 mg/kg/day x 21 days)
150
75
0
Spikes/10 s
1 min
WAY 100635
(100 µg/kg, i.v.)
D
GEPIRONE
(15 mg/kg/day x 14 days)
5-HT
10
150
75
0
Spikes/10 s
1 min
WAY 100635
(100 µg/kg, i.v.)
E
250
200
150
100
50
0
50
CHANGE (% ± S.E.M.)
IN FIRING ACTIVITY
*
*
*
*
CONTROL (n=12)
PAROXETINE
(10 mg/kg/day x 21 days, n=7)
BEFLOXATONE
(0.75 mg/kg/day x 21 days, n=8)
MIRTAZAPINE
(5 mg/kg/day x 21 days, n=12)
GEPIRONE
(15 mg/kg/day x 14 days, n=7)

Chapter VIII: General discussion

The present projects were undertaken to further characterize the interactions between two brain neuronal systems involved in the regulation of mood. In support of the 5-HT hypothesis of depression, these results further characterize factors involved in the modulation of the 5-HT system and provide new insight into the mechanism of action of anxiolytic/antidepressant drugs.

In the first study, we have characterized, *in vivo* and *in vitro,* the automodulation of 5-HT system via 5-$HT_{3/4}$ receptor subtypes at the presynaptic, terminal and postsynaptic levels. As reported in section 2.2.3., the activation of 5-HT_3 receptors generally mediates a rapid depolarization of peripheral and central neurons. However, Ashby et al. (1992, 1994) demonstrated that, in the rat medial prefrontal cortex, the action of 5-HT may be mediated by 5-HT_3-like receptors producing a hyperpolarization. At the postsynaptic level, the results obtained *in vivo* show that the current-dependent suppressant effect of both 5-HT and the 5-HT_3 receptor agonist 2-methyl-5-HT, microiontophoretically-applied, on firing activity of both dorsal hippocampal (CA_1 and CA_3) and somatosensory cortical neurons is mediated via 5-HT_{1A} receptor activation and not via 5-HT_3 receptors. At the presynaptic level, the suppressant effect of 5-HT, 2-methyl-5-HT and 8-OH-DPAT applied by microiontophoresis on the firing activity of DR 5-HT neurons was not antagonized by the 5-HT_3 receptor antagonist BRL 46470A. In fact, only that of 8-OH-DPAT was attenuated by the 5-HT_{1A} receptor antagonist (+)WAY 100135. The failure of (+)WAY 100135 to prevent the suppressant effect of 5-HT is still puzzling. However, this discrepancy has been also observed by other groups (Piñeyro et al., 1995; Bel et al., 1994). In addition, Sprouse and Aghajanian (1987) have previously shown that the $5HT_{1A}$/ß-adrenergic antagonist (-)propranolol prevented the suppressant effect of 8-OH-DPAT or ipsapirone but not that of 5-HT, all drugs were microiontophoretically-applied. Moreover, *in vitro*, Adrien et al. (1992) have shown that the suppressant effect of 2-methyl-5-HT on the firing activity of DR 5-HT neurons was blocked by the 5-HT_{1A} receptor/ß-adrenoceptor antagonist l-propranolol, and we have shown *in vivo* that the suppressant effect of 5-HT was blocked by WAY 100635 (data not shown). It is thus suggested that the suppressant effect of 2-methyl-5-HT on the firing activity of DR 5-HT neurons is mediated via 5-HT_{1A} receptors. This assumption is also supported by the fact that immunolabelled 5-HT_3 receptors have not been detected in raphe nuclei (Morales et al., 1996b). In contrast *in vitro*, previous studies have shown that the activation of 5-HT_3 receptors increases 5-HT release in guinea-pigs and NA release in rats (Blier and Bouchard, 1993; Mongeau et al., 1994c). At the terminal level, only R-zacopride significantly reduced the duration of suppression of firing activity of CA_3 pyramidal neurons induced by the electrical stimulation of the ascending 5-HT pathway, and this attenuating effect was prevented by the three 5-HT_3/5-HT_4 antagonists renzapride, S-zacopride and tropisetron, but not by the selective 5-HT_3 antagonist BRL 46470A. This suggests that 5-HT_4 receptors are involved, as supported by a previous microdialysis study (Ge et al., 1992). On the other hand, *in vitro*, the enhancing action of 2-methyl-5-HT on the electrically-evoked release of [^{3}H]-5-HT in both frontal cortex and hippocampus slices is mediated by 5-HT_3 receptors. As mentioned above, both *in vivo* and *in*

vitro studies have shown that 5-HT_3 receptors are indirectly activated by SSRIs (Martin et al., 1992; Blier and Bouchard, 1994). It is assumed that the indirect activation of 5-HT_3 by SSRIs is responsible for the nausea and emesis observed in a significant percentage of patients using these drugs. While the use of 5-HT_3 receptor antagonists in the antiemetic therapy is well established (Veyrrat-Follet et al., 1997), their use in pharmacotherapy of anxiety and depressive disorders is far from compelling (Greenshaw and Silverstone, 1997). However, there are considerable amounts of preclinical data concerning their potential anxiolytic effects (see Introduction), but the exact mechanism through which these ligands mediate their anxiolytic activity remains unclear. Several possibilities can be envisaged. First, 5-HT_3 receptor antagonists may act through an indirect activation of the NA system. Although the presence of 5-HT_3 receptors on NA fibers has not yet been established, the activation of these receptors increases the release of NA, and this enhancement might contribute to the anxiolytic-like properties of 5-HT_3 receptor antagonists. However, this assumption remains very speculative as such a preponderant role of NA system in anxiety disorders is not generally accepted. Rather, an interaction with the GABA system is more probable. As previously noted, 5-HT_3 receptors are located on both cortical and hippocampal GABA interneurons (Morales et al., 1996a). Hence, it has been proposed that 5-HT_3 receptor antagonists could interact directly with the GABA/benzodiazepine complex rather than their own receptors (Klein et al., 1994). Since it has been suggested that the benzodiazepines produce their anxiolytic effects by enhancing the affinity of the $GABA_A$ receptors for GABA, one may speculate that 5-HT_3 receptor antagonists act as benzodiazepines. However, this has to be taken with caution since 5-HT_3 receptor antagonists possess only a micromolar affinity for the GABA/benzodiazepine complex (Klein et al., 1994). Finally, it has been recently proposed that the MAO inhibitor phenelzine produces anxiolytic effects in animal models by increasing brain GABA levels (Paslawski et al., 1996). This increase of GABA levels has been observed with 5-HT_4 receptor ligands. Indeed, preliminary microdialysis study has shown that the GABA release in the substantia nigra is tonically enhanced by 5-HT_4 receptors activation (Zetterström et al., 1996). By analogy, it may therefore useful to assess the effect of 5-HT_3 receptor antagonists on the GABA levels at least in the cortex and hippocampus (Morales et al., 1996a).

Although very few studies on the interactions between 5-HT and NA systems have been carried out, the characterization of these interactions may be crucial for a better understanding of the mechanism of action of antidepressant drugs. Hence, we were interested in the heteromodulation of 5-HT neurotransmission induced by the α_2-adrenoceptor antagonist mirtazapine, which belongs to a new class of antidepressants. The blockade of presynaptic α_2-adrenoceptors is considered as a possible mechanism for antidepressant activity (see: Pinder and Wieringa, 1993 for review). We have characterized in Chapter III and IV the acute and chronic effects of mirtazapine on 5-HT neurotransmission. Clinical studies have recently shown that short-term therapy with mirtazapine was significantly more effective than placebo, and as effective as amitriptyline, clomipramine, doxepine and trazodone (see: Davis and Wilde, 1996 for review). As mentioned in the introductory section, several classes of antidepressant treatments enhanced

5-HT neurotransmission and this enhancement occurred via different adaptive changes (see Table I). We have seen that antidepressant treatments that increase NA release in the synaptic cleft desensitize the terminal α_2-adrenergic heteroreceptors located on 5-HT fibers in the dorsal hippocampus (Mongeau et al., 1994a, 1994b). These receptors modulate 5-HT release and exert a negative feedback role on this release similar to the terminal 5-HT autoreceptors. Indeed, long-term antidepressant treatment with the reversible monoamine oxidase-A befloxatone and with the selective NA reuptake inhibitor nisoxetine, but not with either the selective 5-HT reuptake inhibitor paroxetine or with electroconvulsive shocks, desensitize the terminal α_2-adrenergic heteroreceptors but not the terminal α_2-adrenergic autoreceptors. Furthermore, it has been shown that a pretreatment with the neurotoxin 6-OHDA followed by a long-term treatment with befloxatone did not modify the function of the α_2-adrenergic heteroreceptors, suggesting that an intact NA input is required for this desensitization to occur (Mongeau et al., 1994b).

Using microdialysis in freely moving rats, systemic administration of mirtazapine increases both 5-HT and DOPAC release in the ventral hippocampus (De Boer et al., 1996). It has been suggested that both the indirect α_1-adrenoceptor-mediated enhancement of 5-HT neuron firing activity and the direct blockade of inhibitory α_2-adrenergic heteroreceptors located on 5-HT terminals are responsible for this increase in extracellular 5-HT. At the level of the NA and 5-HT terminals of the dorsal hippocampus, acute and long-term administration with mirtazapine antagonized both the enhancing effect of a low dose and the attenuating effect of a high dose of the α_2-adrenoceptor agonist clonidine on the effectiveness of the electrical stimulation of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus CA_3 pyramidal neurons. This suggests that acute and long-term administration of mirtazapine blocked both terminal α_2-adrenergic auto- and heteroreceptors in the dorsal hippocampus. After a 48 h washout, only the effect of the high dose of clonidine was attenuated, suggesting a desensitization of the terminal α_2-adrenergic heteroreceptors, but not of the terminal α_2-adrenergic autoreceptors. Moreover, the reduced effectiveness of the stimulation upon increasing its frequency from 1 to 5 Hz (due to a greater activation of terminal 5-HT autoreceptors) was unaltered by the long-term mirtazapine treatment. This lack of effect of long-term mirtazapine on the terminal 5-HT autoreceptor is interesting in the light of the observation that the (-)enantiomer of mirtazapine appears to be endowed with agonistic activity at this autoreceptor. Indeed, (-)mirtazapine (25 μg/kg, i.v.) reduced the duration of suppression of firing of CA_3 pyramidal neurons induced by the stimulation of the 5-HT pathway, and this effect was prevented by the terminal 5-HT autoreceptor antagonist metergoline (Chapter III).

In the DRN, pharmacological studies have shown that, *in vivo*, the firing activity of 5-HT neurons is dependent on a tonic activation by their adrenergic input (Svensson et al., 1975). The inhibitory action of clonidine on these neurons was shown to be due to the activation of α_2-adrenergic autoreceptors decreasing the endogenous NA excitatory input on α_1-adrenergic receptors located on 5-HT neurons (Svensson et al., 1975; Baraban and Aghajanian, 1980; Clement et al., 1992a,b). We have seen that the acute administration of mirtazapine dose-dependently enhanced the firing activity of 5-HT neurons, whereas (-)mirtazapine failed to change the firing rate of DR 5-HT neurons, suggesting that only the (+) enantiomer possess α_2-adrenergic

autoreceptor antagonistic property. Moreover, only (±) mirtazapine antagonized the suppressant effect of clonidine on the firing activity of 5-HT neurons. The enhancement of the firing activity of 5-HT neurons by mirtazapine was abolished in 6-OHDA-pretreated rats revealing the existence of a tonic activation of the terminal α_2-adrenergic autoreceptors in the DRN (see figure 1 for the location of receptors). In contrast with the effects of SSRIs (Artigas et al., 1996), a two-day treatment with (-)pindolol failed to modify the enhancing effect of acutely administered mirtazapine, suggesting that the incremental effect of mirtazapine on the firing activity of DR 5-HT neurons did not result in a physiologically significant activation of somatodendritic 5-HT_{1A} autoreceptors. On the other hand, the spontaneous firing activity of DR 5-HT neurons was markedly increased in long-term mirtazapine-treated rats, and was back to normal 48 h after removal of the minipumps. Taken together, these results suggest that the concomitant administration of pindolol and mirtazapine may not result in a more rapid onset of the antidepressant action of mirtazapine. This assumption is based in the acceleration of the effect of SSRIs by pindolol attributable to the capacity of this drug to prevent the initial decrease of the firing activity of 5-HT neurons produced by SSRIs (see section 5.2.3). Consequently, given that mirtazapine increases the firing activity of 5-HT neurons and (-)pindolol does not modify this effect, it can be expected that these two drugs administered together should not result in a potentiation of the antidepressant effect of mirtazapine. The dose-response curve of the suppressant effect clonidine on the firing activity of 5-HT neurons was not altered significantly in mirtazapine-treated rats with the minipump on board. However, it was markedly shifted to the left after a 48 h washout. Such a sensitization of terminal α_2-adrenergic autoreceptors has also been observed following long-term treatments with the α_2-adrenergic antagonists mianserin and idazoxan (Sugrue, 1980; Cerrito and Raiteri, 1981; Raiteri et al., 1983; Dickinson et al., 1989). One might assume that such changes could result from an increase in the density of terminal α_2-adrenergic autoreceptors as consequence of their sustained blockade.

In the LC, selective α_2-adrenoceptor antagonists increase the spontaneous firing activity of NA neurons whereas clonidine decreases it, with both actions being mediated via somatodendritic α_2-adrenergic autoreceptors (Baraban and Aghajanian, 1980; Marwaha and Aghajanian, 1982; Curtis and Valentino, 1991). Acute administration of mirtazapine significantly increased the spontaneous firing activity of LC NA neurons, and caused a parallel shift to the right on the dose-response curve of the effect of clonidine on the firing activity of NA neurons, suggesting that mirtazapine is an antagonist of the somatodendritic α_2-adrenergic autoreceptors. On the other hand, a 21-day treatment with mirtazapine increased in a sustained manner the spontaneous firing activity of LC NA neurons. Their firing activity was back to normal 48 hours after removing the minipumps. However, this treatment did not modify the dose-response curve of the α_2-adrenoceptor agonist clonidine on the firing activity of NA neurons. Although further experiments are needed to clarify this phenomenon, one may assume that if this treatment, by the sustained blockade of the somatodendritic α_2-adrenergic autoreceptors, produces an up-regulation of these autoreceptors, as speculated in the DRN, then this should induce an hypersensitization of the latter receptors to clonidine. The fact that the suppressant effect of clonidine does not change during and after mirtazapine treatment is puzzling, but one possible explanation could be a modification of the

number and/or the affinity of the NAT in the LC (see section 3.4.1.). Thus, it will be useful to assess the sensitivity and/or the number of the NAT in LC following mirtazapine treatment to clarify this discrepancy.

At the postsynaptic level, the acute, but not the long-term, administration of mirtazapine antagonized the activation of postsynaptic α_2-adrenergic receptors in the dorsal hippocampus. This might be due to the lower brain concentration of mirtazapine during the long-term treatment since acute administration of a relatively high dose of mirtazapine (0.5 mg/kg, i.v.) was required to antagonize postsynaptic α_2-adrenergic receptors.

In conclusion, the sustained enhancement of the firing activity of 5-HT and NA neurons produced by mirtazapine and the desensitization of α_2-adrenergic heteroreceptors on 5-HT terminals might underlie, at least in part, the antidepressant activity of this drug. One may assume that the tonic activation of postsynaptic 5-HT_{1A} receptors is enhanced after a 21-day treatment with mirtazapine (see chapter VII), as a result of a sustained increase in 5-HT neuronal firing activity in the presence of inactivated α_2-adrenergic heteroreceptors on 5-HT terminals. Since mirtazapine, as for all antidepressant drugs, has a delayed onset of action, it is possible that the development of this latter adaptive change may be related to its antidepressant activity. Finally, these studies have allowed us to consider a new therapeutic strategy: the combination of mirtazapine and paroxetine. Preliminary results in rats showed that this combination accelerates the recovery time of firing activity of 5-HT neurons, presumably by increasing the rate of desensitization of somatodendritic 5-HT_{1A} receptors (data not shown). This polypharmacotherapy will also be undertaken in humans in the near future and we hope that the combination of mirtazapine and paroxetine will reduce the latency of the antidepressant response.

Another study was undertaken in an attempt to further characterize the interactions between 5-HT and NA systems. As mentioned in section 4.2.1., several lines of evidence support the notion that the 5-HT system also influences brain NA neurons. An important drawback of various pharmacological agents used to study the interactions between 5-HT-NA systems is the lack of selectivity of these drugs. We have seen that both local (by microiontophoresis) and systemic (100 µg/kg, i.v.) administration of WAY 100635 antagonized the suppressant effect of microiontophoretically-applied 5-HT on the firing activity of dorsal hippocampus CA_3 pyramidal neurons, indicating its antagonistic effect on postsynaptic 5-HT_{1A} receptors. In the LC, WAY 100635 and 5-HT failed to modify the spontaneous firing activity of NA neurons when applied by microiontophoresis. However, the intravenous injection of WAY 100635 (100 µg/kg) readily suppressed the spontaneous firing activity of LC NA neurons. Lesion of 5-HT neurons with the neurotoxin 5,7-DHT increased the spontaneous firing activity of LC NA neurons and abolished the suppressant effect of WAY 100635 on the firing activity of LC NA neurons. These data support the notion that the 5-HT system modulates tonically the firing activity of NA neurons since the suppressant effect of WAY 100635 on the firing activity of LC NA neurons was abolished by the lesion of 5-HT neurons. Taking into account the affinities for the different 5-HT receptor subtypes of the drugs used in this study and the fact that prazosin prevented the effect of WAY 100635, it has been suggested that the suppressant effect of WAY 100635 on the firing

activity of LC NA neurons is mediated via 5-HT_{1A} receptors, but not necessarily, located presynaptically to the NA neurons. It is also possible that postsynaptic 5-HT_{2A} receptors may be involved in the suppressant effect of WAY 100635 on the firing activity of LC NA neurons. Using microdialysis in freely moving rats, Bosker et al. (1996) have recently shown that WAY 100635 (> 50 μg/kg, s.c) tended to increase extracellular 5-HT in the median raphe and the dorsal hippocampus and we have shown that WAY 100635 (100 μg/kg, i.v.) increased the duration of suppression of firing (corresponding to the endogenous 5-HT release) of dorsal hippocampus CA_3 pyramidal neurons induced by the electrical stimulation of the ascending 5-HT pathway. It is thus conceivable that the effect of WAY 100635 on the firing activity of LC NA neurons could be due to a direct blockade of presynaptic 5-HT_{1A} receptors leading to an increase of 5-HT in the LC, which results ultimately in the activation of postsynaptic 5-HT_{2A} receptors. It is also possible that this suppressant effect of WAY 100635 on the LC NA neurons firing could be achieved through the 5-HT-mediated suppression of the excitatory glutamatergic input from the paragigantocellularis nucleus to LC neurons, as previously documented by Aston-Jones et al. (1991). Finally, the fact that WAY 100635 inhibits the firing activity of LC NA neurons and produces in turn a reduction of the NA excitatory input on DR 5-HT neurons may account for its lack of effect on the firing rate of 5-HT neurons. We have observed that the systemic administration of WAY 100635 prevented the reducing action of clonidine on the firing activity of DR 5-HT neurons. One may assume that the reduction of NA excitatory input on DR 5-HT neurons could mask the tonic activation of somatodendritic 5-HT_{1A} receptors by endogenous 5-HT after systemic administration of WAY 100635. To confirm this possibility, it will be useful to determine whether systemic administration of WAY 100635 increases the firing activity of DR 5-HT neurons in 6-OHDA-pretreated rats.

As mentioned in the introduction, because of the non negligible side-effects (cheese-effect) of classical MAOIs (Murphy et al., 1987), a second-generation of reversible MAO-A inhibitors has been recently developed (Thase et al., 1995). At the somatodendritic level, we have seen that befloxatone (0.75 mg/kg/day x 2 days) decreased the firing activity of DR 5-HT neurons. This suggests that short-term treatment with befloxatone produces an inhibition of MAO-A in the DR and consequently enhances the synaptic availability of 5-HT with subsequent activation of somatodendritic 5-HT_{1A} autoreceptors. Moreover, an increase in the firing activity of 5-HT neurons was observed in rats treated with both befloxatone and (-)pindolol and after i.v administration of the 5-HT_{1A} receptor antagonist WAY 100635 in rats treated with befloxatone alone for 2 days. Although, the reason for the increase in firing activity above baseline is still unclear, preliminary data from Fornal et al. (1995) suggest that WAY 100635 blocks the action of endogenous 5-HT at 5-HT_{1A} autoreceptors in the cat DRN, and also reverses the inhibition of the firing rate of 5-HT neurons produced by the 5-HT reuptake blocker fluoxetine above baseline value. After the 2-day treatment with befloxatone, the decrease in firing activity of 5-HT neurons was two times lower in comparison to the reduction of 5-HT firing activity using either phenelzine (nonselective MAO inhibitor), clorgyline (irreversible MAO-A inhibitor) or amiflamine (reversible MAO-A inhibitor) (Blier et al., 1986a, 1986b; Blier and de Montigny,

1987a). These results may be related to the fact that 5-HT_{1A} autoreceptors on DRN 5-HT neurons are perhaps already inactivated only after a 2-day treatment with befloxatone. In fact, the responsiveness of the somatodendritic 5-HT_{1A} autoreceptors to the administration of the agonists LSD, 5-HT and 8-OH-DPAT was decreased by about two-fold. A possible explanation for this early inactivation could be related to the interaction between α_1-adrenoceptors and 5-HT_{1A} receptors, as observed in vitro by Hensler et al. (1996). Using P11 cells expressing 5-HT_{1A}, 5-HT_{2A} and α_1-adrenergic receptors, this group showed that the activation of α_1-adrenoceptors, but not that of 5-HT_{2A} receptors, desensitizes 5-HT_{1A} receptors, as revealed by the decrease of potency of 8-OH-DPAT to inhibit forskolin-stimulated cAMP accumulation (Hensler et al., 1996). However, this assumption should be extrapolated with caution since the somatodendritic 5-HT_{1A} receptor activation does not modify the forskolin-stimulated adenylate cyclase activity (Clarke et al., 1996). Similar results have also been obtained by Le Poul et al. (1995) using SSRIs. This group has shown that after 3 days of treatment with fluoxetine or paroxetine, followed by a one day washout period, the potency of 8-OH-DPAT to suppress the firing of DR 5-HT neurons (neurons activated with α_1-adrenoceptor agonist phenylephrine) recorded from brain slices was significantly reduced. Moreover, these treatments failed to modify the specific 5-HT_{1A} binding sites in the DRN or in other brain areas as measured with either [^{3}H]8-OH-DPAT or with [^{3}H]WAY 100635. The authors have suggested that, already after a 3-day treatment with SSRIs, an adaptive desensitization of the somatodendritic 5-HT_{1A} autoreceptors within the DRN had already taken place without any change in the number of 5-HT_{1A} binding sites. The latter phenomenon (*i.e.* desensitization of DR 5-HT_{1A} autoreceptors without any reduction in the number of the 5-HT_{1A} binding sites which is likely due to uncoupling of the receptor from its transduction system) has also been observed following long-term treatment with the partial 5-HT_{1A} receptors agonist ipsapirone (Schechter et al., 1990). On the other hand, it has been recently shown that the levels of G_o and G_{i2} proteins in the midbrain are reduced by a 3-day treatment with fluoxetine and that this decrease remained at the same level for 22 days of treatment. Because G_o proteins are coupled with 5-HT_{1A} autoreceptors, the decrease of their levels may be associated with the desensitisation of these autoreceptors (Li et al., 1996). However, this reduction of G proteins level is unlikely due exclusively to these autoreceptors, as other receptors coupled to $G_{o/i}$ proteins present in the midbrain could be also involved. For instance, although short-term treatments have not been performed, it has been recently shown that long-term treatments with paroxetine and befloxatone desensitize midbrain 5-HT_{1D} receptors (Piñeyro and Blier, 1996). In contrast to long-term treatment with SSRIs (see Blier and de Montigny, 1994), we have seen that the early inactivation of the somatodendritic 5-HT_{1A} autoreceptors is reversible since the 21-day treatment with befloxatone modified neither the suppressant effect of the autoreceptor agonist LSD administered intravenously, nor 5-HT and 8-OH-DPAT applied by microiontophoresis. This unexpected result is not due to the incomplete inhibition of MAO-A by befloxatone since the extent of MAO inhibition was similar in rats treated for 2- and 21 days with the drug. Furthermore, an increase in brain levels of 5-HT was also present in rats treated for 21 days with befloxatone, although the magnitude of this increase was somewhat lower than after a two-day treatment. One possible explanation for such normalized 5-HT neuronal firing activity and

normosensitive 5-HT_{1A} autoreceptors following sustained MAO inhibition may be the adaptive change of the α_2-adrenergic receptors in the DRN controlling the firing activity of 5-HT neurons. As previously noted, the inhibitory action of the α_2-adrenergic agonist clonidine on the firing activity of 5-HT neurons is suggested to be due to the activation of somatodendritic and terminal α_2-adrenergic autoreceptors decreasing the endogenous NA excitatory drive onto α_1-adrenergic receptors located on 5-HT neurons (Fig. 1). Thus, in rats treated with befloxatone for 21 days, the suppressant effect of clonidine on the firing activity of 5-HT neurons was markedly attenuated, suggesting a desensitization of α_2-adrenergic autoreceptors. However, it was previously shown that the MAO-A inhibitors clorgyline and amiflamine do not alter the responsiveness of NA neurons in the LC to systemic administration of clonidine (Blier et al., 1986a, 1986b). This suggests that the sustained blockade of MAO-A activity by befloxatone, as is the case with clorgyline and amiflamine, decreased the activity of NA neurons by maximally 40% (O. Curet, personal communication), consequently leaving little modulation to be exerted by clonidine. The latter possibility is all the more likely as an attenuated NA activation of 5-HT neurons, resulting from the lesion of NA neurons or the blockade of α_1-adrenoceptors, initially suppresses the firing activity of 5-HT neurons, but this parameter returns to normal within a few days (Baraban and Aghajanian, 1980, Blier et al., 1986a).

In the CA_3 region of the dorsal hippocampus, the short- and long-term treatment with befloxatone, as well as the 2-day treatment with both befloxatone and (-)pindolol, did not modify the responsiveness of postsynaptic 5-HT_{1A} receptors to microiontophoretic application of 5-HT, indicating that the sensitivity of these receptors remains unchanged following such treatments. However, WAY 100635 (100 μg/kg, i.v.), in contrast to (-)pindolol (Romero et al., 1996), antagonized the suppressant effect of microiontophoretically-applied 5-HT onto CA_3 pyramidal neurons in both control and treated rats, thus showing its capacity to block 5-HT_{1A} receptors on the cell body of CA_3 pyramidal neurons.

At the terminal level, we have seen that WAY 100635 (100 μg/kg, i.v.) failed to decrease the duration of suppression of firing of dorsal hippocampus CA_3 pyramidal neurons induced by the electrical stimulation of the ascending 5-HT pathway in control rats and in rats treated with befloxatone alone and in combination with (-)pindolol for both 2 days, as well as after a 21-day treatment with befloxatone. This suggests that WAY 100635 failed to block the intrasynaptic 5-HT_{1A} receptors of CA_3 pyramidal neurons, as is the case with the lack of effect of pertussis toxin on this population of postsynaptic 5-HT_{1A} receptors (Blier et al., 1993; Hadrava et al., 1994). WAY 100635 (100 μg/kg, i.v.), in fact, even increased the efficacy of the stimulation. Another contributory factor to this unexpected effect of WAY 100635 could be due to its suppressant effect on the spontaneous firing activity of LC NA neurons (Chapter IV). This suppressant effect of WAY 100635 on the firing of LC NA neurons induces presumably a decrease of NA release in the synaptic cleft in the dorsal hippocampus. Consequently, as demonstrated in previous studies (Mongeau et al., 1993, 1994a,b), the decrease of endogenous NA release reduces the tonic activation of α_2-adrenergic heteroreceptors, located on 5-HT fibers in the dorsal hippocampus, which in turn leads to an enhancement of the endogenous release of 5-HT induced by the electrical stimulation of the ascending 5-HT pathway. To confirm this assumption it will be useful

to assess the effect of systemic administration of WAY 100635 on the effectiveness of the stimulation of the 5-HT pathway in 6-OHDA-pretreated rats.

Long-term treatment with befloxatone does not affect the responsiveness of terminal 5-HT autoreceptors in the hippocampus and hypothalamus of guinea-pigs measured *in vitro* in brain slices (Blier and Bouchard, 1994). In contrast, we have seen that the decrease in the effectiveness of the stimulation upon increasing its frequency from 1 to 5 Hz (due to the activation of terminal 5-HT autoreceptors) was attenuated after a 2-day treatment with befloxatone alone. As was the case with the somatodendritic 5-HT_{1A} autoreceptors, these results suggest that befloxatone administration produced an early inactivation of terminal 5-HT_{1B} autoreceptors. This contention is further supported by the loss of the enhancing effect of the 5-HT autoreceptor antagonist metergoline on the efficacy of the stimulation in rats treated with befloxatone for 2 days. This inactivation was transient, as indicated by the return to a normal pattern of the 1-5 Hz stimulations after 21 days of treatment with befloxatone. The reason of the lack of persistence of the inactivation of the terminal 5-HT_{1B} autoreceptors remains to be explained.

In rats treated for 2 days with befloxatone alone or in combination with (-)pindolol, as well as in rats treated with befloxatone for 21 days, the i. v. administration of WAY 100635 enhanced the firing activity of CA_3 pyramidal neurons. When WAY 100635 is injected systemically, it does not enhance the firing rate of 5-HT neurons in control anesthetized rats (Fletcher et al., 1994; Fletcher et al., 1996; data not shown), but it will reverse the suppressant effect of an enhanced activation of 5-HT_{1A} autoreceptors resulting from an increased synaptic availability of 5-HT itself or of an exogenous 5-HT_{1A} receptor agonist. Consequently, in rats treated with befloxatone for two days, one cannot conclude that there is an enhanced activation of postsynaptic 5-HT_{1A} receptors, which exert an inhibitory effect on the firing of CA_3 pyramidal neurons, since the firing activity of 5-HT neurons is attenuated before the administration of WAY 100635 in these rats. However, in rats treated with befloxatone and (-)pindolol for two days, as well as in rats treated with befloxatone alone for 21 days, the firing activity of 5-HT neurons is not affected, and WAY 100635 markedly enhanced the firing activity of CA_3 pyramidal neurons. Therefore, it can be concluded that the latter two treatments produced an enhanced tonic activation of postsynaptic 5-HT_{1A} and possibly of other 5-HT receptors.

In conclusion, the short-term administration of befloxatone reduces the firing activity of 5-HT neurons which is followed by a recovery after 21 days of sustained administration. This sequence of events correlates well with the delayed onset of MAOIs in major depression. Given that the firing activity of 5-HT neurons is even above the normal value following a two-day treatment with befloxatone and (-)pindolol, this combined treatment could be expected to produce the same effect on 5-HT neurotransmission as after long-term treatment with befloxatone alone.

The last series of experiments were undertaken to assess the tonic activation of postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus following chronic antidepressant administration. We have chosen several classes of antidepressants drugs which enhance 5-HT neurotransmission via different adaptive changes (see table I). As describes in section 5.2., the enhancement of 5-HT neurotransmission following long-term antidepressant treatments has been

demonstrated *in vivo* by both microdialysis and electrophysiological (by the stimulation of 5-HT pathway paradigm) studies. We report here the first direct functional evidence of an increase activation of postsynaptic 5-HT_{1A} receptors by antidepressant treatments, thus confirming the enhancement of 5-HT neurotransmission by several classes of antidepressant treatments. At least *in vivo*, none of the drugs used in this study modified the sensitivity of the dorsal hippocampus postsynaptic 5-HT_{1A} receptors. Moreover, neither befloxatone, mirtazapine nor gepirone modified the effectiveness of the electrical stimulation of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus CA_3 pyramidal neurons, whereas paroxetine treatment increase this effectiveness, presumably as a result of the desensitisation of terminal 5-HT_{1B} autoreceptors (Blier and de Montigny, 1987b; Chaput et al., 1991; See chapter IV and VI). In rats treated for 3 weeks with either paroxetine, befloxatone, mirtazapine or for 2 weeks with gepirone, WAY 100635 induced a marked increase in the firing activity of dorsal hippocampus pyramidal neurons, while in control rats this firing remain unchanged. It is suggested that, in rats treated with paroxetine, this enhancement of the 5-HT neurotransmission is due to an increase of synaptic 5-HT concentration in the dorsal hippocampus resulting from the desensitization of somatodendritic and terminal 5-HT autoreceptors (Blier and de Montigny, 1994), as well as the down-regulation of the neuronal 5-HT carrier (Piñeyro et al., 1994). In the case of rats treated with befloxatone, this could be due to an increase in the synaptic availability of 5-HT in the dorsal hippocampus induced by the inhibition of the MAO-A or due to the release of 5-HT not being dampened as a result of the desensitization of terminal α_2-adrenoceptors located on 5-HT fibers (Blier and Bouchard, 1994; Mongeau et al., 1994b). In the case of rats treated with mirtazapine, this could be the consequence of increased 5-HT neurotransmission due to the sustained increase of the firing activity of 5-HT neurons and the desensitization of terminal α_2-adrenoceptors located on 5-HT fibers, leading to an increase of the endogenous 5-HT release in the dorsal hippocampus (see chapter IV). Finally, in the case of rats treated with gepirone, this could be due to a normalization of the synaptic availability of 5-HT, resulting from desensitization of somatodendritic 5-HT_{1A} autoreceptors, in the presence of exogenous 5-HT_{1A} receptor agonist activating normosensitive postsynaptic 5-HT_{1A} receptors (Blier and de Montigny, 1994).

In conclusion, it has been demonstrated that chronic treatments with either the selective and reversible MAO-A inhibitor befloxatone, the SSRI paroxetine, the α_2-adrenergic antagonist mirtazapine, or with the 5-HT_{1A} receptor agonist gepirone enhance the tonic activation of postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus, as demonstrated by the enhancing effect of the administration of the 5-HT_{1A} receptor antagonist WAY 100635. These results constitute further direct evidence that an enhanced 5-HT neurotransmission may underlie the antidepressant response in humans. Positron emission tomography studies, using [^{11}C]WAY 100635, have been recently performed in humans in order to evaluate the distribution of brain 5-HT_{1A} receptors (Pike et al., 1996). Hence, PET studies evaluating brain activations (such cerebral blood flow) will be useful to determine whether such tonic activation of postsynaptic 5-HT_{1A} receptors is enhanced following antidepressant treatments in humans.

Figure 1: Hypothetical schema of the anatomical connections and functional interactions of dorsal raphe nucleus (5-HT), locus coeruleus (NE) and dorsal hippocampus (CA_3) in the rat. The activity of dorsal raphe 5-HT neurons is dependent on the tonic activation by the adrenergic input from LC via α_2-adrenergic autoreceptors on NE terminals (although there is no definite evidence that α_1-adrenoceptors are located on somatodendritic level of dorsal raphe 5-HT neurons) and tonic autoinhibition by somatodendritic 5-HT_{1A} autoreceptors. The activity of LC NE neurons is mainly dependent on the tonic autoinhibition by somatodendritic α_2-adrenergic autoreceptors and the tonic inhibitory input from 5-HT fibers. At the level of dorsal hippocampus, the amount of NE and 5-HT is negatively regulated by terminal α_2-adrenergic and 5-HT_{1B} autoreceptors respectively and endogenous NE dampers 5-HT release through tonic activation of α_2-adrenergic heteroreceptors.

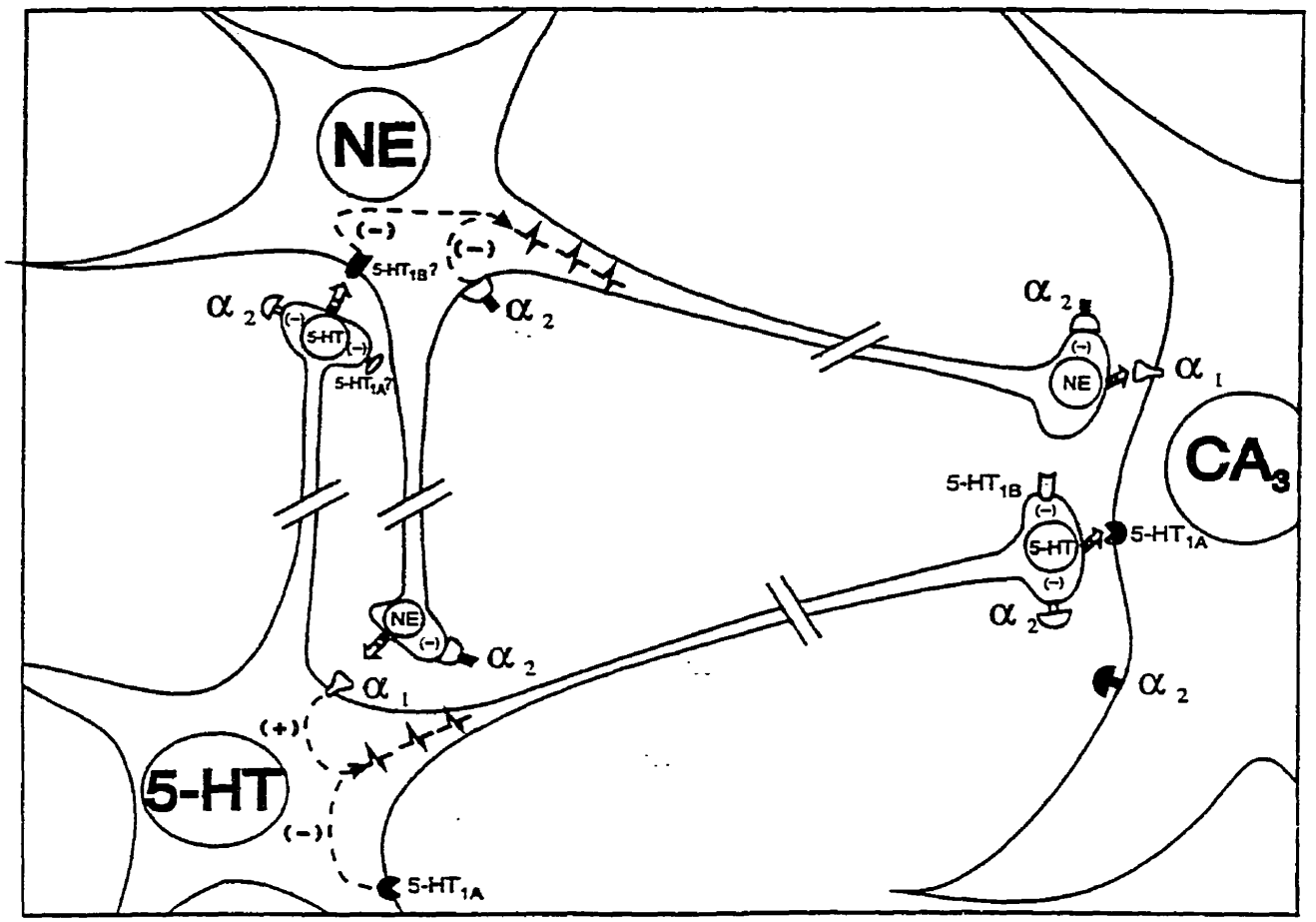


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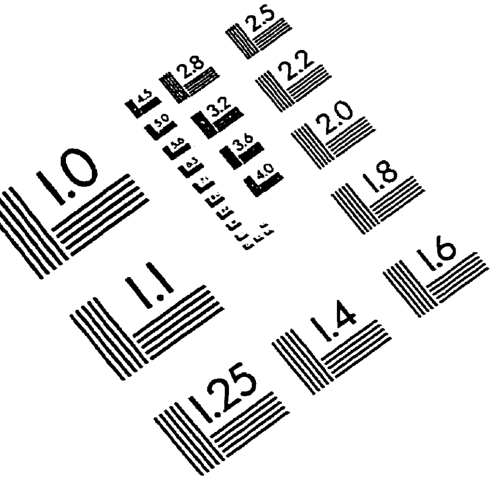
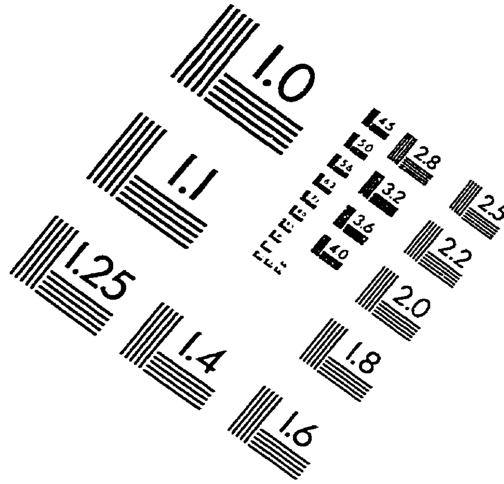
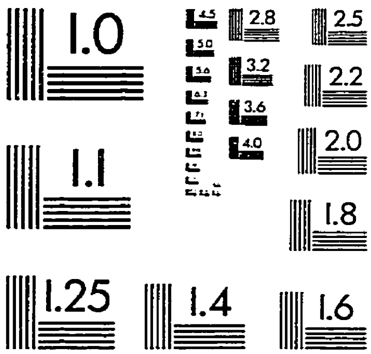
IMAGE EVALUATION TEST TARGET (QA-3)







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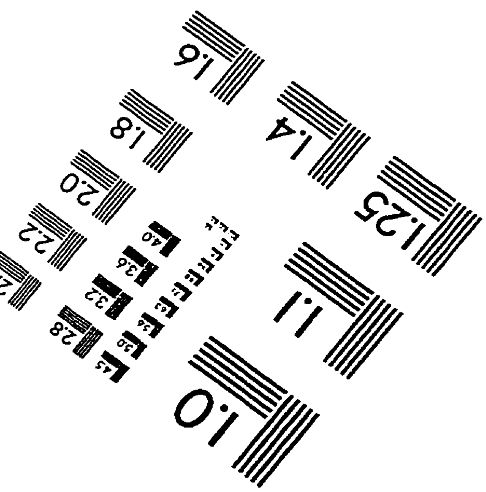

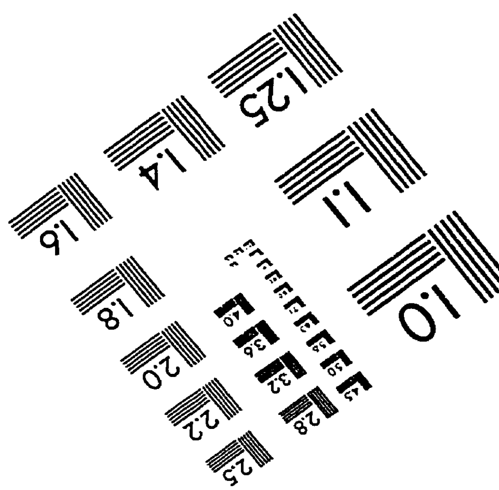

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