The Journal of Neuroscience, May 1994, 14(5): 3036–3047

Desensitization of the Neuronal 5-HT Carrier following Its Long-Term Blockade

Graciela Piñeyro, Pierre Blier, Trevor Dennis, and Claude de Montigny

Neurobiological Psychiatry Unit, McGill University, Department of Psychiatry, Montréal, Québec, Canada H3A 1A1

***In vivo* extracellular unitary recordings, *in vitro* ^{3}H-5-hydroxytryptamine (5-HT) uptake, and ^{3}H-paroxetine binding assays were used to assess the effect of acute and long-term administration of the 5-HT reuptake inhibitor paroxetine on the neuronal 5-HT transporter in the rat dorsal hippocampus. Recovery time of the firing activity of CA_3 hippocampus pyramidal neurons following microiontophoretic application of 5-HT was used as an index of *in vivo* reuptake activity. In a first series of experiments, the acute intravenous administration of paroxetine and 5-HT denervation with the neurotoxin 5,7-dihydroxytryptamine produced a marked prolongation of the suppressant effect of 5-HT, indicating that reuptake into 5-HT terminals plays a significant role in terminating the action of microiontophoretically applied 5-HT. In a second series of experiments, rats were treated with paroxetine (10 mg/kg/d, s.c.) for 2 or 21 d. In both treatment groups, there was a marked prolongation of the effect of microiontophoretically applied 5-HT; however, in rats treated for 2 d, the prolongation was significantly greater than that observed in rats treated for 21 d. After the 21 d treatment with paroxetine and a 48 hr washout, the prolongation of the effect of microiontophoretically applied 5-HT by acute intravenous paroxetine was significantly reduced, suggesting a decrease in the number of 5-HT carriers. In keeping with this interpretation, following the same treatment regimen, there was a 50% and 60% reduction of the *in vitro* ^{3}H-5-HT uptake in hippocampal and dorsal raphe slices, respectively, and a reduced effectiveness of paroxetine in blocking ^{3}H-5-HT uptake in both regions. The determination of the binding parameters of ^{3}H-paroxetine revealed that, in rats treated for 21 d with paroxetine (10 mg/kg/d, s.c.), following a 48 hr washout K_d values were unchanged but B_{max} values were reduced by 70% and 60% in hippocampal and cortical membranes, respectively.**

[Key words: 5-HT uptake, 5-HT transporter, 5-HT carrier, ^{3}H-paroxetine binding, downregulation, rat hippocampus]

Received June 29, 1993; revised Oct. 18, 1993; accepted Oct. 26, 1993.

We thank Raymond Mongeau for friendly advice throughout this work. We are grateful to Claude Bouchard and Normand Lavoie for technical assistance, Than Vo for computer programming, and Helen Cameron and Lise Martin for secretarial assistance. This work was supported by grants from the Medical Research Council of Canada (MRC) and Fonds de la Recherche en Santé du Québec (FRSQ). G.P. is a receipt of a Royal Victoria Hospital Research Institute Fellowship, P.B. of an MRC Scholarship, and T.D. of an FRSQ Scholarship.

Correspondence should be addressed to Graciela Piñeyro, Neurobiological Psychiatry Unit, McGill University, Department of Psychiatry, 1033 Pine Avenue West, Montréal, Québec, Canada H3A 1A1.

Copyright © 1994 Society for Neuroscience 0270-6474/94/143036-12$05.00/0

The 5-hydroxytryptamine (5-HT) reuptake process and 5-HT transporter have been widely studied *in vitro* using both ^{3}H-5-HT uptake and radioligand binding techniques (Marcusson and Ross, 1990). In contrast, the characterization of 5-HT reuptake activity *in vivo* has not been so extensive, as studies have been mostly concerned with physiological, behavioral, or neurochemical consequences of reuptake inhibition (Fuller and Wong, 1990; Johnson, 1991). Using an *in vivo* electrophysiological paradigm, Wang et al. (1979) reported that 5-HT reuptake plays a significant role in terminating the action of microiontophoretically applied 5-HT onto lateral geniculate and amygdaloid neurons. However, the 5-HT reuptake process did not appear to play an important role in terminating the suppressant effect of microiontophoretically applied 5-HT onto CA_3 pyramidal neurons (de Montigny et al., 1980).

Reports on the occurrence of a downregulation of the 5-HT transporter following long-term antidepressant treatment are controversial. Radioligand binding studies indicate that the neuronal 5-HT transporter is associated with ^{3}H-imipramine (Langer and Raisman, 1983) and ^{3}H-paroxetine binding sites (Habert et al., 1985; Mellerup and Plenge, 1986) and that repeated administration of several classes of antidepressant drugs downregulate ^{3}H-imipramine (Plenge and Mellerup, 1982; Brunello et al., 1987), but not ^{3}H-paroxetine, binding sites (Graham et al., 1987; Cheetham et al., 1991; Foy et al., 1991). This controversy may stem in part from the observation that while ^{3}H-paroxetine binds to a single population of sites selectively located on 5-HT neurites (Marcusson et al., 1988; Hrdina et al., 1990), ^{3}H-imipramine has been shown to label a heterogeneous population of sites in brain tissue (Reith et al., 1983; Marcusson et al., 1985). Recently, Kovachich et al. (1992), using ^{3}H-cyanoimipramine to label the 5-HT transporter, have concluded that different types of antidepressants do not exert consistent effects on the density of 5-HT reuptake sites.

The present study was thus undertaken to address the following issues that remained unsolved: (1) to devise a reliable method for measuring electrophysiologically *in vivo* 5-HT reuptake activity; (2) to assess functionally the possibility of a desensitization of the 5-HT carrier following long-term reuptake blockade; and (3) to correlate functional modifications with changes in the ^{3}H-paroxetine binding parameters following long-term reuptake blockade with paroxetine.

Materials and Methods

Treatments. Male Sprague–Dawley rats (175–200 gm) were implanted subcutaneously with an osmotic minipump (Alza, Palo Alto, CA) that delivered 10 or 20 mg/kg/d of paroxetine (SmithKline Beecham, Harlow, England) for 2 or 21 d. The drug was dissolved in a 50% ethanol: water solution, and control rats were implanted with a minipump containing the vehicle. Unless otherwise specified, the experiments were

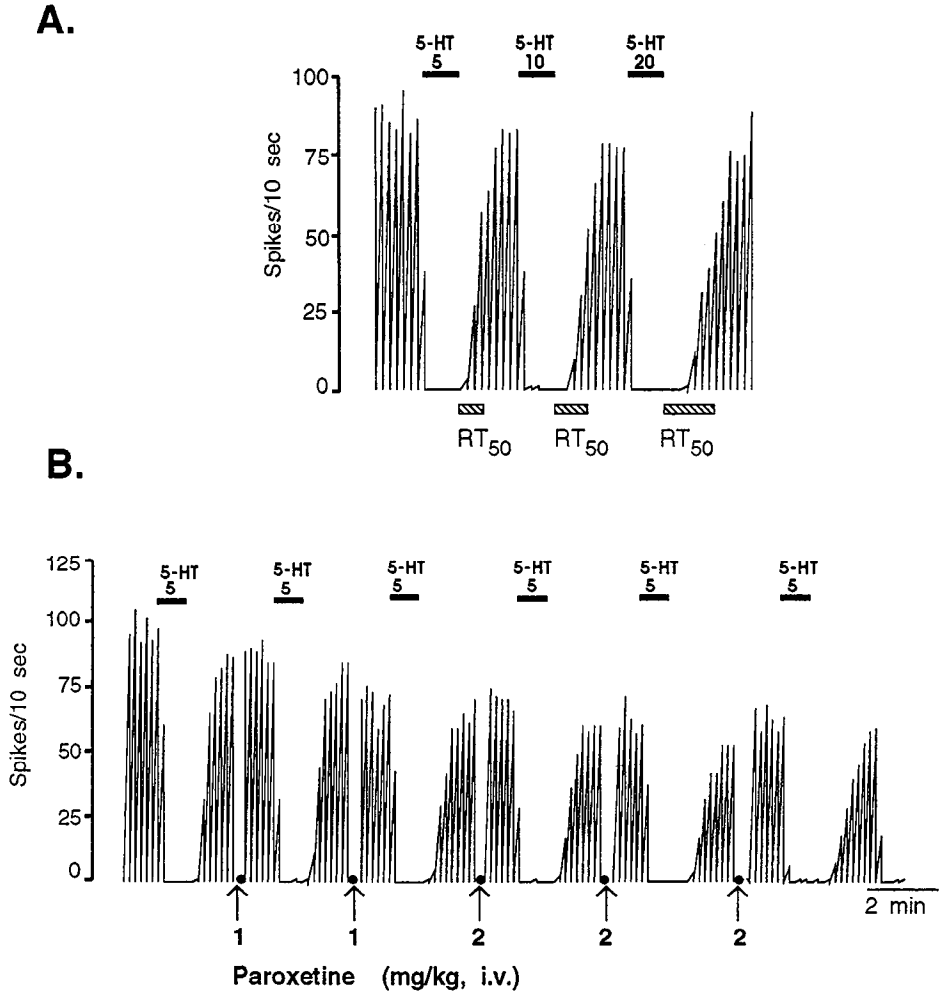

Figure 1. Integrated firing rate histograms showing the response of a representative dorsal hippocampus CA_3 pyramidal neuron to increasing microiontophoretic currents of 5-HT (*A*), and the effect of successive injections of paroxetine at 10 min intervals on the response to microiontophoretic application of 5-HT (*B*). The *solid bars above each trace* indicate the duration of application for which the ejection currents are given in nA. The *hatched bars below the histogram in A* indicate RT_{50}. This value is the time in seconds required by a neuron to recover by 50% its initial firing frequency calculated from the termination of the microiontophoretic application of drugs (*left edge of the hatched bar*). The *dots at the bottom of the histogram in B* represent periods of 10 min. Time scale applies to both traces.

carried out with the osmotic minipump in place. Two weeks before the experiments, another series of rats received an intracerebroventricular injection of 5,7-dihydroxytryptamine (5,7-DHT; 200 μg of free base in 20 μl of 0.9% NaCl and 0.1% ascorbic acid). This treatment has been shown to reduce 5-HT content in CA_3 region by at least 90% (Gerson and Baldessarini, 1975). Desipramine (25 mg/kg, i.p.) was administered 1 hr before 5,7-DHT to protect noradrenaline neurons (Bjorklund et al., 1975). Control rats were injected with saline. All experiments were carried out under chloral hydrate anesthesia (400 mg/kg, i.p.).

Electrophysiological experiments. Extracellular unitary recordings were obtained from pyramidal neurons in the CA_3 region of dorsal hippocampus. The microelectrode was descended 4 mm lateral and 4 mm anterior to lambda. Pyramidal neurons were identified by their high-amplitude (0.5–1.2 mV), long-duration (0.6–1 msec) complex spike discharges alternating with simple spike activity (Kandel and Spencer, 1961). A leak or a small current of ACh (0–5 nA) was used to activate the neurons within their physiological range (8–14 Hz; Ranck, 1975). Microiontophoretic applications were performed with five-barreled glass micropipettes that were pulled in a conventional manner and their tips broken back to 10–15 μm under microscopic control. The central barrel, filled with 2 M NaCl solution, was used for extracellular unitary recording. Three of the side barrels contained three of the following solutions: 5-HT creatinine sulfate (5 mM in 200 mM NaCl, pH 4; Sigma, St. Louis, MO); norepinephrine (NE) (50 mM in 200 mM NaCl, pH 4; Sigma); gepirone (25 mM in 200 mM NaCl, pH 4; Bristol-Myers Squibb, Wallingford, CT); or ACh (20 mM in 200 mM NaCl, pH 4; Sigma). The fourth side barrel, containing a 2 M NaCl solution, was used for automatic current balancing. Ejection periods were always of 50 sec.

The responsiveness of CA_3 pyramidal neurons to microiontophoretic application of drugs was assessed using $I \cdot T_{50}$ and RT_{50} values calculated by on-line computer with a 0.1 sec resolution. The $I \cdot T_{50}$ value represents the charge in nanocoulombs (1 nC = 1 nA × 1 sec) required to obtain a 50% decrease of the firing rate of the neuron recorded and has been shown to provide an index of the neuronal responsiveness to the microiontophoretically applied drug (de Montigny and Aghajanian, 1977; de Montigny et al., 1980). The presynaptic component of neuronal responsiveness to microiontophoretic application of drugs was evaluated using the RT_{50} method. RT_{50} is defined as the time in seconds required by the neuron to recover 50% of its initial firing frequency from the termination of microiontophoretic application. The RT_{50} value has been shown to provide a reliable index of the *in vivo* activity of the NE reuptake process in the rat hippocampus (de Montigny et al., 1980; Gravel and de Montigny, 1987) and of the 5-HT carrier in the rat amygdala and lateral geniculate body (Wang et al., 1979).

Determination of in vitro *^{3}H-5-HT uptake.* For determination of *in vitro* ^{3}H-5-HT uptake, animals were decapitated, their brains rapidly removed, and dissected on an ice-cold plate. Slices of 0.4 mm thickness from hippocampus or raphe region were prepared using a McIlwain chopper. They were incubated for 3 min at 37°C in a Krebs solution with various concentrations (0–1000 nM) of paroxetine, and bubbled with a mixture of 95% O_2 and 5% CO_2. The composition of the Krebs solution was 118 mM NaCl, 4.7 mM $CaCl_2$, 1 mM NaH_2PO_4, 25 mM $NaHCO_3$, 11.1 mM glucose, 0.004 mM Na_2EDTA, and 0.11 mM ascorbic acid. After the incubation period, ^{3}H-5-HT (specific activity, 22.7 Ci/mmol; New England Nuclear Research Products, Mississauga, Ontario, Canada) was added at a final concentration of 5 nM, 20 nM, or 100 nM. Following a 3 min incubation period, uptake was terminated by transferring the slices to 5 ml of ice-cold buffer, and they were then solubilized in 0.5 ml of Soluene 350 (Packard Instruments, Downers Grove, IL). Radioactivity in the slices and the incubation medium was determined by liquid scintillation spectroscopy. Parallel experiments were carried out at 0°C as control for passive diffusion. All experiments were performed in duplicate and the amount of tritium actively captured by the tissue (C_A) was calculated according to the formula $C_A = C_T - C_P$, where

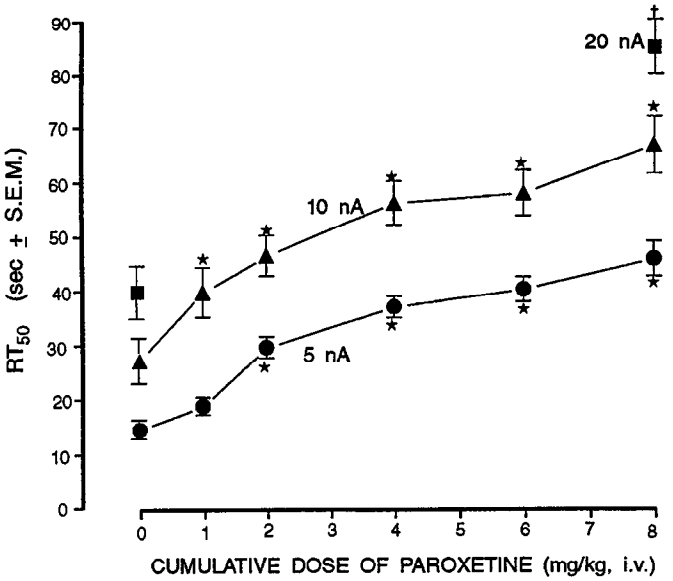

Figure 2. Effect of successive intravenous injections of paroxetine on the recovery time, expressed as RT_{50} values (means ± SEM), from microiontophoretic applications of 5-HT with 5 nA (●), 10 nA (▲), and 20 nA (■) ($N = 9$ for all currents used). *, $p < 0.001$, compared to preinjection value using the Student's paired t test.

C_T and C_P are the tissue≥dium ratios of ^{3}H-5-HT at 37°C and 0°C, respectively. Inhibition of uptake was calculated by means of the formula % of inhibition = $[1 - C_{AC}/C_{AP}] \times 100$, where C_{AC} and C_{AP} are the amounts of actively captured ^{3}H-5-HT in a medium with or without paroxetine, respectively.

IC_{50} values for the uptake of ^{3}H-5-HT were determined by computer analysis (GRAPHPAD, Graphpad Software, San Diego, CA) from concentration–effect curves based on four concentrations of paroxetine.

^{3}H-paroxetine binding assays. Binding assays using ^{3}H-paroxetine (25 Ci/mmol; New England Nuclear Research Products) were performed according to a previously described protocol (Marcusson et al., 1988). Forty-eight hours after the removal of the osmotic minipump rats were decapitated, brains dissected, and cortex and hippocampus immediately frozen and kept at −70°C until the binding assays were carried out. Membranes were prepared by homogenizing brain tissue in 15 ml of ice-cold buffer (50 mM Tris HCl, 120 mM NaCl, 5 mM KCl; pH 7.4) and centrifuging at 48,000 × g for 10 min at 4°C. The resulting pellet was suspended in 15 ml of buffer and centrifuged. The final pellet was suspended to a final tissue concentration of 40–60 μg protein/ml (approximately 0.75 mg wet weight/ml) in the binding assay. The homogenates were incubated with ^{3}H-paroxetine (0.03–2 nM) at 22°C in a final volume of 1600 μl for 60 min. Incubations were terminated by addition of 4 ml ice-cold buffer and filtration through Whatman GF/C filters using a 24-channel cell harvester. Filters were then washed with four 4 ml rinses in cold buffer. Radioactivity trapped by the filters was determined by liquid scintillation spectroscopy (Beckman Counter LS 6000 SE, Beckman Instruments, Fullerton, CA). Nonspecific binding was estimated in the presence of 100 μM 5-HT. Binding was analyzed using the curve-fitting program LIGAND (G. A. McPherson, Elsevier-Biosoft, Cambridge, UK).

Statistical analysis. Results were expressed throughout as means ± SEM. When two means were compared, statistical significance of their difference was assessed using the two-tailed paired or nonpaired Student's t tests as indicated. For multiple comparisons, one-way or two-way ANOVA was used for independent and paired samples, respectively.

Results

Effect of acute intravenous administration of paroxetine on the recovery time from microiontophoretic applications of 5-HT

The duration of suppression following 5-HT applications was assessed in naive rats using increasing currents for applying

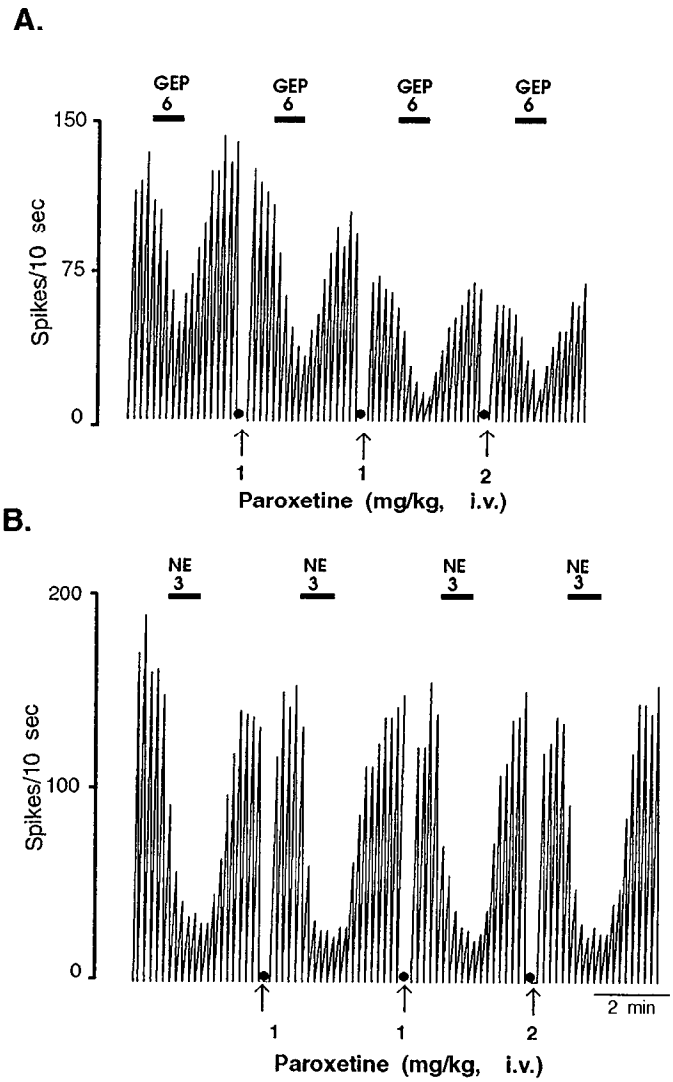

Figure 3. Integrated firing rate histograms of dorsal hippocampus CA_3 pyramidal neurons showing the effect of successive injections of paroxetine at 10 min intervals on the response of a dorsal hippocampus pyramidal neuron to microiontophoretic application of gepirone (*A*) and NE (*B*). *Dots at the bottom of each histogram* represent periods of 10 min. Time scale applies to both tracings.

5-HT. The recovery time (RT_{50} value) was proportional to the current used: 5, 10, and 20 nA applications yielded RT_{50} values of 16 ± 2, 27 ± 4, and 41 ± 5 sec, respectively ($n = 9$; Fig. 1*A*). Following intravenous administration of successive 1–2 mg/kg doses of paroxetine (cumulative doses of 2, 4, 6, and 8 mg/kg), the effect of microiontophoretically applied 5-HT was dose-dependently prolonged (Figs. 1*B*, 2). At the highest dose of paroxetine used (8 mg/kg, i.v.), the RT_{50} values for 5 nA, 10 nA, and 20 nA applications were increased by 206%, 145%, and 110%, respectively.

In order to confirm the selectivity of paroxetine for the 5-HT carrier in this paradigm, the effect of paroxetine was assessed on the recovery from microiontophoretic applications of the 5-HT_{1A} agonist gepirone and NE, neither of which are substrates for the 5-HT transporter. In contrast to the prolongation of the effect observed for 5-HT, the RT_{50} values for these two compounds were not affected by successive doses of paroxetine (Fig. 3, Table 1). While in the dose range of 1–4 mg/kg, intravenous

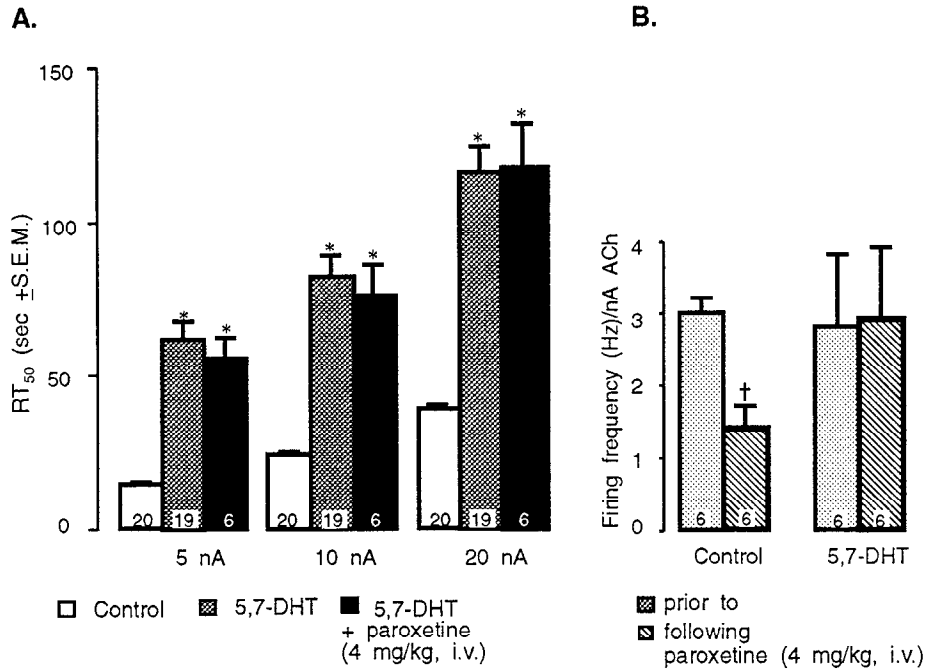

Figure 4. *A*, Recovery time, expressed as RT_{50} values (means ± SEM), of dorsal hippocampus CA_3 pyramidal neurons from microiontophoretic applications of 5-HT in control rats and in rats treated with 5,7-DHT, before and after intravenous administration of 4 mg/kg of paroxetine. *, $p < 0.001$ compared to control values using two-tailed Student's *t* test. *B*, Effect of intravenous paroxetine on pyramidal neuron firing frequency in control and 5,7-DHT–lesioned rats. †, $p < 0.01$ using two-tailed paired Student's *t* test. The number of neurons tested is given at the bottom of each column.

administration of paroxetine dose-dependently increased RT_{50} values of 5-HT applications ($r = 0.8$, $p < 0.001$), RT_{50} values for NE and gepirone were not correlated with the dose of paroxetine administered ($r = 0.01$, $p = 0.8$).

Effect of 5-HT denervation on the recovery from microiontophoretic applications of 5-HT

The prolongation of the recovery time from microiontophoretic applications of 5-HT, following acute 5-HT reuptake blockade, in the absence of a modification of its initial effectiveness [$I \cdot T_{50}$ values: 23 ± 3 nC prior to, and 25 ± 4 nC following, paroxetine (4 mg/kg, i.v.; $n = 14$)], suggested a pure presynaptic effect of paroxetine. This interpretation relied, however, on the assumption that the recovery of the firing rate of CA_3 pyramidal neurons from microiontophoretic applications of 5-HT is exerted mainly by the 5-HT transporters located on 5-HT terminals. To verify this hypothesis, the recovery from 5-HT applications was assessed in 5,7-DHT–lesioned rats. As expected, 5-HT denervation markedly prolonged the effect of 5-HT: the RT_{50} values were increased by 324%, 262%, and 197% for 5 nA, 10 nA, and 20 nA applications, respectively (Fig. 4*A*). Interestingly, as shown in Figure 4*A*, the injection of paroxetine (4 mg/kg, i.v.) in 5,7-DHT–lesioned rats did not produce a further increase in RT_{50}, indicating that pretreatment with 5,7-DHT had produced a complete denervation and that the effect of paroxetine observed in intact rats (Figs. 1, 2) was entirely attributable to the blockade of the 5-HT transporters located on 5-HT terminals.

In intact rats, intravenous administration of paroxetine per se reduced pyramidal neuron firing frequency. Consequently, the microiontophoretic current of the ACh had to be increased from 4 ± 0.7 to 9 ± 0.8 nA ($n = 6$) to restore the firing rate to its preinjection level. Brunel and de Montigny (1987) have previously demonstrated that ACh does not alter the response of pyramidal neurons to 5-HT. In 5,7-DHT–lesioned rats, paroxetine had no effect on the firing frequency of pyramidal neurons ($n = 6$; Fig. 4*B*).

It is also noteworthy that 5,7-DHT lesion and administration of paroxetine (10 mg/kg/d, s.c.) for 2 d yielded nearly identical RT_{50} values. When the RT_{50} values from 5-HT applications were

Table 1. Effect of acute intravenous paroxetine on the recovery from microiontophoretic applications of gepirone, norepinephrine and serotonin

Cumulative dose of paroxetine (mg/kg, i.v.)	RT_{50} (s ± SEM) Gepirone ($n = 3$)	NE ($n = 3$)	5-HT ($n = 3$)
0	23 ± 10	35 ± 12	10 ± 2
1	18 ± 4	35 ± 15	14 ± 5
2	18 ± 5	37 ± 12	26 ± 4*
4	20 ± 11	35 ± 9	39 ± 4*

* $P < 0.01$ using the two-tailed Student's *t* test.

Table 2. Effect of short-term paroxetine administration and 5,7-DHT lesion on the recovery from microiontophoretic applications of 5-HT

Current (nA)	RT_{50} (s ± SEM) Control ($n = 19$)	5,7-DHT ($n = 19$)	Paroxetine *low* dose* ($n = 15$)	Paroxetine *high* dose** ($n = 17$)
5	14.5 ± 1	61.5 ± 6***	62.3 ± 6***	66.2 ± 3***
10	24.3 ± 1	82.6 ± 7***	88.8 ± 7***	85.8 ± 3***
20	39.0 ± 2	116.0 ± 8***	122.0 ± 9***	109.4 ± 4***

In paroxetine treated rats the experiments were performed with the osmotic pump in place.

* 10 mg/kg/day, s.c. × 2 days.

** 20 mg/kg/day, s.c. × 2 days.

*** $p < 0.001$ using two-tailed Student's *t* test.

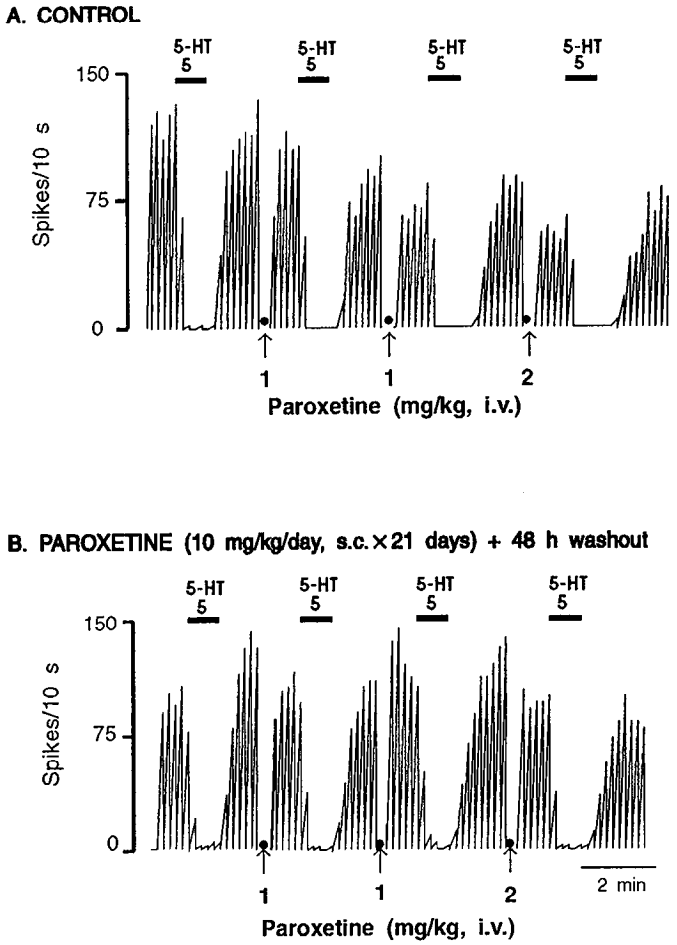

Figure 5. Integrated firing rate histograms showing the response of dorsal hippocampus CA_3 pyramidal neurons to microiontophoretic applications of 5-HT in a control rat (*A*) and a long-term paroxetine-treated rat (*B*), following successive intravenous injections of paroxetine at 10 min intervals. Rats were implanted subcutaneously with an osmotic minipump which delivered either vehicle or paroxetine. After 21 d the minipump was removed and a 48 hr washout period was allowed. Time scale applies to both traces.

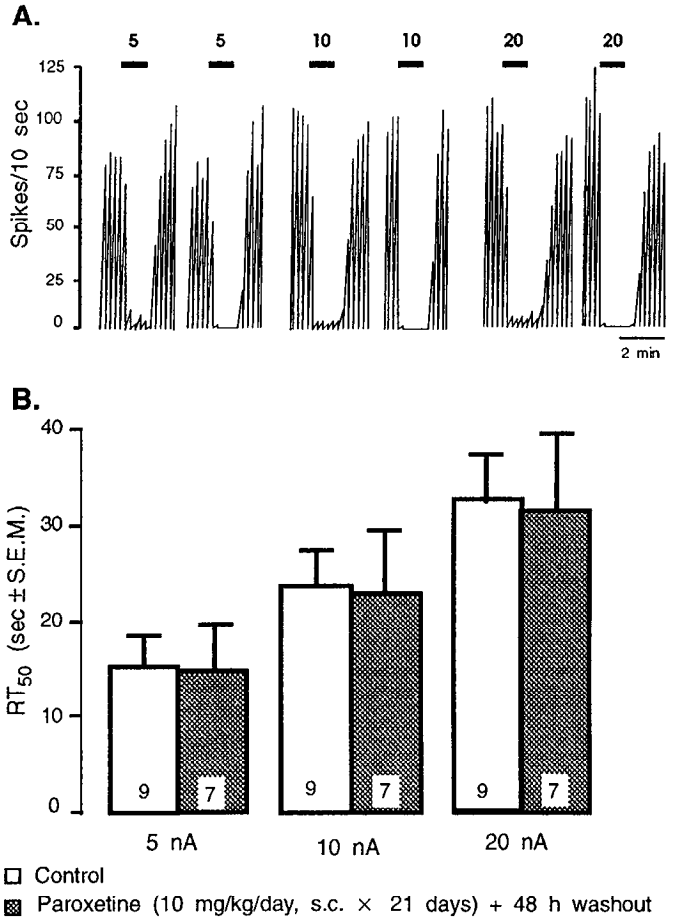

Figure 6. *A*, Integrated firing rate histograms illustrating the response of dorsal hippocampus CA_3 pyramidal neurons in a control and a long-term paroxetine-treated rat 48 hr after the removal of the osmotic pump. Each current is applied alternatively in the control rat and the paroxetine-treated rat. *B*, Recovery time expressed as RT_{50} values (mean ± SEM) of dorsal hippocampus pyramidal neurons from microiontophoretically applied 5-HT in control rats and in rats treated with paroxetine for 21 d. Note that RT_{50} values for the 5 nA current are significantly smaller than for the 20 nA current and this difference is maintained after long-term paroxetine administration ($p < 0.001$). Number of neurons tested is given at the bottom of each column.

assessed in rats that were still carrying the osmotic minipump during the experiment, this parameter was increased by 320%, 270%, and 212% for 5, 10, and 20 nA currents, respectively (as compared to 324%, 262%, and 197% for the same currents in 5,7-DHT-lesioned rats). This suggests that, at a dose of paroxetine of 10 mg/kg/d, all the 5-HT transporters were blocked, thus simulating denervation. Furthermore, when the dose of paroxetine was doubled (20 mg/kg/d × 2 d), RT_{50} values did not further increase (Table 2), thereby confirming that the 10 mg/kg/d dose of paroxetine maximally inhibits 5-HT reuptake.

Table 3. Effect of long-term paroxetine treatment on the ED_{50} of acute intravenous paroxetine for increasing the recovery from 5-HT applications*

Current (nA)	Dose of paroxetine (mg/kg, i.v.) Control ($n = 9$)	Paroxetine** ($n = 7$)	p***
5	1.7 ± 0.2	3.2 ± 0.6	<0.03
10	2.4 ± 0.2	4.1 ± 0.4	<0.01
20	2.4 ± 0.3	4.0 ± 0.7	<0.05

* ED_{50} was calculated as the dose of paroxetine necessary to increase RT_{50} by 50%.
** 10 mg/kg/day, s.c. × 21 days.
*** Using the two-tailed Student's t test.

Effect of long-term administration of paroxetine on the recovery time from microiontophoretic applications of 5-HT

To test the effect of long-term reuptake blockade on the 5-HT transporter, the effectiveness of successive intravenous doses (1–2 mg/kg) of paroxetine to prolong RT_{50} values was assessed in control and paroxetine-treated rats (10 mg/kg/d, s.c. × 21 d) following a 48 hr washout period to ensure complete drug elimination. As exemplified in Figure 5, the effectiveness of acute intravenous paroxetine was reduced in the paroxetine-treated group. Before the injection of paroxetine, the RT_{50} values were similar in treated and in control rats (Fig. 6), indicating, first, that the reuptake process was no longer blocked after the washout period, and second, that at the concentrations attained with the different microiontophoretic currents of 5-HT used, the ability of 5-HT terminals to take up 5-HT was unchanged after

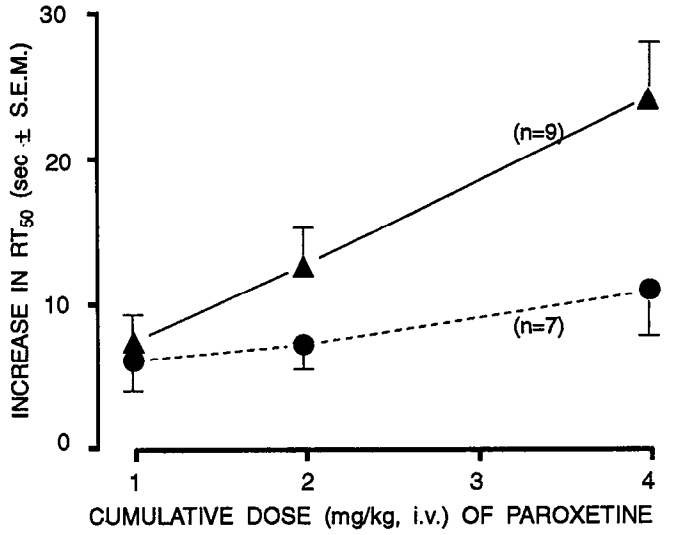

Figure 7. Effect of successive injections of paroxetine on the recovery time from 5 nA microiontophoretic applications of 5-HT in control rats (▲) and in rats treated with paroxetine (10/mg/kg/d, s.c. × 21 d) following 48 hr washout (●). Values are expressed as increase in RT_{50} (mean ± SEM). $p < 0.05$, comparing the two curves by ANOVA.

long-term blockade by paroxetine. The first dose of paroxetine (1 mg/kg, i.v.) increased the RT_{50} values to a similar extent in control and long-term paroxetine-treated rats. However, subsequent cumulative doses of 2 and 4 mg/kg produced smaller effects in long-term paroxetine-treated rats than in controls (Figs. 5, 7), indicating a decreased efficacy of intravenous paroxetine in blocking 5-HT reuptake following long-term paroxetine treatment. For all currents used, the dose of paroxetine required to increase RT_{50} values by 50% (ED_{50}) was doubled in long-term paroxetine-treated rats (Table 3).

To investigate further the induction of a "tolerance" by long-term paroxetine administration, the suppressant effect of 5-HT was determined in control rats and in rats treated with paroxetine (10 mg/kg/d, s.c.) for 2 or 21 d without removing the osmotic pump. For all currents used, recovery from microiontophoretic applications of 5-HT was prolonged in both treated groups, but to a significantly lesser extent in long-term– than in short-term–treated rats (Fig. 8). The RT_{50} values from 5, 10, and 20 nA applications of 5-HT were increased by 377%, 300%, and 198% in 2 d paroxetine-treated rats, and by 254%, 196%, and 149% in 21 d paroxetine-treated rats (Fig. 9).

Recovery from microiontophoretic applications of gepirone was unchanged by long-term administration of paroxetine, in keeping with the fact that it is not a substrate for the 5-HT transporter. RT_{50} values from 4 and 10 nA applications of gepirone were 28 ± 4 sec and 53 ± 8 sec in control rats, and 24 ± 4 sec and 50 ± 6 sec in rats treated with paroxetine for 21 d. The initial responsiveness ($I \cdot T_{50}$ values) to microiontophoretic applications of gepirone and of 5-HT was also unchanged following long-term paroxetine treatment, ruling out the possibility that the reduced effectiveness of acute paroxetine to prolong the effect of 5-HT could be ascribed to a decrease in neuronal responsiveness to the activation of postsynaptic 5-HT_{1A} receptors. For 5-HT, respective $I \cdot T_{50}$ values for 5 and 10 nA applications were 24 ± 3 nC and 39 ± 4 nC in control rats (n = 18), and 23 ± 2 nC and 42 ± 3 nC following the 21 d paroxetine treatment (n = 18).

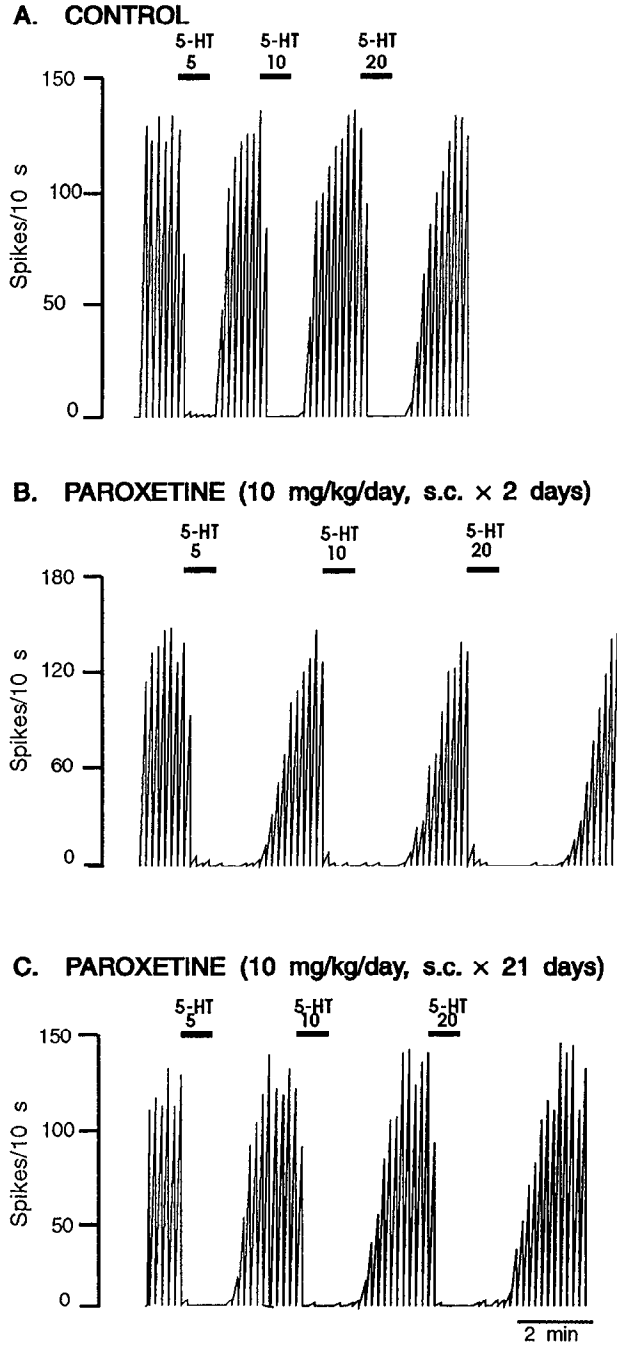

Figure 8. Integrated firing rate histograms showing the response of CA_3 dorsal hippocampus pyramidal neurons to microiontophoretically applied 5-HT in a control rat (*A*) and in rats treated with paroxetine (10 mg/kg/d, s.c.) for 2 d (*B*) or 21 d (*C*). In *B* and *C*, the experiments were carried out with the minipump in place.

Effect of long-term paroxetine administration on in vitro ^{3}H-5-HT uptake

The following series of experiments were carried out to verify *in vitro* the induction of desensitization of the 5-HT transporter by long-term paroxetine administration. In control experiments, increasing the concentration of paroxetine in the incubation medium caused a concentration-dependent reduction in the amount of radioactivity captured by the slices. In slices from 5,7-DHT–lesioned rats, the uptake of ^{3}H-5-HT was reduced by

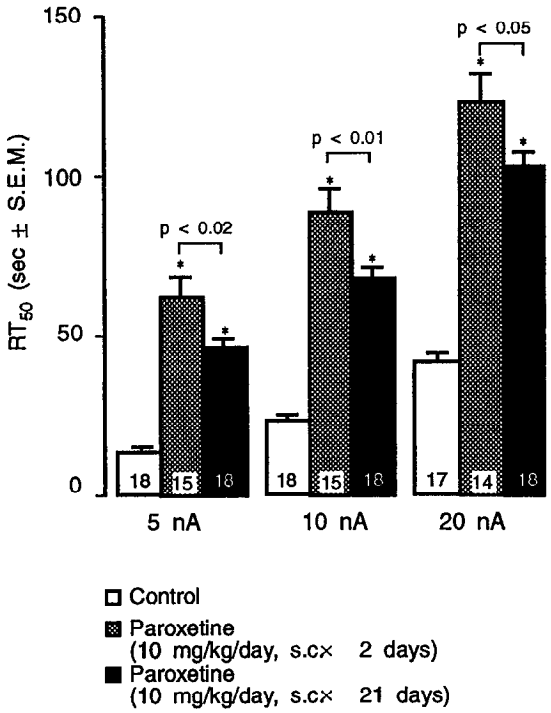

Figure 9. Recovery time, expressed as RT_{50} values (mean ± SEM), of dorsal hippocampus pyramidal neurons from microiontophoretically applied 5-HT in control rats and in rats treated with paroxetine (10 mg/kg/d, s.c.) for 2 or 21 d. The number of neurons tested is given at the bottom of each column. *, $p < 0.001$ compared to control values using two-tailed Student's t test. p values indicated on the figure also were obtained using the nonpaired Student's t test.

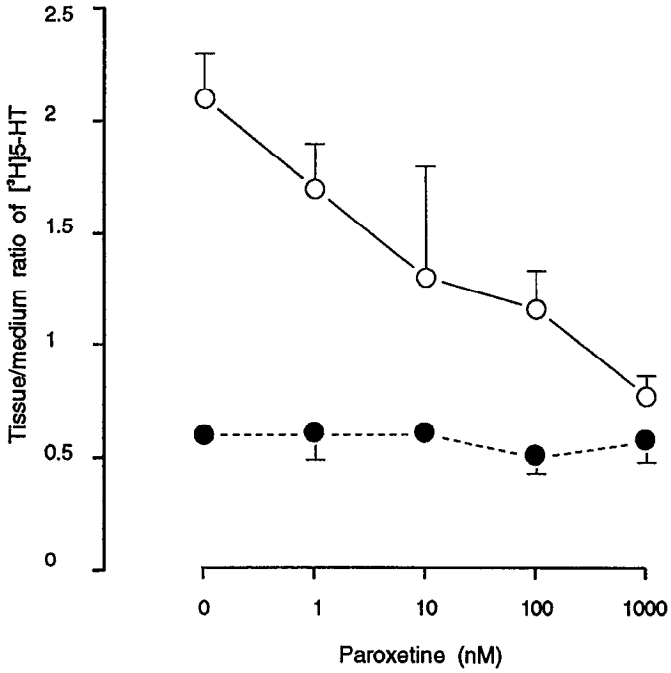

Figure 10. Effect of increasing concentrations of paroxetine on ^{3}H-5-HT uptake in hippocampal slices of control (○) and 5,7-DHT–lesioned rats (●), $n = 3$ in each group. $p < 0.001$ comparing both curves by ANOVA.

70% (Fig. 10). Moreover, the incubation with increasing paroxetine concentrations did not produce further inhibition (Fig. 10). As ^{3}H-5-HT uptake in control slices incubated with the highest paroxetine concentration (1000 nM) was not significantly different from that observed after 5,7-DHT lesion, it can be assumed that maximal blockade of 5-HT uptake sites was attained at this concentration.

In slices from control rats, paroxetine in the incubation medium was more effective in blocking reuptake using a 100 nM than a 5 nM concentration of ^{3}H-5-HT (Fig. 11*D,F*). From each of these paroxetine concentration–effect curves, the concentration of paroxetine inducing 50% of the maximal inhibition (attained with 1000 nM paroxetine) was determined. Paroxetine was six times more potent in blocking reuptake when the ^{3}H-5-HT concentration in the incubation medium was high (100 nM) than when it was low (5 nM): respective calculated IC_{50} values were 18 nM and 107 nM.

Following a 48 hr washout period, in slices incubated with 100 nM ^{3}H-5-HT, radioactivity taken up by the tissue was reduced by 50% in the long-term paroxetine-treated group. This effect was significant in slices incubated with 100 nM ^{3}H-5-HT and with paroxetine concentrations lower than 1000 nM (Fig. 11*A*).

Following long-term treatment with paroxetine, the *in vitro* effectiveness of paroxetine to block 5-HT uptake was reduced by 43%, thus confirming results from electrophysiological experiments reported above. However, this *in vitro* effect was only seen when incubation was carried out in the presence of 5 nM ^{3}H-5-HT (Fig. 11*F*). When slices were incubated with 20 nM ^{3}H-5-HT, long-term paroxetine treatment did not have any detectable effect (Fig. 11*B,E*). Moreover, it is noteworthy that after long-term paroxetine treatment the difference in IC_{50} for paroxetine at different ^{3}H-5-HT concentrations was abolished. Calculated IC_{50} values were 14 nM and 13 nM for ^{3}H-5-HT concentrations of 5 nM and 100 nM, respectively.

The effect of long-term reuptake blockade was also assessed in slices from the raphe region. It induced changes similar to those observed in the hippocampus: ^{3}H-5-HT uptake was reduced by 60% (Fig. 12*A*) and 10 nM, but not 100 nM, of paroxetine produced a much smaller inhibition of ^{3}H-5-HT uptake than in slices from control rats (Fig. 12*B*).

Effect of long-term paroxetine administration on ^{3}H-paroxetine binding parameters

Specific saturable binding of ^{3}H-paroxetine to rat cortical and hippocampal membranes was concentration dependent (28% specific binding at 2 nM ^{3}H-paroxetine). Scatchard transformation of the binding data produced linear plots in all cases (Fig. 13) and fitted better a one- rather than a two-site model. Mean K_d and B_{max} values in control and long-term paroxetine-treated rats for both regions are summarized in Table 4. After long-term paroxetine administration, the total number of binding sites was reduced by 60% in cortex and by 70% in hippocampus, thus confirming a downregulation of the 5-HT transporter. The fact that K_d values remained unchanged after the treatment ruled out the possibility that the changes observed could be due to a competitive inhibition of binding by residual paroxetine after the 48 hr washout period.

Discussion

Electrophysiological data obtained in the present study confirm and extend previous observations that RT_{50} values following

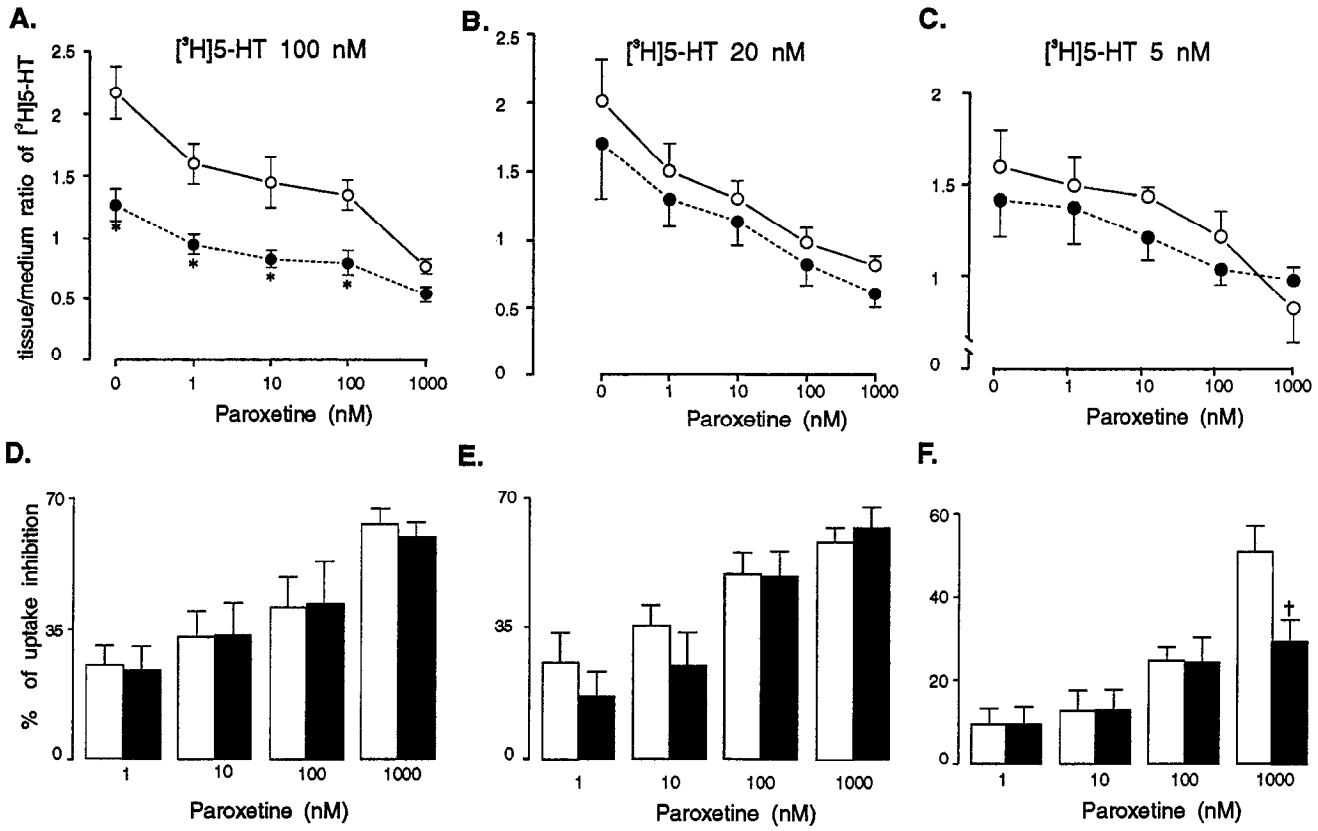

Figure 11. ³H-5-HT reuptake activity (*A–C*) and effectiveness of paroxetine to inhibit ³H-5-HT reuptake, expressed as percentage of uptake inhibition (*D–F*) in hippocampal slices from control ($n = 5$; ○) and long-term paroxetine-treated rats ($n = 5$; ●) (10 mg/kg/d, s.c. × 21 d + 48 hr washout). *, $p < 0.02$ as compared to control values using nonpaired Student's *t* test; †, $p < 0.05$ as compared to control using nonpaired Student's *t* test.

microiontophoretic applications of 5-HT provide a reliable index of the *in vivo* activity of the 5-HT reuptake process, since it is prolonged three- to fourfold by acute, short-term, and long-term administration of paroxetine (Figs. 2, 9) as well as by lesioning 5-HT terminals (Fig. 4). The fact that a selective 5-HT reuptake inhibitor (SSRI) prolongs recovery from 5-HT applications, leaving unaffected the effects of gepirone and NE (Table 1), which are not substrates for the 5-HT transporter, strongly suggests that the recovery of the firing frequency of pyramidal neurons following microiontophoretic applications of 5-HT depends on the activity of the 5-HT carrier. In a previous report we had concluded that, in dorsal hippocampus, 5-HT reuptake did not play a significant role in terminating the action of microiontophoretically applied 5-HT (de Montigny et al., 1980). The apparent contradiction between the latter report and the present one is probably due to the fact that, herein, microiontophoretic applications were carried out using higher currents of a more concentrated solution of 5-HT to ensure that it would diffuse from the ejection site (near the cell body) to strata radiatum and oriens, where the majority of 5-HT terminals are located (Oleskevich and Descarries, 1990).

In keeping with previous observations that lesioning of 5-HT terminals abolishes ³H-paroxetine binding (de Souza and Kuyatt, 1987; Hrdina et al., 1990; Dewar et al., 1991), in our electrophysiological paradigm the 5,7-DHT pretreatment completely abolished the effect of acute paroxetine on the duration of suppression of firing activity by the microiontophoretic application of 5-HT (Fig. 4*A*). A similar effect was observed *in vitro* where paroxetine failed to decrease ³H-5-HT uptake in slices obtained from 5,7-DHT–lesioned rats (Fig. 10). Even if 5-HT transporters have been found in primary astrocyte cultures from neonatal rat brain (Kimelberg and Katz, 1985), the majority of

Table 4. Binding parameters of [³H]paroxetine in control and long-term paroxetine-treated rats

	Kd (nM)		Bmax (fmol/mg of prot.)	
Brain region	Control	Paroxetine*	Control	Paroxetine*
Hippocampus	0.3 ± 0.05	0.2 ± 0.08	114 ± 8	31 ± 9**
Cortex	0.3 ± 0.04	0.2 ± 0.06	138 ± 19	55 ± 14***

* 10 mg/kg/day, s.c. × 21 days + 48 h washout.

** $p < 0.001$, using two-tailed Student's *t* test.

*** $p < 0.01$, using two-tailed Student's *t* test ($n = 4$).

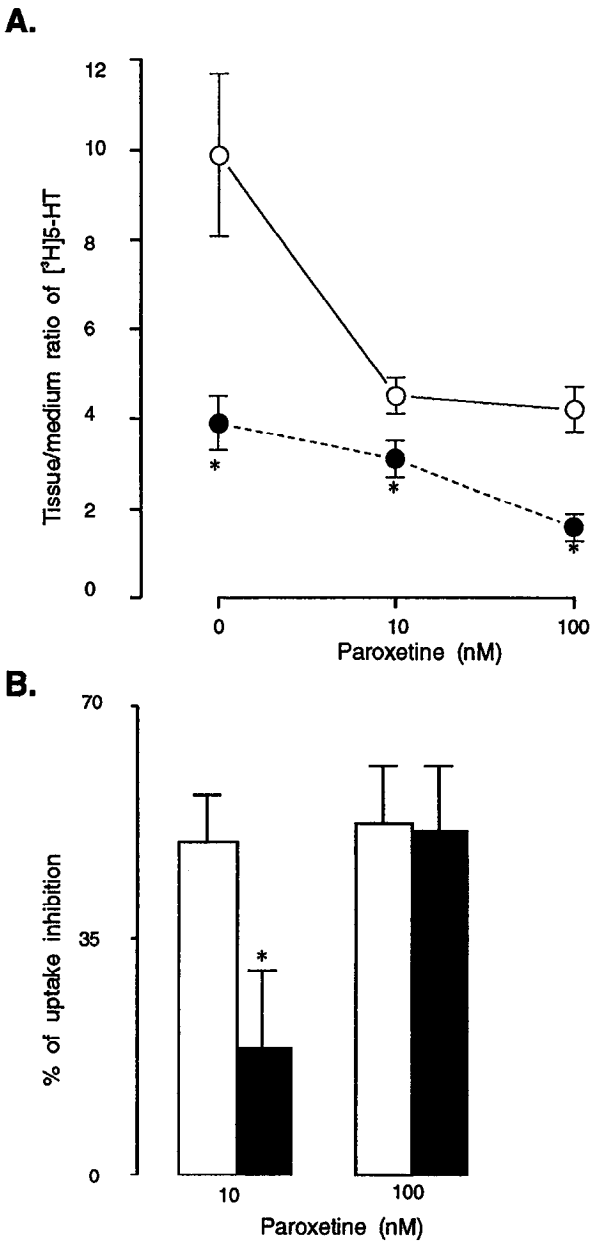

Figure 12. ^{3}H-5-HT reuptake (*A*) and effectiveness of paroxetine to inhibit 5-HT reuptake (*B*) in dorsal raphe slices from control (○) and long-term paroxetine-treated rats (●) (10 mg/kg/d, s.c. × 21 d + 48 hr washout; $N = 6$ in each group). *, $p < 0.05$ as compared to corresponding control values using nonpaired Student's t test. The concentration of ^{3}H-5-HT in the incubation medium was 100 nM.

autoradiographic studies of ^{3}H-5-HT reuptake in adult rat brain show that 5-HT uptake by astrocytes is minimal relative to that by 5-HT terminals (Katz and Kimelberg, 1985). Our results further support the notion that functionally relevant transporters are confined to 5-HT terminals.

The electrophysiological paradigm we devised was not only sensitive enough to detect the effect of paroxetine on exogenously applied 5-HT through changes in RT_{50}, but it could also detect the effect of 5-HT reuptake blockade on endogenously released 5-HT via the reduction of pyramidal neuron firing frequency (Figs. 1*B*, 4*B*, 5*A*). Since denervation abolished the consistent suppressant effect produced by paroxetine (Fig. 4*B*) on pyramidal neuron firing frequency, the latter observation clearly demonstrates the physiological role played by the 5-HT transporter in terminating the action of endogenously released 5-HT.

Long-term paroxetine treatment results in adaptive changes of the 5-HT transporter. These changes were detected not only in hippocampus (Figs. 7, 9, 11) but also in cerebral cortex (Fig. 13) and in the raphe region (Fig. 12), indicating that 5-HT transporters in multiple pre- and postsynaptic regions of the rat CNS share the ability to adapt to their sustained occupation.

Though previous studies using ^{3}H-imipramine have reported a decreased number of binding sites following long-term antidepressant treatment (Barbaccia et al., 1983; Arora and Meltzer, 1986; Brunello et al., 1987), these studies did not take into account the heterogeneity of ^{3}H-imipramine binding sites. In the present study, the use of paroxetine as the tritiated ligand allows us to conclude that the changes observed are directly related to the 5-HT transporter as it has been shown to selectively label this carrier. In contrast with our observations, Graham et al. (1987), Brunswick et al. (1991), and Cheetham et al. (1991), using ^{3}H-paroxetine, and Hrdina et al. (1990), taking into account high- and low-affinity ^{3}H-imipramine binding sites, did not detect any change in B_{max} following long-term antidepressant treatment. These divergent results could be explained by the fact that in the aforementioned studies reuptake blockers were administered by intraperitoneal injections. Since SSRIs have been shown to be rapidly metabolized in rodents (Buus Lassen, 1978; Fredricson, 1982), intraperitoneal injections, given usually every 12 or 24 hr, lead to large fluctuations in drug plasma levels, and consequently in the degree of reuptake blockade. For example, for the SSRI citalopram, which in rats has a half-life of 3 hr (Fredricson, 1982), its suppressant effect on the firing activity of 5-HT neurons is no longer detectable 15 hr after the injection of a high dose (Chaput et al., 1986). The observation of the lack of 5-HT reuptake blockade 48 hr after a 21 d treatment (Fig. 6) is thus fully consistent with the rapid metabolism of paroxetine in rodents (Buus Lassen, 1978). In the present study, to avoid fluctuations and produce stable plasma levels throughout the treatment period, paroxetine was administered by sustained subcutaneous infusion. Interestingly, Lesch et al. (1993) have recently reported the induction of downregulation of 5-HT transporter mRNA following long-term subcutaneous administration of fluoxetine, imipramine, and chlorimipramine. It therefore seems that stable plasma concentrations of the reuptake blocker administered are essential for the induction of an adaptive response at the level of the 5-HT transporter. Moreover, Kovachich et al. (1992) have reported that intraperitoneal administration of sertraline, but not that of citalopram, can reduce ^{3}H-cyanoimipramine binding in different limbic regions. The fact that desmethylsertraline is more potent and has a longer half-life than mono- and dimethylated citalopram metabolites (Boyer and Feighner, 1991) further supports the notion that a sustained occupation is required to induce a downregulation of the neuronal 5-HT transporter. Since Lesch et al. (1993), using 5-HT reuptake inhibitors less potent than paroxetine, still observed a downregulation of the mRNA encoding the 5-HT transporter, it is improbable that uptake blocking capacity of the different SSRI might account for the differences between this and previous studies (Graham et al., 1987; Hrdina et al., 1990; Brunswick et al., 1991; Cheetham et al., 1991).

In the present electrophysiological experiments, desensitization of the 5-HT transporter in long-term paroxetine-treated rats was evidenced by a reduction in the effectiveness of acute

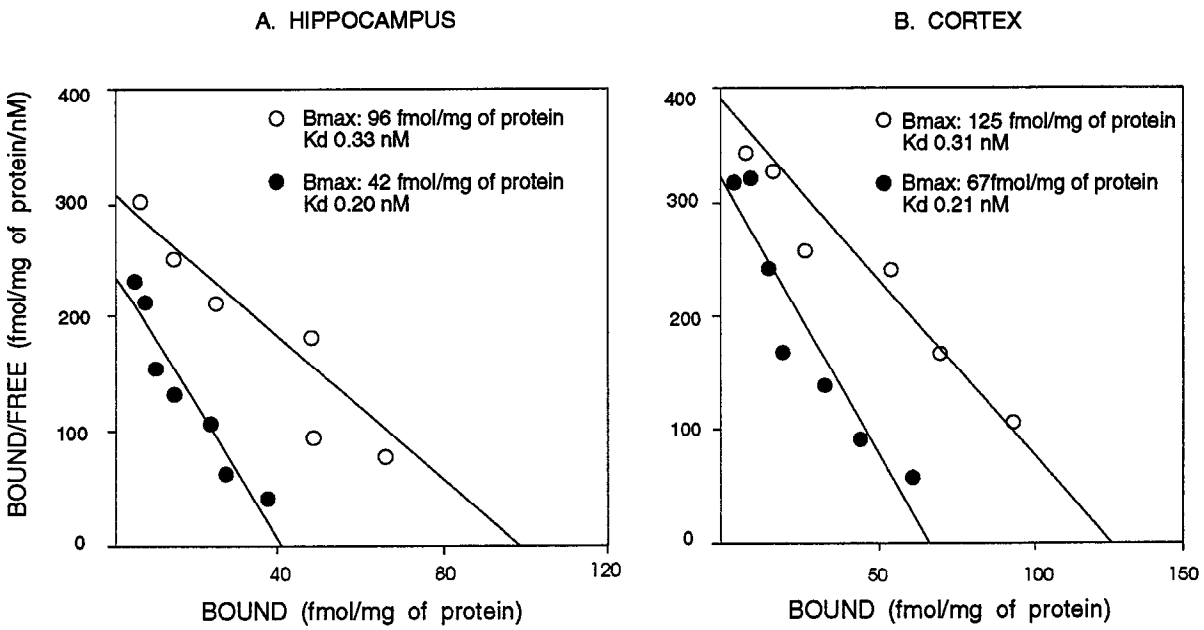

Figure 13. Representative Scatchard plots of specific ^{3}H-paroxetine binding to hippocampal (*A*) and cortical (*B*) membranes in a control rat (○) and in a rat treated with paroxetine (●) (10 mg/kg/d, s.c. × 21 d + 48 hr washout).

paroxetine to prolong the duration of suppression of firing by microiontophoretic applications of 5-HT (Fig. 7). Indeed, the ED_{50} of intravenous paroxetine to prolong RT_{50} was doubled in long-term paroxetine-treated rats (Table 3). Furthermore, the effect of subcutaneous administration of paroxetine on RT_{50} was significantly smaller in rats treated for 21 d than in those treated for 2 d (Fig. 9). Since the prolongation of RT_{50} values produced by 2 d subcutaneous administration of 10/mg/kg/d of paroxetine was similar in magnitude to that produced by a dose of 20 mg/kg/d, it can be assumed that, at the dose of 10/mg/kg/d, paroxetine blocked all available uptake sites. The fact that the effect of the 2 d treatment with 10/mg/kg/d of paroxetine was greater than that of the 21 d treatment suggests that the mechanism underlying this tolerance could be a reduction in the number of 5-HT transporters. This assumption was confirmed by radioligand binding studies that showed a 70% decrease in the number of ^{3}H-paroxetine binding sites in hippocampal membranes following long-term paroxetine administration (Fig. 13*A*).

The current model for the 5-HT transporter proposes that 5-HT, tricyclic, and nontricyclic uptake inhibitors all bind to the same or overlapping sites on the carrier (Marcusson et al., 1989; Marcusson and Ross, 1990). There is an apparent discrepancy between the reduced effectiveness of acute injection of paroxetine (Fig. 7), the reduced ^{3}H-5-HT uptake (Fig. 11), and the reduced number of ^{3}H-paroxetine binding sites (Fig. 12) 48 hr after the long-term paroxetine treatment, indicating a decrease in the number of 5-HT transporters, and the observation that, at this time point, the effect of microiontophoretically applied 5-HT was not prolonged (Fig. 6). This could be explained by the presence of spare 5-HT transporters. Hence, even if the long-term paroxetine treatment downregulates 5-HT transporter sites, the number of remaining sites would be sufficient to take up all of the microiontophoretically applied 5-HT, even when applied with the highest current (20 nA) used. This explanation is all the more likely since 5-HT is applied at the level of the soma, so that only minute amounts of the neurotransmitter would actually reach the 5-HT terminals, located mostly on remote dendritic trees. The existence of spare transporters could also explain the observation that, although paroxetine is a competitive antagonist for 5-HT reuptake, its IC_{50} was six times higher at 5 nM than at 100 nM ^{3}H-5-HT concentrations. At the low ^{3}H-5-HT concentration (5 nM), before interfering with ^{3}H-5-HT uptake, paroxetine would first need to occupy a large number of sites, thus explaining the high IC_{50} values observed in these conditions. At the high ^{3}H-5-HT concentration (100 nM), all (or most) of the transporters being operant, even low doses of paroxetine can interfere with reuptake. A reduction in the number of 5-HT transporters could also explain the fact that IC_{50} values for paroxetine at different ^{3}H-5-HT concentrations did not differ any longer following long-term paroxetine treatment since the number of spare sites would be minimal.

The proportion of occupied/free transporters could then explain the different ways in which desensitization was expressed in the *in vitro* reuptake experiments (Fig. 11). At 100 nM ^{3}H-5-HT (when all transporters are operant) a reduction in the number of sites was detected by reduction in ^{3}H-5-HT uptake capacity, whereas at the low concentration of ^{3}H-5-HT (5 nM), even in the presence of a reduced number of transporters, the remaining ones were in sufficient number to take up as much radioactivity as the control slices. Thus, in the latter condition, the reduction in the number of transporters was reflected by a decreased efficacy of the maximally effective paroxetine concentration (1000 nM).

The reduction in ^{3}H-paroxetine binding sites after long-term paroxetine administration confirms the downregulation of 5-HT transporters. The fact that K_d values were unchanged after treatment rules out the possibility of affinity changes being involved in the desensitization mechanism. This is in agreement with the observation that, even though paroxetine and 5-HT show different affinity for the 5-HT transporter, the present functional electrophysiological and uptake studies show that 5-HT reuptake activity and paroxetine effectiveness were reduced to a similar extent (45–60%) after long-term reuptake blockade.

Though K_d values found with ^{3}H-paroxetine in the present study were in the range of those previously reported by several groups, B_{max} values found in control rats were lower than those initially reported by Marcusson et al. (1988) for rat cortical membranes. However, values similar to those obtained in the present study were subsequently reported by Marcusson's group in the human cortex (Anderson et al., 1992) and by Foy et al. (1991) in rat diencephalon.

Blakely et al. (1991) and Haber and Goldman (1992) have cloned and expressed a 5-HT transporter from rat brain. Comparison of amino acid sequences demonstrates a 70% similarity between this protein and the NE transporter. In keeping with this observation, functional (Lacroix et al., 1991) and binding (Bauer and Tejani-Butt, 1992) assays of the NE transporter after long-term desipramine administration showed results similar to the ones observed for the 5-HT transporter in the present study. Therefore, monoaminergic transporters seem to possess common adaptive mechanisms elicited by their sustained occupation. Since long-term blockade of the 5-HT transporter induces a decrease in dorsal raphe mRNA codifying this protein (Lesch et al., 1993), the regulatory process most probably takes place at the transcriptional level.

In conclusion, the data presented in this study show (1) that the 5-HT reuptake process can play a significant role in terminating the action of microiontophoretically applied 5-HT in the rat hippocampus and that the RT_{50} method provides a reliable index of the *in vivo* activity of this process; and (2) that the 5-HT transporter, though not a receptor per se, shares with some of them the characteristic of adapting to its sustained occupation through a downregulation mechanism. Thus, it is possible that the enhancement of the efficacy of 5-HT synaptic transmission following long-term administration of SSRIs would not only be due to desensitization of somatodendritic and terminal autoreceptors (Chaput et al., 1986), but also to a decrease in 5-HT reuptake activity.

References

Anderson A, Eriksson A, Marcusson J (1992) Unaltered number of brain serotonin uptake sites in suicide victims. J Psychopharmacol 6:509–513.

Arora RC, Meltzer HY (1986) Effect of subchronic treatment with imipramine, chlorpromazine and the combination on [^{3}H]imipramine binding in rat blood platelets and frontal cortex. Life Sci 39:2289–2296.

Barbaccia ML, Gandolfi O, Chuang D, Costa E (1983) Modulation of neuronal serotonin uptake by a putative endogenous ligand of imipramine recognition sites. Proc Natl Acad Sci USA 80:5134–5138.

Bauer ME, Tejani-Butt S (1992) Effects of repeated administration of desipramine or electroconvulsive shock on norepinepherine uptake sites measured by [^{3}H]nisoxetine autoradiography. Brain Res 582: 208–214.

Bjorklund A, Baumgarten HG, Rensch A (1975) 5,7-Dihydroxytryptamine: improvement of its selectivity for serotonin neurons in the CNS by pretreatment with desipramine. J Neurochem 24:833–835.

Blakely RD, Berson HE, Fremeau RT, Caron MG, Peek MM, Prince HK, Bradley CC (1991) Cloning and expression of a functional serotonin transporter from rat brain. Nature 354:66–70.

Boyer WF, Feighner JP (1991) Pharmacokinetics and drug interactions. In: Selective serotonin reuptake inhibitors (Feighner JP, Boyer WF, eds), pp. 81–88. New York: Wiley.

Brunel S, de Montigny C (1987) Diurnal rhythms in the responsiveness of hippocampal pyramidal neurons to serotonin, norepinephrine, γ-aminobutyric acid and acetylcholine. Brain Res Bull 18:205–212.

Brunello N, Riva M, Volterra A, Racagni G (1987) Effect of some tricyclic and nontricyclic antidepressants on [^{3}H]imipramine binding and serotonin uptake in rat cerebral cortex after prolonged treatment. Fundam Clin Pharmacol 1:327–333.

Brunswick DJ, Kovachich GB, Aronson CE, Frazer A (1991) Effects of repeated administration on serotonin uptake sites measured using [^{3}H]cyanoimipramine autoradiography In: Presynaptic receptors and neuronal transporters (Langer SZ, Galzin AM, Costentin J, eds), pp 233–234. Oxford: Pergamon.

Buus Lassen J (1978) Potent and long-lasting potentiation of two 5-hydroxytryptophan-induced effects in mice by three selective uptake inhibitors. Eur J Pharmacol 47:351–358.

Chaput Y, de Montigny C, Blier P (1986) Effects of a selective 5-HT reuptake blocker, citalopram, on the sensitivity of 5-HT autoreceptors: electrophysiological studies in the rat brain. Naunyn Schmiedebergs Arch Pharmacol 333:342–348.

Cheetham SC, Viggers JA, Bucket WR (1991) Repeated antidepressant drug and electroconvulsive shock administration does not alter [^{3}H]paroxetine binding to rat frontal cortical membranes. Br J Pharmacol 102:242P.

de Montigny C, Aghajanian GK (1977) Preferential action of 5-methoxydimethyltryptamine on presynaptic serotonin receptors: a comparative iontophoretic study with LSD and 5-HT. Neuropharmacology 16:811–818.

de Montigny C, Wang RY, Reader TA, Aghajanian GK (1980) Monoaminergic denervation of the rat hippocampus: microiontophoretic studies on pre- and postsynaptic supersensitivity to norepinephrine and serotonin. Brain Res 200:363–376.

de Souza EB, Kuyatt BL (1987) Autoradiographic localization of [^{3}H]paroxetine-labelled serotonin uptake sites in rat brain. Synapse 1:488–496.

Dewar KM, Reader TA, Grondin L, Descarries L (1991) [^{3}H]paroxetine binding and serotonin content of rat and rabbit cortical areas, hippocampus, neostriatum, ventral mesencephalic tegmentum, and midbrain raphe nuclei region. Synapse 9:14–26.

Foy B, Hepner E, Hrdina PD (1991) Relationship of [^{3}H]paroxetine binding and 5-HT recognition sites on the neuronal serotonin transporter. In: Presynaptic receptors and neuronal transporters (Langer SZ, Galzin AM, Costentin J, eds), pp 231–232. Oxford: Pergamon.

Fredricson Overo K (1982) Kinetics of citalopram in test animals, drug exposure in safety studies. Prog Neuropsychopharmacol Biol Psychiatr 6:297–309.

Fuller RW, Wong DT (1990) Serotonin uptake and serotonin uptake inhibition. Ann NY Acad Sci 600:68–80.

Gerson S, Baldessarini RJ (1975) Selective destruction of serotonin terminals in rat forebrain by high doses of 5,7 dihydroxytryptamine. Brain Res 85:140–145.

Graham D, Tahraoui L, Langer SZ (1987) Effect of chronic treatment with selective monoamine oxidase inhibitors and specific 5-hydroxytryptamine uptake inhibitors on [^{3}H]paroxetine binding to cerebral cortical membranes of the rat. Neuropharmacology 26:1087–1092.

Gravel P, de Montigny C (1987) Noradrenergic denervation prevents sensitization of rat forebrain neurons to tricyclic antidepressant treatment. Synapse 1:133–139.

Haber R, Goldman D (1992) Expression of size-selected RNA encoding brain serotonin transporter in *Xenopus laevis* oocytes. J Neurochem 58:1165–1167.

Habert E, Graham D, Tahraoui L, Claustre Y, Langer SZ (1985) Characterization of [^{3}H]paroxetine binding to rat cortical membranes. Eur J Pharmacol 118:107–114.

Hrdina PD, Foy B, Hepner A, Summers RJ (1990) Antidepressant binding sites in brain: autoradiographic comparison of [^{3}H]imipramine localization and relationship to serotonin transporter. J Pharmacol Exp Ther 252:410–418.

Johnson AM (1991) The comparative pharmacological properties of selective serotonin re-uptake inhibitors. In: Selective serotonin reuptake inhibitors (Feighner JP, Boyer WF, eds), pp 37–70. New York: Wiley.

Kandel ER, Spencer WA (1961) Electrophysiology of hippocampal neurons. II. After-potentials and repetitive firing. J Neurophysiol 24: 243–259.

Katz DM, Kimelberg HK (1985) Kinetics and autoradiography of high affinity uptake of serotonin by primary astrocyte cultures. J Neurosci 5:1901–1908.

Kimelberg HK, Katz DM (1985) High affinity uptake of serotonin into immunocytochemically identified astrocytes. Science 228:889–891.

Kovachich GB, Aronson CE, Brunswick DJ (1992) Effect of repeated administration of antidepressants on serotonin uptake sites in limbic

and neocortical structures of rat brain determined by quantitative autoradiography. Neuropsychopharmacology 7:317–324.

Lacroix D, Blier P, Curet O, de Montigny C (1991) Effects of long-term desipramine administration on noradrenergic neurotransmission: electrophysiological studies in the rat brain. J Pharmacol Exp Ther 257:1081–1090.

Langer SZ, Raisman R (1983) Binding of [^{3}H]imipramine and [^{3}H]desipramine as biochemical tools for studies in depression. Neuropharmacology 22:407–413.

Lesch KP, Aulakh CS, Wolozin BJ, Tolliver TJ, Hill JL, Murphy DL (1993) Regional brain expression of serotonin transporter mRNA and its regulation by reuptake inhibiting antidepressants. Mol Brain Res 17:31–35.

Marcusson JO, Ross SB (1990) Binding of some antidepressants to the 5-hydroxytryptamine transporter in brain and platelets. Psychopharmacology 102:145–155.

Marcusson JO, Fowler CJ, Hall H, Ross SB, Winblad B (1985) "Specific" binding of [^{3}H]imipramine to protease sensitive and protease resistant sites. J Neurochem 44:705–711.

Marcusson JO, Bergstrom M, Eriksson K, Ross SB (1988) Characterization of [^{3}H]paroxetine binding in rat brain. J Neurochem 50: 1783–1790.

Marcusson JO, Andersson A, Backstrom I (1989) Drug inhibition indicates a single-site model of the 5-HT uptake site/antidepressant binding site in rat and human brain. Psychopharmacology 99:17–21.

Mellerup E, Plenge P (1986) High affinity binding of [^{3}H]paroxetine and [^{3}H]imipramine to rat neuronal membranes. Psychopharmacology 89:436–439.

Oleskevich S, Descarries L (1990) Quantified distribution of the serotonin innervation in adult rat hippocampus. Neuroscience 34:19–33.

Plenge P, Mellerup ET (1982) [^{3}H]imipramine high-affinity binding sites in rat brain. Effects of imipramine and lithium. Psychopharmacology 77:94–98.

Ranck JB (1975) Behavioral correlates and firing repertoires of neurons in the dorsal hippocampal formation of unrestrained rats. In: The hippocampus (Isaacson I, Robert L, eds), p 207. New York: Plenum.

Reith MEA, Sershen H, Allen D, Lajtha A (1983) High and low affinity binding of [^{3}H]imipramine in mouse cerebral cortex. J Neurochem 102:389–395.

Wang RY, de Montigny C, Gold BI, Roth RH, Aghajanian GK (1979) Denervation supersensitivity to serotonin in rat forebrain: single cell studies. Brain Res 178:479–497.