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Chronic fluoxetine treatment selectively uncouples raphe 5-HT$_{1A}$ receptors as measured by [^{35}S]-GTPγS autoradiography

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1 Selective Serotonin Reuptake Inhibitors (SSRIs) are thought to have a delay in therapeutic efficacy because of the need to overcome the inhibitory influence of raphe 5-HT$_{1A}$ autoreceptors. Prolonged SSRI administration has been reported to desensitize these autoreceptors. We have used [^{35}S]-GTPγS autoradiography to determine whether this desensitization occurs at the level of receptor/G protein coupling.

2 Male mice were injected intraperitoneally once a day with saline or 20 mg kg^{-1} fluoxetine for either 2 days or 14 days. 5-HT$_{1A}$ receptor binding and coupling to G proteins were assessed using [^{3}H]-8-OH-DPAT and [^{35}S]-GTPγS autoradiography, respectively.

3 The 5-HT receptor agonist 5-carboxamidotryptamine (5-CT) stimulated [^{35}S]-GTPγS binding in the substantia nigra, as well as in hippocampus and dorsal raphe nucleus. The 5-HT$_{1A}$ receptor antagonist p-MPPF (4-fluoro-N-(2-[4-(2-methoxyphenyl)1-piperazinyl]ethyl)-N-(2-pyridinyl)benzamide) blocked this effect in the latter regions, whereas the 5-HT$_{1B/D}$ antagonist GR-127,935 (2′-methyl-4′-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazin-l-yl)-phenyl]-amide) only decreased labelling in substantia nigra.

4 Fourteen-day fluoxetine treatment decreased 5-CT-stimulated [^{35}S]-GTPγS binding in dorsal raphe (saline: 112±12% stimulation; fluoxetine: 66±13%), but not in substantia nigra (99±14% vs 103±7%) or hippocampus (157±3% vs 148±18%). Two-day fluoxetine treatment did not alter 5-CT-stimulated [^{35}S]-GTPγS binding in any of the brain areas investigated.

5 Decreased [^{35}S]-GTPγS binding was not due to receptor down-regulation, since the density of raphe [^{3}H]-8-OH-DPAT binding sites was unaffected by fluoxetine treatment.

6 These results suggest that the desensitization of presynaptic 5-HT$_{1A}$ receptor function occurs at the level of receptor-G protein interaction on dorsal raphe neurons, and may underlie the therapeutic efficacy of long-term SSRI treatment.

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Abbreviations: Gpp(NH)p, guanylylimidodiphosphate; GTPγS, guanosine-5′-*O*-(3-thio)triphosphate; SSRI, Selective Serotonin Reuptake Inhibitors

Introduction

Several neurotransmitters have been implicated in the pathophysiology of major depression, including the serotonin (5-HT) and noradrenaline systems, but the neurobiological causes of the disease remain unclear (Blier & de Montigny, 1994). More is known on the mechanism of action of antidepressant drugs and several lines of evidence suggest that an enhancement of 5-HT neurotransmission might underlie the therapeutic response to different types of antidepressant treatment. Selective Serotonin Reuptake Inhibitors (SSRIs), including fluoxetine (Prozac®) are among the most effective anti-depressants and SSRIs belonging to different chemical families are thought to exert their effect by increasing extracellular 5-HT concentrations through their blocking of the serotonin reuptake carrier (Perry & Fuller, 1992; Kreiss & Lucki, 1995; Blier *et al*., 1990).

Microdialysis studies have shown that both acute and repeated fluoxetine administration can increase extracellular 5-HT in striatum, hippocampus and other forebrain areas (Perry & Fuller, 1992; Rutter & Auerbach, 1993; Kreiss & Lucki, 1995). However, the increase in forebrain extracellular 5-HT elicited by acute SSRI administration is limited by a negative feedback involving raphe autoreceptors, with resulting decrease in raphe neuronal activity and 5-HT release (Artigas *et al*., 1996). Whereas the SSRI-induced increase in extracellular 5-HT is immediate, there is a 2 to 6 week delay in therapeutic efficacy of SSRIs. This delay suggests that 5-HT reuptake inhibition *per se* is unlikely to account for the therapeutic activity, but that some kind of long term neuro-adaptive change underlies the antidepressant action of SSRIs. The slow onset of therapeutic response to SSRIs is hypothesized to involve the progressive desensitization of somatodendritic 5-HT$_{1A}$ receptors with repeated treatment (Blier & de Montigny, 1994). Such an effect would gradually allow for a return of raphe neuronal activity and increased release of 5-HT in

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forebrain targets of the dorsal raphe which provides the major serotonergic innervation of the neocortex (O'Hearn & Molliver, 1984). This is consistent with the observation that co-administration of a 5-HT_{1A} receptor antagonist decreases the time required for SSRIs to achieve therapeutic efficacy (Artigas *et al*., 1996).

Of the ~14 receptors identified to date, the 5-HT_{1A} receptor is one of the best-characterized (for a recent review see Raymond *et al*., 1999). It shows high affinity for 5-HT, 8-OH-DPAT, as well as for anxiolytic drugs such as buspirone, ipsapirone and gepirone. 5-HT_{1A} receptors are located both presynaptically on the cell bodies and dendrites of serotonergic neurons in the raphe nuclei, and postsynaptically in forebrain areas including hippocampus, entorhinal and prefrontal cortex. Single unit recording (Blier *et al*., 1988) and microdialysis (Kreiss & Lucki, 1995) studies in rats have shown that 5-HT_{1A} autoreceptors in the dorsal raphe are desensitized following chronic fluoxetine treatment. Chronic fluoxetine therapy lessens the ability of a 5-HT_{1A} agonist, 8-OH-DPAT, to suppress 5-HT release (Kreiss & Lucki, 1995). Most studies have shown that chronic treatment with fluoxetine does not alter 5-HT_{1A} receptor density (Welner *et al*., 1989; Larsson *et al*., 1990; Schechter *et al*., 1990; Hensler *et al*., 1991, Wieland *et al*., 1993; Le Poul *et al*., 1995, Hervás *et al*., 2001), although not all studies agree (Fanelli & McMonagle-Strucko, 1992). It therefore seems likely that desensitization occurs downstream of the receptor and reflects altered coupling of agonist binding to cellular response.

5-HT_{1A} receptors are coupled to G proteins of the G_i family, which include G_{i1}, G_{i2}, G_{i3}, G_o and G_z proteins (Raymond *et al*., 1993; Barr *et al*., 1997). We have previously shown that activation of this type of receptors can be visualized on frozen brain sections of normal rats and guinea-pigs using [35S]-GTPγS labelling (Waeber & Moskowitz, 1997), a technique developed by Sim *et al*. (1995). Chronic treatment with the full 5-HT_{1A} receptor agonist 8-OH-DPAT has been shown to decrease [35S]-GTPγS labelling induced by 5-HT_{1A} receptor activation in rat raphe nucleus (Hensler & Durgam, 2001). A related study using the azapirone anxiolytic buspirone, a partial 5-HT_{1A} receptor agonist, reported decreased 8-OH-DPAT induced [35S]-GTPγS labelling in lateral septum as well as raphe nucleus (Sim-Selley *et al*., 2000). Interestingly, electrophysiological experiments have shown that chronic treatment with other azapirone drugs, gepirone and ipsapirone, did not affect 5-HT_{1A} receptor activity in hippocampus but led to desensitization of 5-HT_{1A} autoreceptors in dorsal raphe nucleus, in a way very similar to the effect of chronic treatment with SSRIs (Blier & de Montigny, 1987; Schechter *et al*., 1990). We therefore hypothesized that 5-HT_{1A} autoreceptor desensitization in the raphe following chronic fluoxetine treatment was likely to occur at the level of receptor-G protein interaction.

In the present study, we used agonist-stimulated [35S]-GTPγS receptor autoradiography to demonstrate that chronic treatment with the SSRI fluoxetine affects receptor coupling, but not density, in the same way as chronic treatment with 5-HT_{1A} receptor agonists (Sim-Selley *et al*., 2000; Hensler & Durgam, 2001).

Methods

Materials

[35S]-GTPγS was obtained from New England Nuclear (Boston, MA, U.S.A.) (specific activity 1000–1500 Ci mmol^{-1}). [3H]-8-OH-DPAT was obtained from Amersham (Arlington Heights, IL, U.S.A.) (specific activity, 205 Ci mmol^{-1}). GDP was purchased from Sigma Chemical (St. Louis, MO, U.S.A.). 5-carboxamidotryptamine, p-MPPF dihydrochloride (4-fluoro-N-(2-[4-(2-methoxyphenyl)1-piperazinyl]ethyl)-N-(2-pyridinyl)benzamide) and DPCPX (8-cyclopentyl-1,3-dipropylxanthine) were from Sigma-RBI (Natick, MA, U.S.A.). GR-127,935 (2′-methyl-4′-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-amide) was provided by Glaxo.

Tissues

Adult male Swiss Webster mice (25–30 g, Taconic, Germantown, NY, U.S.A.) received either 2-day or 14-day treatment consisting of intraperitoneal injections once a day (1000–1100 h) with saline or 20 mg kg^{-1} fluoxetine (dissolved at a concentration of 2 mg ml^{-1}). Twenty-four hours after the last injection, they were sedated with inhaled CO_2 and decapitated. The whole brain and upper cervical spinal cord were dissected and frozen in isopentane cooled at −40°C. Hindbrains (posterior to bregma-2.5 mm) were cut into 14 μm sections using a cryostat-microtome (Microm HM505E). Sections were thaw-mounted onto Superfrost Plus glass slides (Fisher Scientific, Pittsburgh, PA, U.S.A.) and stored at −80°C for less than 3 weeks.

Autoradiography

[35S]-GTPγS binding was visualized as previously described (Sim *et al*., 1995; Waeber & Moskowitz, 1997; Happe *et al*., 2001) with minor modifications. Sections were thawed to room temperature 15 min before the experiment and incubated for 15 min at room temperature in 50 mM glycylglycine buffer (pH 7.4) containing 150 mM NaCl and 1 mM EGTA, and for a further 30 min in the same buffer supplemented with 3 mM $MgCl_2$, 0.2 mM dithiothreitol, and 2 mM GDP. In order to observe agonist-induced binding, sections were incubated in the appropriate concentration of agonist for 60 min at 30°C in buffer containing 2 mM GDP, 100 nM DPCPX (to block adenosine A_1 receptors stimulated by endogenously formed adenosine; Laitinen, 1999), 10 μM pargyline, and 0.1 nM [35S]-GTPγS. Slides were then washed for 5 min twice in ice-cold buffer (without GDP), dipped briefly in ice-cold deionized water, dried under a stream of cold air, and exposed to Kodak Bio-Max film along with 14C-labelled polymer standards (Amersham) for 24 h.

5-HT_{1A} receptor binding sites were visualized in sections consecutive to those used for [35S]-GTPγS autoradiography as follows. Sections were thawed to room temperature 15 min before the experiment and incubated for 15 min at room temperature in 0.17 M Tris·HCl (pH 7.4) containing 4 mM $CaCl_2$. Sections were then incubated for 60 min in buffer supplemented with 2 nM [3H]-8-OH-DPAT 10 μM pargylin and 0.01% ascorbic acid, washed twice for 5 min each in ice-

cold buffer, briefly dipped in ice-cold deionized water, dried under a stream of cold air and exposed to Amersham ^{3}H-Hyperfilm for 2 weeks along with ^{3}H-labelled polymer standards (Amersham).

Image analysis

[^{3}H]-8-OH-DPAT and [^{35}S]-GTPγS binding in selected brain regions were assessed by measuring the optical density of the autoradiograms using a computerized image analysis system (M4; Imaging Research, St. Catharines, Ontario, Canada). Agonist-induced [^{35}S]-GTPγS binding is expressed as percentage of basal binding.

Data analysis

Concentration-effect curves were obtained from fitting autoradiographic measurement data points by non-linear regression using Grafit (Erithacus Software, Staines, U.K.). The equation used was $\text{Stim} = E_{max}/(1 + EC_{50}/\text{Ago})$, where Stim is the stimulated binding (in per cent over basal), E_{max} is the maximal binding, EC_{50} is the concentration of agonist resulting in half-maximal [^{35}S]-GTPγS binding, and Ago is the agonist concentration.

To compare concentration/effect curves in saline and treated animals, the improvement of fit using two different curves (to fit data from saline- and fluoxetine-treated mice separately) over the simpler model using a single curve (to fit the pooled values from both saline- and fluoxetine-treated mice) was assessed by calculating the F statistics:

$$F = \frac{(SS_1 - SS_2)/(df_1 - df_2)}{SS_2/df_2}$$

where the subscript 1 refers to the simplest model (single concentration/effect curves fitted to pooled data), SS_i is the sum of squares of the respective residuals and df_i the respective degrees of freedom.

Basal [^{35}S]-GTPγS binding in different regions were compared within the 2-day and 14 day treatment groups with a *t*-test and a Bonferroni correction (since sections from the short- and long-term treatment groups were labelled in separate experiments, no attempt was made to compare basal [^{35}S]-GTPγS labelling between these groups).

Results

As previously described in the rat and guinea-pig (Waeber & Moskowitz, 1997), basal [^{35}S]-GTPγS binding was heterogeneous, with relatively higher levels in interpeduncular nucleus, central grey and dorsal raphe (Figure 1). At variance with these two species however, little basal binding was found in mouse superficial grey layer of the superior colliculus. This difference is unlikely to be related to the different buffer used in the present study (Happe *et al*., 2001), since rat sections incubated together with mouse section did shown relatively intense basal [^{35}S]-GTPγS labelling in the superior colliculus (data not shown).

No significant differences in the level of basal [^{35}S]-GTPγS binding were observed in the different treatment groups in the dorsal raphe and hippocampus (Table 1). Long-term (14-day), but not short-term (2-day) fluoxetine treatment significantly decreased basal [^{35}S]-GTPγS labelling in substantia nigra.

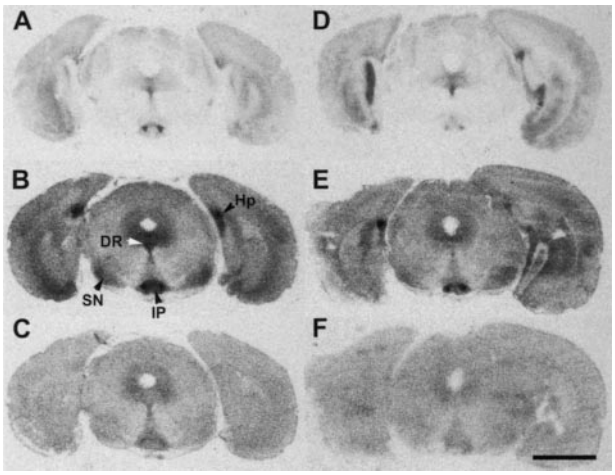

Figure 1 Distribution of binding sites for the selective 5-HT$_{1A}$ radioligand [^{3}H]-8-OH-DPAT in representative coronal sections of a mouse treated for 14 days with saline (A) or 20 mg kg^{-1} fluoxetine (D). Note the similar density of binding site over the dorsal raphe (DR). (B and E) Show 5-carboxamidotryptamine (5-CT) induced [^{35}S]-GTPγS labelling in sections consecutive to those shown in (A and D). Note the marked decrease in labelling in the dorsal raphe, but the similar density of labelling in the substantia nigra (SN), interpeduncular nucleus (IP) and hippocampus (Hp). (C and F) Show basal [^{35}S]-GTPγS binding, relatively homogeneous at this brain level, with slightly higher intensity in the central grey and interpeduncular nucleus. Quantification revealed significantly decreased basal [^{35}S]-GTPγS binding in the substantia nigra, but not dorsal raphe of mice treated with fluoxetine for 14 days (see Table 1). Scale bar is 2 mm.

The potent non-selective 5-HT receptor agonist 5-carboxamidotryptamine (5-CT) stimulated [^{35}S]-GTPγS binding in hippocampus (molecular layer of dentate gyrus and strata oriens and radiatum of CA_3), dorsal raphe, interpeduncular nucleus, and substantia nigra (Figure 1). In the presence of the specific 5-HT$_{1A}$ receptor antagonist p-MPPF (1 μM), 5-CT-stimulated [^{35}S]-GTPγS binding virtually disappeared in hippocampus and dorsal raphe (Figure 2). This finding indicates that 5-CT-enhanced [^{35}S]-GTPγS labelling is accounted for by 5-HT$_{1A}$ receptors in dorsal raphe and hippocampus, confirming previous reports in rats (Waeber & Moskowitz, 1997; Sim *et al*., 1997; Alper & Nelson, 1998; Dupuis *et al*., 1998), and in agreement with the distribution of binding sites for the specific 5-HT$_{1A}$ ligand [^{3}H]-8-OH-DPAT in consecutive sections of the same mice (Figure 1A,D). In contrast, 5-CT-induced [^{35}S]-GTPγS binding in substantia nigra was markedly decreased by the 5-HT$_{1B/1D}$ receptor antagonist GR-127,935 (1 μM), but was unaffected by the 5-HT$_{1A}$ receptor antagonist p-MPPF, suggesting that the relevant receptors in this region belong to the 5-HT$_{1B/1D}$ subtype.

There was no significant difference in the EC_{50} value for 5-CT in the different regions or treatment groups (Table 1). In contrast, 14-day fluoxetine treatment significantly decreased the maximal [^{35}S]-GTPγS binding in dorsal raphe nucleus, but not in the other brain regions investigated (Figure 3). Short-term fluoxetine treatment (2 days) did not affect EC_{50} values or maximal [^{35}S]-GTPγS binding in any brain area (Table 1).

Table 1 Effect of short-term (A: 2 days) and long-term (B: 14 days) treatment with fluoxetine in basal and 5-CT stimulated [^{35}S]-GTPγS binding in different regions of the mouse brain

A. Short-term treatment

Region	*pEC50* (−log nM)	*2-day saline* Basal binding (nCi g^{-1} tissue)	*Emax* (% of basal)	*pEC50* (−log nM)	*2-day fluoxetine* Basal binding (nCi g^{-1} tissue)	*Emax* (% of basal)
Hippocampus	7.81±0.16	297±51	93±10	7.93±0.07	297±30	97±4
Raphe	7.93±0.24	389±59	81±14	8.17±0.10	421±21	76±5
Substantia Nigra	7.69±0.11	338±51	108±7	7.79±0.06	362±4	86±3

B. Long-term treatment

Region	*pEC50* (−log nM)	*14-day saline* Basal binding (nCi g^{-1} tissue)	*Emax* (% of basal)	*pEC50* (−log nM)	*14-day fluoxetine* Basal binding (nCi g^{-1} tissue)	*Emax* (% of basal)
Hippocampus	7.95±0.04	455±24	157±3	7.99±0.18	421±18	148±18
Raphe	7.92±0.15	694±41	112±12	7.77±0.30	650±26	66±13[a]
Substantia Nigra	7.82±0.20	603±17	99±14	7.76±0.11	490±13[b]	103±7

[a]$P<0.05$, F statistics; [b]$P<0.001$, *t*-test.

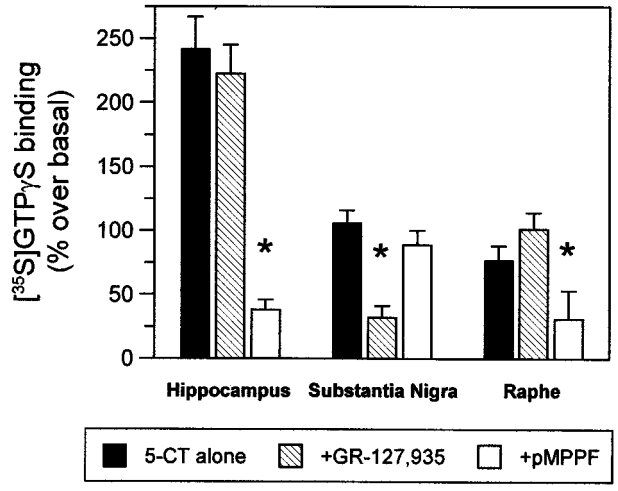

Figure 2 Pharmacological profile of [^{35}S]-GTPγS binding induced by 10 μM 5-CT in mouse hippocampus, substantia nigra and dorsal raphe (bars represent mean±s.e.mean, $n=5$. Representative of two independent experiments). The 5-HT$_{1A}$ receptor antagonist p-MPPF (1 μM) virtually abolished 5-CT-induced [^{35}S]-GTPγS binding in hippocampus and dorsal raphe, regions where the 5-HT$_{1B/1D}$ receptor antagonist GR-127,935 (1 μM) showed no effect. In contrast, labelling in substantia nigra was markedly attenuated by GR-127,935, but unaffected by p-MPPF. *$P<0.05$ (one-way analysis of variance, followed by Dunnett's *post hoc* test).

None of the treatments altered the density of [^{3}H]-8-OH-DPAT binding sites in the regions studied (Table 2).

Discussion

The main finding of the present study is that long-term (14-day) treatment with the SSRI fluoxetine selectively uncouples 5-HT$_{1A}$ receptors from G proteins in the dorsal raphe nucleus, but not in hippocampus. This effect is not observed after short-term (2-day) treatment. In addition, 5-HT$_{1B}$ receptor coupling in the substantia nigra is not altered after either short-term or long-term treatment.

The decrease in 5-CT induced [^{35}S]-GTPγS labelling in dorsal raphe nucleus is unlikely to be due to a decrease in 5-HT$_{1A}$ receptor density, since the density of [^{3}H]-8-OH-DPAT labelling in the raphe of fluoxetine-treated mice was not significantly different from that found in the raphe of vehicle-treated mice. Numerous previous studies have found that chronic fluoxetine administration does not alter 5-HT$_{1A}$ receptor binding (see Introduction for references). In addition, Hensler & Durgam (2001) have recently reported decreased 5-HT$_{1A}$ receptor coupling in raphe nucleus without alteration in receptor density after 14-day treatment with the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT.

Interestingly, in a study very similar to ours, Li *et al.* (1997) failed to observe any effect of chronic fluoxetine treatment on the G protein coupling of raphe 5-HT$_{1A}$ receptors. In their study, 5-HT$_{1A}$ receptors were labelled in frozen brain sections with [^{3}H]-8-OH-DPAT in the absence or presence of the GTP analogue guanylylimidodiphosphate (Gpp(NH)p). Differences in species and treatment regimen could account for the contrasting outcomes, but it is more likely that most 5-HT$_{1A}$ receptors in dorsal raphe exist in an uncoupled, low-affinity state, insensitive to Gpp(NH)p. Indeed, Li *et al.* (1997) found that less than 25% of [^{3}H]-8-OH-DPAT binding in dorsal raphe and hippocampus was inhibited by this GTP analogue. Direct activation of [^{35}S]-GTPγS binding therefore seems to be a more powerful technique to detect alteration in 5-HT$_{1A}$ receptor coupling after chronic treatment with serotonergic agents.

The fluoxetine dose that we chose has been shown to increase 5-HT content in microdialysate from mouse striatum and hippocampus for well over 200 min (Knobelman *et al.*, 2001). We therefore hypothesize that dorsal raphe 5-HT$_{1A}$ receptor uncoupling results from the long-term receptor stimulation by 5-HT released from the serotonergic neurons. However, it is unlikely that the differential effect of fluoxetine treatment on raphe 5-HT$_{1A}$ receptors vs hippocampal 5-HT$_{1A}$ receptors is due to a smaller elevation in extracellular 5-HT in the latter area, since the same region-specific pattern has been observed after chronic systemic treatment with a full (Hensler & Durgam, 2001) and a partial (Sim-Selley *et al.*, 2000) 5-HT$_{1A}$ receptor agonist.

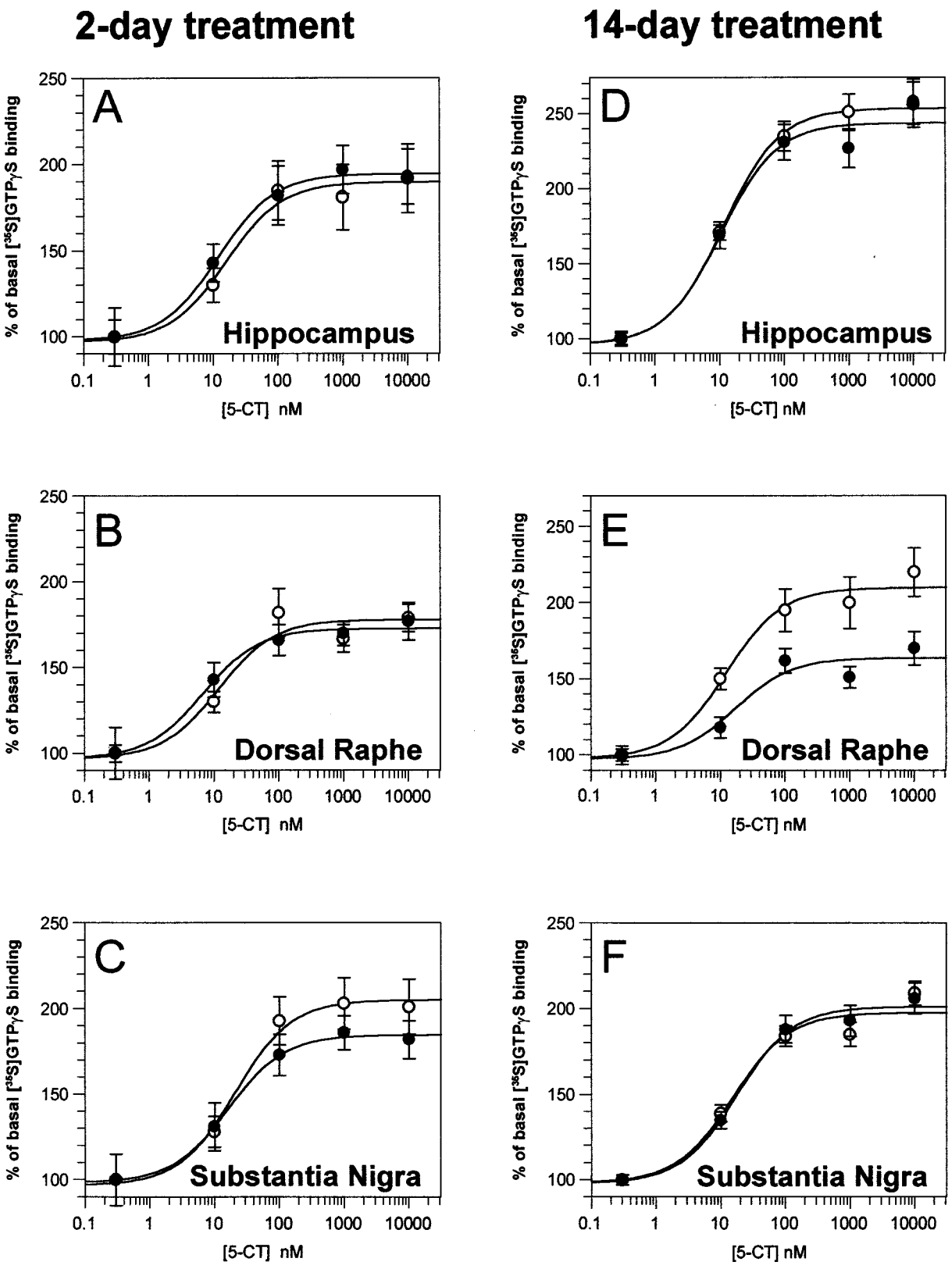

Figure 3 Concentration/response curves for the 5-carboxamidotryptamine (5-CT) induced [^{35}S]-GTPγS binding in different brain regions of mice treated with saline (empty circle) or 20 mg kg^{-1} fluoxetine (full circles) for 2 days (left column) or 14 days (right column). Data points are the means ± s.e.mean of 8–10 different animals per group. No attempt was made to compare data from the 2-day treatment group with data from the 14-day treatment group, because tissue sections were ^{35}S-GTPγS-labelled in different experiments. The only significant treatment-related difference was found in the dorsal raphe of mice treated with fluoxetine for 14 consecutive days, in which [^{35}S]-GTPγS was significantly decreased (see Table 1).

Table 2 Density of [3H]-8-OH-DPAT binding sites in dorsal raphe and hippocampus of brain sections consecutive to those used for [35S]-GTPγS autoradiography. Tissues were processed as described in Methods and incubated in the presence of 2 nM [3H]-8-OH-DPAT. Densities are given as mean ± s.e.mean (fmol mg^{-1} tissue) and represent the amount of receptors occupied at this radioligand concentration

	2-Day saline	*2-Day fluoxetine*	*14-Day saline*	*14-Day fluoxetine*
Hippocampus	123 ± 4	116 ± 5	113 ± 5	108 ± 5
Dorsal Raphe	114 ± 2	109 ± 4	104 ± 5	95 ± 5

The mechanism mediating the uncoupling of raphe 5-HT_{1A} receptors following treatment with fluoxetine or 5-HT_{1A} receptor agonists and the reason for its regional selectivity are unclear. Raphe 5-HT_{1A} receptors are localized presynaptically to both dendritic processes and somata, whereas hippocampal 5-HT_{1A} receptors are postsynaptic and associated exclusively with dendritic spines (Kia *et al*., 1996). It is possible that sub-cellular localization influences the protein environment of the receptors (e.g. G protein subtypes, kinases), which could modify the development of desensitization. [35S]-GTPγS autoradiography can be best applied to receptors coupled to Pertussis-sensitive G proteins, but there is no indication that [35S]-GTPγS binds differentially to G_{i1}, G_{i2}, G_{i3}, or G_o (Waeber & Moskowitz, 1997). Although 5-HT_{1A} receptors preferentially couple to G_{i3} proteins, they can activate all inhibitory G proteins (Raymond *et al*., 1999). It is conceivable that 5-HT_{1A} receptors in dorsal raphe are coupled to a particular G protein subtype more prone to down-regulation. Indeed, using immunoaffinity chromatography followed by immunoblotting with subtype-specific anti-Gα antibodies, Mannoury-La-Cour *et al*. (2001) found that 5-HT_{1A} receptors are physically coupled to G_o in hippocampus, and to G_{i3} in raphe nuclei. Although we did not observe any treatment-related difference in basal [35S]-GTPγS labelling in the latter region, this observation was made at a single [35S]-GTPγS concentration. Further studies will be useful to quantify the level of specific G proteins in dorsal raphe after chronic fluoxetine treatment. Li *et al*. (1996) have shown that chronic fluoxetine treatment reduces the concentration of G_{i1} and G_{i3} in hypothalamus, and G_o and G_{i2} in midbrain, while the latter two protein were unaltered in frontal cortex despite 22 days of treatment. The decrease that they observed in the midbrain could reflect the reduction in basal [35S]-GTPγS binding that we found in the substantia nigra of fluoxetine-treated mice.

A study of the time course of 5-HT_{1A} receptor desensitization using electrophysiological recordings showed that the potency of 8-OH-DPAT to depress the firing of raphe neurons was reduced as early as after a 3-day fluoxetine treatment (Le Poul *et al*., 1995). Although we did not study the time course of 5-HT_{1A} receptor uncoupling, our results are compatible with this study, since we do not observe any decrease in 5-CT induced [35S]-GTPγS binding after 2 days of fluoxetine treatment. Chronic treatment with the full 5-HT_{1A} receptor agonist 8-OH-DPAT significantly attenuated 5-HT_{1A} receptor-stimulated [35S]-GTPγS binding in dorsal raphe after both 7 and 14 days (Hensler & Durgam, 2001). It is therefore quite possible that changes in [35S]-GTPγS binding could have been observed after less than the full 14-day fluoxetine treatment that we employed.

It is interesting to note that the effects of long-term blockade of 5-HT re-uptake sites with fluoxetine is similar to those of chronic treatment with 5-HT_{1A} receptor agonists (Sim-Selley *et al*., 2000; Hensler & Durgam, 2001), but not to those of genetic deletion of the 5-HT transporter. Studies in 5-HT transporter knockout mice showed that the decreased agonist induced [35S]-GTPγS binding in dorsal raphe was due to a reduced of 5-HT_{1A} receptor density, but not to altered G-protein coupling or G-protein levels (Fabre *et al*., 2000; Li *et al*., 2000). Similar changes in 5-HT_{1B} receptors were observed in the substantia nigra of these mice (Fabre *et al*., 2000). It is possible that the absence of the transporter during development produces more complex changes than transient pharmacological blockade in adult brain.

Similar results have recently been obtained in Substance P (Neurokinin-1) receptor knockout mice (Froger *et al*., 2001). These mice show decreased 5-HT_{1A} receptor density and agonist-induced [35S]-GTPγS binding in dorsal raphe, but not in hippocampus (see above). This observation, taken together with the fact that the substance P receptor antagonist MK-869 shows therapeutic efficacy in depressed patients (Kramer *et al*., 1998), substantiates the hypothesis that enhancement of 5-HT neurotransmission underlies the therapeutic response to different types of antidepressant treatments (Blier & de Montigny, 1994).

In conclusion, the time course and regional selectivity of the changes as well as the lack of effect on the receptor density observed in the present study are similar to previous observations following chronic administration of antidepressant drugs (Blier & de Montigny, 1994). Although the present study does not rule out the occurrence of changes downstream of the G protein level or in regions other than the ones investigated, the present findings suggest that uncoupling between raphe 5-HT_{1A} receptors and their signalling G proteins plays an important role in the delayed therapeutic effects of SSRIs. Further studies are needed to determine whether these alterations are brought about by post-translational modification of the receptors (e.g. phosphorylation), changes in the G protein population, or in another interacting molecule. Understanding specific mechanisms contributing to long-term fluoxetine-induced 5-HT_{1A} receptor desensitization on dorsal raphe neurons could lead to the development of more effective antidepressants with a reduced delay in therapeutic activity.

References

ALPER, R.H. & NELSON, D.L. (1998). Characterization of 5-HT_{1A} receptor-mediated [^{35}S]GTPγS binding in rat hippocampal membranes. *Eur. J. Pharmacol.,* **343,** 303–312.

ARTIGAS, F., ROMERO, L., DE MONTIGNY, C. & BLIER, P. (1996). Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT_{1A} antagonists. *Trends Neurosci.,* **19,** 378–383.

BARR, A.J., BRASS, L.F. & MANNING, D.R. (1997). Reconstitution of receptors and GTP-binding regulatory proteins (G proteins) in Sf9 cells. A direct evaluation of selectivity in receptor G protein coupling. *J. Biol. Chem.,* **272,** 2223–2229.

BLIER, P., CHAPUT, Y. & DE MONTIGNY, C. (1988). Longterm 5-HT reuptake blockade, but not monoamine oxidase inhibition, decreases the function of terminal 5-HT autoreceptors: an electrophysiological study in the rat brain. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **337,** 246–254.

BLIER, P. & DE MONTIGNY, C. (1987). Modification of 5-HT neuron properties by sustained administration of the 5-HT_{1A} agonist gepirone: electrophysiological studies in the rat brain. *Synapse,* **1,** 470–480.

BLIER, P. & DE MONTIGNY, C. (1994). Current advances and trends in the treatment of depression. *Trends Pharmacol. Sci.,* **15,** 220–226.

BLIER, P., DE MONTIGNY, C. & CHAPUT, Y. (1990). A role for the serotonin system in the mechanism of action of antidepressant treatments: preclinical evidence. *J. Clin. Psychiatry,* **51** (Suppl.): 14–20.

DUPUIS, D.S., PALMIER, C., COLPAERT, F.C. & PAUWELS, P.J. (1998). Autoradiography of serotonin 5-HT_{1A} receptor-activated G proteins in guinea pig brain sections by agonist-stimulated [^{35}S]GTPγS binding. *J. Neurochem.,* **70,** 1258–1268.

FANELLI, R.J. & MCMONAGLE-STRUCKO, K. (1992). Alteration of 5-HT_{1A} receptor binding sites following chronic treatment with ipsapirone measured by quantitative autoradiography. *Synapse,* **12,** 75–81.

FABRE, V., BEAUFOUR, C., EVRARD, A., RIOUX, A., HANOUN, N., LESCH, K.P., MURPHY, D.L., LANFUMEY, L., HAMON, M. & MARTRES, M.P. (2000). Altered expression and functions of serotonin 5-HT_{1A} and 5-HT_{1B} receptors in knock-out mice lacking the 5-HT transporter. *Eur. J. Neurosci.,* **12,** 2299–2310.

FROGER, N., GARDIER, A.M., MORATALLA, R., ALBERTI, I., LENA, I., BONI, C., DE FELIPE, C., RUPNIAK, N.M., HUNT, S.P., JACQUOT, C., HAMON, M. & LANFUMEY, L. (2001). 5-Hydroxytryptamine (5-HT)$_{1A}$ Autoreceptor Adaptive Changes in Substance P (Neurokinin 1) Receptor Knock-Out Mice Mimic Antidepressant-Induced Desensitization. *J. Neurosci.,* **21,** 8188–8197.

HAPPE, H.K., BYLUND, D.B. & MURRIN, L.C. (2001). Agonist-stimulated [^{35}S]GTPγS autoradiography: optimization for high sensitivity. *Eur. J. Pharmacol.,* **422,** 1–13.

HENSLER, J.G., COVACHICH, A. & FRAZER, A. (1991). A quantitative autoradiographic study of 5-HT_{1A} receptor regulation. Effect of 5,7-dihydroxytryptamine and antidepressant treatments. *Neuropsychopharmacology,* **4,** 131–144.

HENSLER, J.G. & DURGAM, H. (2001). Regulation of 5-HT_{1A} receptor-stimulated [^{35}S]GTPγS binding as measured by quantitative autoradiography following chronic agonist administration. *Br. J. Pharmacol.,* **132,** 605–611.

HERVAS, I., VILARO, T., ROMERO, L., SCORZA, C., MENGOD, G. & ARTIGAS, F. (2001). Desensitization of 5-HT_{1A} autoreceptors by a low chronic fluoxetine dose. Effect of the concurrent administration of WAY-100635. *Neuropsychopharmacology,* **24,** 11–20.

KIA, H.K., BRISORGUEIL, M.J., HAMON, M., CALAS, A. & VERGÉ, D. (1996). Ultrastructural localization of 5-hydroxytryptamine$_{1A}$ receptors in the rat brain. *J. Neurosci. Res.,* **46,** 697–708.

KNOBELMAN, D.A., HEN, R. & LUCKI, I. (2001). Genetic regulation of extracellular serotonin by 5-hydroxytryptamine$_{1A}$ and 5-hydroxytryptamine$_{1B}$ autoreceptors in different brain regions of the mouse. *J. Pharmacol. Exp. Ther.,* **298,** 1083–1091.

KRAMER, M.S., CUTLER, N., FEIGHNER, J., SHRIVASTAVA, R., CARMAN, J., SRAMEK, J.J., REINES, S.A., LIU, G., SNAVELY, D., WYATT-KNOWLES, E., HALE, J.J., MILLS, S.G., MACCOSS, M., SWAIN, C.J., HARRISON, T., HILL, R.G., HEFTI, F., SCOLNICK, E.M., CASCIERI, M.A., CHICCHI, G.G., SADOWSKI, S., WILLIAMS, A.R., HEWSON, L., SMITH, D., RUPNIAK, N.M. *et al.* (1998). Distinct mechanism for antidepressant activity by blockade of central substance P receptors. *Science,* **281,** 1640–1645.

KREISS, D.S. & LUCKI, I. (1995). Effects of acute and repeated administration of antidepressant drugs on extracellular levels of 5-hydroxytryptamine measured in vivo. *J. Pharmacol. Exp. Ther.,* **274,** 866–876.

LAITINEN, J.T. (1999). Selective detection of adenosine A_1 receptor-dependent G-protein activity in basal and stimulated conditions of rat brain [^{35}S]guanosine 5′-(gamma-thio)triphosphate autoradiography. *Neuroscience,* **90,** 1265–1279.

LARSSON, L.G., RENYI, L., ROSS, S.B., SVENSSON, B. & ANGEBY-MOLLER, K. (1990). Different effects on the responses of functional pre- and postsynaptic 5-HT_{1A} receptors by repeated treatment of rats with the 5-HT_{1A} receptor agonist 8-OH-DPAT. *Neuropharmacology,* **29,** 86–91.

LE POUL, E., LAARIS, N., DOUCET, E., LAPORTE, A.M., HAMON, M. & LANFUMEY, L. (1995). Early desensitization of somato-dendritic 5-HT_{1A} autoreceptors in rats treated with fluoxetine or paroxetine. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **352,** 141–148.

LI, Q., BATTAGLIA, G. & VAN DE KAR, L.D. (1997). Autoradiographic evidence for differential G-protein coupling of 5-HT_{1A} receptors in rat brain: lack of effect of repeated injections of fluoxetine. *Brain Res.,* **769,** 141–151.

LI, Q., MUMA, N.A. & VAN DE KAR, L.D. (1996). Chronic fluoxetine induces a gradual desensitization of 5-HT_{1A} receptors: Reductions in hypothalamic and midbrain G_i and G_o proteins and in neuroendocrine responses to a 5-HT_{1A} agonist. *J. Pharmacol. Exp. Ther.,* **279,** 1035–1042.

LI, Q., WICHEMS, C., HEILS, A., LESCH, K.P. & MURPHY, D.L. (2000). Reduction in the density and expression, but not G-protein coupling, of serotonin receptors (5-HT_{1A}) in 5-HT transporter knock-out mice: gender and brain region differences. *J. Neurosci.,* **20,** 7888–7895.

MANNOURY-LA-COUR, C., EL MESTIKAWY, S., RUMAJOGEE, P., BERNARD, R., MIQUEL, M.-C., HAMON, M. & LANFUMEY, L. (2001). Regional differences in G proteins coupled to 5-HT_{1A} receptors in the rat brain. *Soc. Neurosci.,* **27,** 380.7 (abstract).

O'HEARN, E. & MOLLIVER, M.E. (1984). Organization of raphe-cortical projections in rat: a quantitative retrograde study. *Brain Res. Bull.,* **13,** 709–726.

PERRY, K.W. & FULLER, R.W. (1992). Effect of fluoxetine on serotonin and dopamine concentration in microdialysis fluid from rat striatum. *Life Sci.,* **50,** 1683–1690.

RAYMOND, J.R., MUKHIN, Y.V., GETTYS, T.W. & GARNOVSKAYA, M.N. (1999). The recombinant 5-HT_{1A} receptor: G protein coupling and signalling pathways. *Br. J. Pharmacol.,* **127,** 1751–1764.

RAYMOND, J.R., OLSEN, C.L. & GETTYS, T.W. (1993). Cell-specific physical and functional coupling of human 5-HT_{1A} receptors to inhibitory G protein alpha-subunits and lack of coupling to Gs alpha. *Biochemistry,* **32,** 11064–11073.

RUTTER, J.J. & AUERBACH, S.B. (1993). Acute uptake inhibition increases extracellular serotonin in the rat forebrain. *J. Pharmacol. Exp. Ther.,* **265,** 1319–1324.

SCHECHTER, L.E., BOLANOS, F.J., GOZLAN, H., LANFUMEY, L., HAJ-DAHMANE, S., LAPORTE, A.M., FATTACCINI, C.M. & HAMON, M. (1990). Alterations of central serotonergic and dopaminergic neurotransmission in rats chronically treated with ipsapirone: biochemical and electrophysiological studies. *J. Pharmacol. Exp. Ther.,* **255,** 1335–1347.

SIM, L.J., SELLEY, D.E. & CHILDERS, S.R. (1995). *In vitro* autoradiography of receptor-activated G proteins in rat brain by agonist-stimulated guanylyl 5′-[gamma-[^{35}S]thio]-triphosphate binding. *Proc. Natl. Acad. Sci. U.S.A.,* **92,** 7242 – 7246.

SIM, L.J., XIAO, R. & CHILDERS, S.R. (1997). *In vitro* autoradiographic localization of 5-HT$_{1A}$ receptor-activated G-proteins in the rat brain. *Brain Res. Bull.,* **44,** 39 – 45.

SIM-SELLEY, L.J., VOGT, L.J., XIAO, R., CHILDERS, S.R. & SELLEY, D.E. (2000). Region-specific changes in 5-HT$_{1A}$ receptor-activated G-proteins in rat brain following chronic buspirone. *Eur. J. Pharmacol.,* **389,** 147 – 153.

WAEBER, C. & MOSKOWITZ, M.A. (1997). 5-Hydroxytryptamine$_{1A}$ and 5-Hydroxytryptamine$_{1B}$ receptors stimulate [^{35}S] Guanosine-5′-O-(3-thio)triphosphate binding to rodent brain sections as visualized by *in vitro* autoradiography. *Mol. Pharmacol.,* **52,** 623 – 631.

WELNER, S.A., DE MONTIGNY, C., DESROCHES, J., DESJARDINS, P. & SURANYICADOTTE, B.E. (1989). Autoradiographic quantification of serotonin$_{1A}$ receptors in rat brain following antidepressant drug treatment. *Synapse,* **4,** 347 – 352.

WIELAND, S., FISCHETTE, C.T. & LUCKI, I. (1993). Effect of chronic treatments with tandospirone and imipramine on serotonin-mediated behavioral responses and monoamine receptors. *Neuropharmacol.,* **32,** 561 – 573.

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