# Altered Glucocorticoid Rhythm Attenuates the Ability of a Chronic SSRI to Elevate Forebrain 5-HT: Implications for the Treatment of Depression

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Both glucocorticoids and selective serotonin reuptake inhibitors (SSRIs) alter aspects of 5-HT function including somatodendritic 5-HT$_{1A}$ autoreceptor sensitivity. Many depressed patients prescribed SSRIs have pre-existing flattened diurnal gluococorticoid rhythm. In these patients, interactions between flattened glucocorticoid rhythm and chronic SSRIs, which impact on the SSRI's ability to elevate forebrain 5-HT, may alter clinical efficacy. To address this issue rats underwent implantation of slow-release corticosterone (75 mg pellet s.c.) (to flatten the glucocorticoid rhythm) or sham surgery, and injection of fluoxetine (10 mg/kg/day i.p., 12 days) or vehicle. Using microdialysis in the frontal cortex we found that (21 h after the last injection) extracellular 5-HT was elevated in fluoxetine- or corticosterone-treated animals, but not in those treated with corticosterone plus fluoxetine. In fluoxetine-treated animals, blockade of terminal reuptake by local perfusion of fluoxetine increased 5-HT to the same level as it did in controls, suggesting normal terminal 5-HT release after chronic fluoxetine. However, 5-HT levels following local reuptake blockade in both the corticosterone and corticosterone plus fluoxetine groups were lower than controls, suggesting a corticosterone-induced decrease in terminal release. Finally in fluoxetine, corticosterone, and corticosterone plus fluoxetine groups, there was marked 5-HT$_{1A}$ receptor desensitization, evidenced by attenuation of the decrease in 5-HT release following systemic fluoxetine injection. The data indicate that, despite desensitization of 5-HT$_{1A}$ autoreceptors, concurrent flattened glucocorticoid rhythm compromises the ability of SSRIs to elevate forebrain 5-HT. These findings suggest a potential mechanism for the reduced antidepressant efficacy of SSRIs in those patients with pre-existing glucocorticoid abnormalities.
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## INTRODUCTION

An increase in synaptic levels of 5-HT in the forebrain may be necessary for the therapeutic efficacy of antidepressant drugs including selective serotonin reuptake inhibitors (SSRIs) (see Blier, 2001). However, pharmacological studies indicate that SSRIs acutely inhibit 5-HT release by increasing activation of somatodendritic 5-HT$_{1A}$ autoreceptors in the dorsal raphe nucleus (DRN), and this effect limits their ability to increase synaptic 5-HT in the forebrain (Gartside *et al*, 1995; Hjorth and Auerbach, 1996). On

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chronic administration, SSRIs have been shown to desensitize 5-HT$_{1A}$ autoreceptors (De Montigny *et al*, 1990; Le Poul *et al*, 1995; Davidson and Stamford, 1998). This desensitization, which allows levels of synaptic 5-HT in the forebrain to rise (Gardier *et al*, 1996; Dawson *et al*, 2000), shows some temporal correspondence with the onset of antidepressant efficacy (Gardier *et al*, 1996; Le Poul *et al*, 1995) and hence is accepted to be an important feature of the response to chronic administration of SSRIs (see Blier *et al*, 1987, Blier, 2001; Artigas *et al*, 2001).

Somatodendritic 5-HT$_{1A}$ autoreceptors are subject to regulation, not only by chronic SSRI administration but also by glucocorticoids (cortisol in man and corticosterone in rodents) (Young *et al*, 1994; Laaris *et al*, 1997). This may be of particular importance since, in addition to abnormalities of 5-HT function (Drevets *et al*, 2000; Sargent *et al*, 2000; Bhagwagar *et al*, 2002), many depressed patients display abnormal hypothalamo-pituitary–adrenal (HPA) axis function. This is manifest in a flattening of the normal diurnal rhythm of glucocorticoid levels together with an elevation of

nadir levels (Deuschle *et al*, 1997; Wong *et al*, 2000). We have recently shown in rats, that flattening the glucocorticoid rhythm, with an elevation of the nadir, causes a desensitization of somatodendritic 5-HT$_{1A}$ autoreceptors (Leitch *et al*, 2003). Hence the response of 5-HT$_{1A}$ receptors to flattened glucocorticoid rhythm in some part resembles their response to SSRIs. Indeed it has been suggested, that the HPA axis changes observed in depression may represent a 'natural antidepressant response' (Young, 1994). However, corticosterone has also been shown in previous studies to regulate 5-HT synthesis (Rastogi and Singhal, 1978; Miller *et al*, 1980; Culman *et al*, 1984) and may have other effects on 5-HT neurotransmission (see Chaouloff, 2000).

Given that both altered glucocorticoid rhythm and chronic SSRI treatment have been shown to alter components of 5-HT neurotransmission including 5-HT$_{1A}$ autoreceptor sensitivity, it seems possible that these two factors may interact. Any such interaction may have the result of either enhancing or attenuating the ability of SSRIs to elevate forebrain 5-HT and hence may affect antidepressant efficacy. Since many depressed patients with a pre-existing flattening of their glucocorticoid rhythm will be prescribed SSRIs as a treatment for their depression, this potential interaction may be of therapeutic importance.

The present study was designed to examine whether the actions of chronic fluoxetine on 5-HT function may be modified by the concurrent presence of flattened glucocorticoid rhythm. Specifically we examined effects of the two treatments, alone and in combination, on the sensitivity of 5-HT$_{1A}$ receptors controlling 5-HT release in the frontal cortex and on extracellular levels of 5-HT in this region. In adrenally intact male rats we used slow-release corticosterone pellets in a paradigm which we have previously shown to flatten the glucocorticoid rhythm without increasing total glucocorticoid exposure (Leitch *et al*, 2003). We combined this corticosterone treatment (or sham surgery) with chronic once daily injection of the SSRI fluoxetine (or vehicle). Following 2 weeks' treatment, aspects of brain 5-HT neurotransmission were examined using *in vivo* microdialysis.

## METHODS

### Animals

Male Sprague–Dawley rats purchased from Bantin and Kingman, UK, were group housed with *ad libitum* access to food and water under controlled environmental conditions (temperature: 20°C; humidity: 30–50%) on a 12 h light/dark cycle (lights on 07.00 h). Animals were allowed to acclimatize for 1 week before undergoing any procedures. This study was carried out in accordance with the *UK Animals (Scientific Procedures) Act*.

### Chronic Treatments

Groups of rats underwent implantation of a corticosterone pellet or sham surgery and chronic injection of fluoxetine or vehicle. Rats (approx. 180 g) were implanted with commercially made corticosterone pellets (Innovative Research of America, USA) designed to release 3.6 mg/day corticosterone. Under isoflurane anesthesia, pellets were implanted

subcutaneously on the right flank and the wound was sutured. Immediately following surgery, the analgesic buprenorphine (0.05 mg/kg s.c.) was administered. Sham rats underwent the same surgical procedure but no pellet was implanted.

Following surgery, rats were housed in groups of two or three. At 2 days following surgery, chronic injections were commenced. Rats were weighed every second day and injected i.p. daily at around 13.00 h with of fluoxetine (10 mg/kg i.p. in sterile water) or water vehicle (1 ml/kg body weight).

### Microdialysis

On day 13 or 14 following surgery (ie 11 or 12 days following commencement of chronic daily injections), animals underwent microdialysis under chloral hydrate anesthesia as described elsewhere (Gartside *et al*, 1995). Briefly, rats were anesthetized with chloral hydrate and a concentric microdialysis probe with a 3–3.5 mm dialysing window (Hospal membrane) was stereotaxically implanted into the right frontal cortex (stereotaxic coordinates: rostral 3.2 mm, lateral 3.0 mm, ventral −4.6 mm from bregma and dura surface, with the incisor bar set 3.3 mm below the interaural line). Coordinates were adjusted for animals under 220 g and probe placement was verified *post hoc* by inspection of coronal brain sections.

The dialysis probe was continuously perfused at 2.3 µl/min with artificial CSF (140 mM NaCl, 3 mM KCl, 2.5 mM CaCl$_2$, 1 mM MgCl$_2$, 1.2 mM Na$_2$HPO$_4$, 0.27 mM NaH$_2$PO$_4$, 7.2 mM glucose, pH 7.4). Dialysates collected over 20-min periods were injected onto a high-performance liquid chromatography (HPLC) system and separation was performed on a 100 mm × 4.6 mm 3 µm microsorb 100 C18 HPLC column (Varian, USA) maintained at 30°C using a column heater (Waters). Mobile phase (127 mM NaH$_2$PO$_4$, 25 µM octane sulfonic acid, 0.045% EDTA, 15% methanol, pH 3.9) was pumped through the system at 1.1 ml/min and 5-HT was detected using a Coulochem II (ESA Inc., USA) electrochemical detector fitted with a 5020 guard cell ($E = 250$ mV) and a 5014B microdialysis cell ($E1 = 60$ mV and $E2 = 200$ mV). The resulting peak height was measured and quantified with reference to external standards containing 50 and 500 fmol 5-HT. The lower limit of detection (2 × noise) of the HPLC assay for 5-HT was around 4 fmol.

Once a stable baseline of dialysate 5-HT was established (2–3 h after implantation), the perfusion medium was switched to one containing 10 µM fluoxetine and samples collected for a further 80 min. Rats were then injected with fluoxetine (10 mg/kg i.p.) and dialysates were collected for a further 120 min. Groups of two or three rats from a single treatment group were used each day. The microdialysis procedure was started at around 08.30 h and fluoxetine was injected around 13.00 h.

### Data Analysis and Statistics

Data shown are mean ± SEM. Time-course dialysate 5-HT data are presented as absolute amounts of 5-HT in the dialysate. The time-course of the response to systemic fluoxetine is also shown as change from predrug baseline (average of two predrug values). Basal 5-HT was calculated

from the mean of the three samples before switching the perfusion medium (samples 1, 2, and 3). 5-HT levels after local perfusion of fluoxetine were calculated from the mean of samples 7 and 8—although fluoxetine was given i.p. before sample 8 was collected, its effects did not become apparent until sample 9 and samples 7 and 8 represented the maximum levels of 5-HT attained. The area under (over) the curve for the response to systemic fluoxetine was calculated as the sum of the changes from baseline (samples 7 and 8) in the postdrug measurement period (samples 9–13). Statistical analysis of these data was carried out using a two-way ANOVA with fluoxetine and corticosterone as between-subject factors, with *post hoc* Student's *t*-tests. Significance at the 95% level and above is reported.

## RESULTS

### General Health Indicators

All animals appeared healthy throughout the treatment period. Although corticosterone-implanted animals lost some weight in the few days following surgery, thereafter their rate of weight gain was similar to that in the sham treatment groups. On the day of the dialysis experiment, sham/vehicle and sham/fluoxetine animals ($275.3 \pm 9.6$ g (6) and $256.2 \pm 3.6$ g (9), respectively) were heavier than both corticosterone/vehicle- and corticosterone/fluoxetine-treated groups ($206.0 \pm 7.6$ g (8) and $225.6 \pm 12.5$ g (8), respectively).

### Effects of Treatments on Extracellular 5-HT in the Frontal Cortex

*Basal 5-HT.* Following implantation of the microdialysis probe into the frontal cortex, levels of 5-HT in the dialysates fell rapidly (data not shown) and attained relatively stable basal levels after 2–3 h (Figure 1). Basal levels of 5-HT were
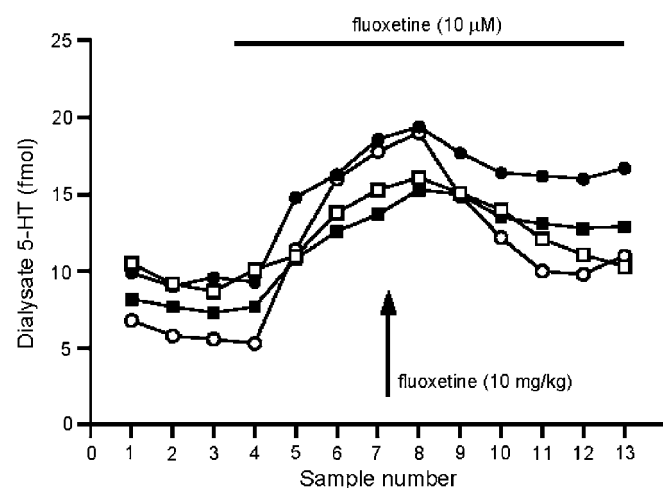
**Figure 1** Time-course of the dialysis experiment in the four treatment groups. Dialysates were collected at 20 min intervals. Bar indicates switching of the perfusion medium to one containing 10 μM fluoxetine, and arrow indicates the injection of 10 mg/kg fluoxetine (i.p.). Open circles: sham/vehicle group ($n = 6$); closed circles: sham/fluoxetine group ($n = 9$); open squares: corticosterone/vehicle group ($n = 8$); closed squares: corticosterone/fluoxetine group ($n = 8$). Data are mean. Error bars omitted for clarity.
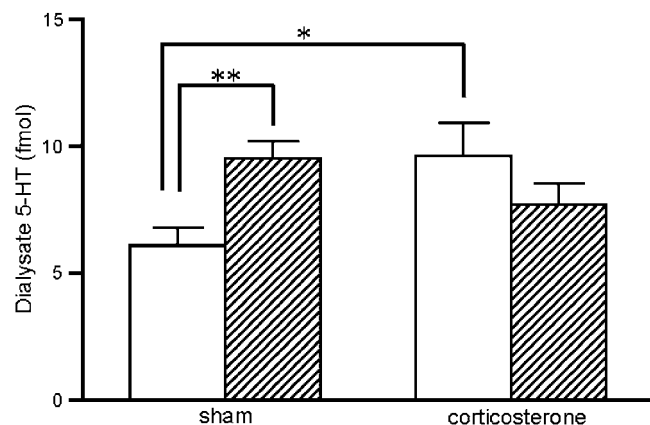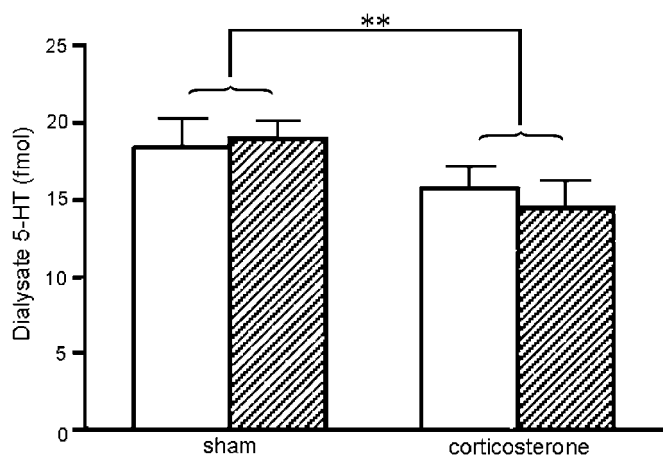
**Figure 2** Basal 5-HT levels in the four treatment groups. Open columns show data from vehicle-treated animals, and shaded columns show data from fluoxetine-treated groups. Data are the mean ± SEM calculated from samples 1–3 (see Figure 1). *$p<0.05$, **$p<0.01$, *post hoc* LSD test.

elevated in both the sham/fluoxetine and the corticosterone/vehicle groups when compared to the sham/vehicle group. In the corticosterone/fluoxetine group the basal 5-HT level was intermediate between the high level in the sham/fluoxetine and the corticosterone/vehicle groups and the lower level in the sham/vehicle group (Figure 1). The basal 5-HT levels in the four treatment groups, calculated as the mean of the last three samples before switching the perfusion medium, are presented in Figure 2. Statistical analysis of these data by two-way ANOVA revealed no significant main effect of either fluoxetine or corticosterone. However, there was a significant interaction between fluoxetine and corticosterone ($F_{1,27} = 7.7$, $p = 0.01$) and a *post hoc* Student's *t*-test showed that 5-HT levels in both the sham/fluoxetine and the corticosterone/vehicle groups were significantly higher than in the sham/vehicle group.

*Effect of local inhibition of 5-HT reuptake.* When the perfusion medium was switched to one containing 10 μM fluoxetine, levels of 5-HT in the dialysates rose over the following 80–100 min (see Figure 1). However, the new 5-HT level attained varied between the treatment groups. Figure 3 shows the mean level in each of the four treatment groups calculated from the average of samples 7 and 8. Local perfusion of fluoxetine raised 5-HT levels to the same levels in the sham/vehicle group and the sham/fluoxetine group. In the corticosterone/vehicle and the corticosterone/fluoxetine groups, the levels of 5-HT also rose following local fluoxetine perfusion; however, in both cases the levels attained were lower than those in the sham surgery groups (Figure 3). Statistical analysis of these data showed that there was a significant main effect of corticosterone ($F_{1,27} = 5.2$, $p<0.05$) but no significant main effect of fluoxetine, and no corticosterone × fluoxetine interaction.

*5-HT$_{1A}$ autoreceptor function.* Following the establishment of new baseline levels of 5-HT in the presence of locally perfused fluoxetine, systemic administration of fluoxetine (10 mg/kg i.p.) reduced dialysate 5-HT (see Figures 1 and 4). In sham/vehicle-treated animals systemic fluoxetine caused a large decrease in dialysate 5-HT. However, in both sham/fluoxetine-treated and corticosterone/vehicle-treated animals,

**Figure 3** 5-HT levels in the four treatment groups after local application of fluoxetine. Open columns show data from vehicle-treated groups, and shaded columns show data from fluoxetine-treated groups. Data are the mean ± SEM calculated from the mean of samples 7 and 8 **$p < 0.01$ main effect of corticosterone treatment (ANOVA).
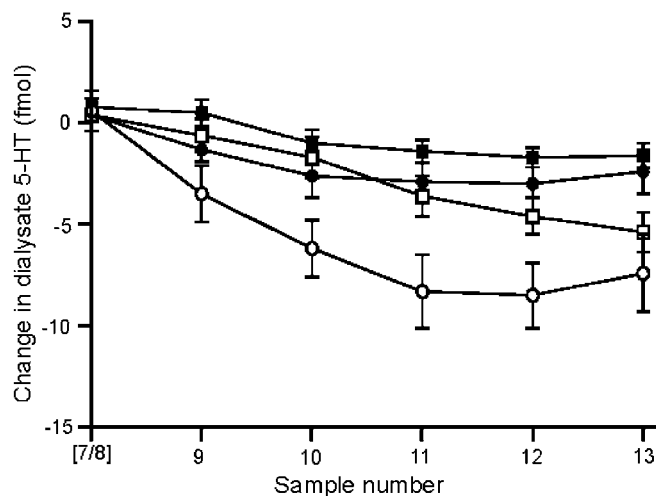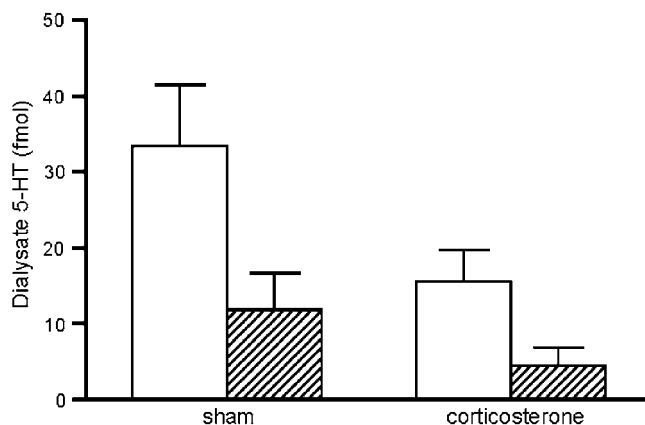


**Figure 4** Time-course of the response to systemic injection of fluoxetine in the four treatment groups. Data are expressed as change from the baseline (mean of samples 7 and 8) and are mean ± SEM.

this response to systemic fluoxetine was markedly attenuated compared to sham/vehicle controls. In animals that had been treated with the combination of corticosterone and fluoxetine (corticosterone/fluoxetine), the response to systemic fluoxetine was also markedly attenuated. In Figure 5 the responses to systemic fluoxetine in the four groups are shown as the area under the curve for the postdrug measurement period. Analysis of variance of the AUC data showed a significant main effect of fluoxetine ($F_{1,27} = 10.9$, $p = 0.003$) and a significant main effect of corticosterone ($F_{1,27} = 6.5$, $p = 0.017$) but no significant interaction.

## DISCUSSION

In the present study we investigated the effects of chronic fluoxetine and flattened corticosterone rhythm, and their



**Figure 5** Response to systemic injection of fluoxetine in the four treatment groups Data are the area under the curve calculated by summing the differences from baseline (mean of samples 7 and 8) in the postinjection samples. For statistical analysis, see text.

possible interactions with respect to central 5-HT neurotransmission. We found that chronic fluoxetine and flattened corticosterone rhythm, both individually and in combination, markedly desensitized 5-HT$_{1A}$ autoreceptors. However, the treatments differed substantially with respect to their net effects on 5-HT levels in the frontal cortex. Thus chronic fluoxetine (sham/fluoxetine) treatment elevated basal extracellular 5-HT levels (measured 21 h after the last dose of fluoxetine) relative to sham/vehicle controls. However, when terminal reuptake was further blocked by local perfusion of fluoxetine, 5-HT attained the same absolute level in sham/fluoxetine-treated animals as in sham/vehicle-treated controls suggesting that terminal 5-HT release was normal. In animals with flattened corticosterone rhythm (corticosterone/vehicle), basal extracellular 5-HT was also elevated compared to sham/vehicle controls but there was no elevation of basal 5-HT in the corticosterone/fluoxetine-treated group. Furthermore, following local reuptake blockade the cortical 5-HT levels attained in both corticosterone/vehicle- and corticosterone/fluoxetine-treated groups were markedly lower than those in the sham/vehicle (and sham/fluoxetine) controls, consistent with there being decreased terminal release in corticosterone-treated animals.

### Chronic Fluoxetine

Our present data showing an elevation in basal dialysate 5-HT are in accord with previous *in vivo* microdialysis studies in which chronic treatment with fluoxetine has been reported to increase extracellular 5-HT in the frontal cortex (Rutter *et al*, 1994; Invernizzi *et al*, 1996). Although basal 5-HT was elevated approximately 21 h after the last of the chronic injections of fluoxetine, we found that local application of fluoxetine further increased dialysate 5-HT, suggesting that at this time 5-HT reuptake in the frontal cortex is not fully inhibited. However, in the chronic fluoxetine-treated animals the level of dialysate 5-HT attained following local perfusion of fluoxetine was similar to that attained in vehicle-treated controls. These latter data, which suggest that terminal 5-HT release and hence 5-HT firing is normal(ized) in the frontal cortex of the chronic

fluoxetine group after 12 days' treatment, are consistent with electrophysiological studies showing normalization of 5-HT firing after 2 weeks' fluoxetine treatment (Le Poul *et al*, 2000).

When terminal 5-HT reuptake is blocked, systemic administration of SSRIs has been shown to inhibit 5-HT release via activation of $5\text{-HT}_{1A}$ receptors (Hjorth and Auerbach, 1996). Here we used this decrease in dialysate 5-HT as an index of $5\text{-HT}_{1A}$ receptor sensitivity. We observed that in the chronic fluoxetine-treated (sham/ fluoxetine) animals, the magnitude of the inhibitory response to systemic fluoxetine was markedly diminished compared to that seen in the sham/vehicle-treated controls. This finding is consistent with chronic fluoxetine treatment having induced a functional desensitization of $5\text{-HT}_{1A}$ autoreceptors and again is in line with previous reports (Le Poul *et al*, 1995; Invernizzi *et al*, 1996).

### Flattened Glucocorticoid Rhythm

Like chronic fluoxetine, chronic flattening of the glucocorticoid rhythm (by clamping levels at a point midway between the normal nadir and zenith (Leitch *et al*, 2003)) resulted in an increase in basal dialysate 5-HT. Indeed the levels of 5-HT in the corticosterone/vehicle group were very similar to those in the sham/fluoxetine group 21 h after the last injection. However, when terminal 5-HT reuptake was blocked by local perfusion of fluoxetine, the level of dialysate 5-HT attained in the corticosterone/vehicle-treated animals was significantly lower than that in either the sham/ fluoxetine or the sham/vehicle group. Finally, corticosterone-treated animals showed evidence of a $5\text{-HT}_{1A}$ receptor desensitization, which was of the same degree of magnitude as that in chronic fluoxetine-treated animals. This is fully consistent with our own recent data in which we found that the 8-OHDPAT-induced inhibition of 5-HT release was markedly reduced in animals treated with this regime of corticosterone (Leitch *et al*, 2003).

The mechanism underlying the effects of flattening the glucocorticoid rhythm on basal 5-HT is not clear, but would be consistent with either a decrease in reuptake and/or an increase in terminal 5-HT release. There are few data available on the effects of glucocorticoids on the 5-HT transporter, but what evidence is available suggests that flattened glucocorticoid rhythm is unlikely to decrease 5-HT reuptake (Kulikov *et al*, 1997; Fernandez *et al*, 2001). An increase in 5-HT release is possible; however it is unlikely that this is secondary to the $5\text{-HT}_{1A}$ receptor desensitization as pharmacological blockade of these receptors with WAY100635 has been shown to have no effect on 5-HT levels in frontal cortex dialysates (Gartside *et al*, 1995; Sharp *et al*, 1997; Invernizzi *et al*, 1996). A more likely mechanism underlying increased 5-HT release would be an increase in the activity of tryptophan hydroxylase, an enzyme that has previously been shown to be increased by high corticosterone and stress (Rastogi and Singhal, 1978; Culman *et al*, 1984). Whatever the mechanism of this increase in basal 5-HT, this effect of corticosterone is difficult to reconcile with the reduction in absolute 5-HT levels when terminal 5-HT reuptake was blocked. One possibility is that the corticosterone treatment increases 5-HT synthesis and release but, at the same time, increases the sensitivity of

terminal $5\text{-HT}_{1B}$ autoreceptors. Evidence suggests that the negative influence of $5\text{-HT}_{1B}$ autoreceptors on 5-HT release is only manifest when synaptic levels are relatively high (Sharp *et al*, 1997; Roberts *et al*, 1999). Hence, in corticosterone-treated animals increased $5\text{-HT}_{1B}$ sensitivity could limit release when terminal reuptake is blocked while having no effect on (lower) basal levels. To our knowledge, the effects of glucocorticoids on the function of $5\text{-HT}_{1B}$ terminal autoreceptors (as opposed to postsynaptic $5\text{-HT}_{1B}$ receptors) has not been reported. Although corticosteroids have been shown to be without influence on $5\text{-HT}_{1B}$ receptor mRNA expression in the DRN (Neumaier *et al*, 2000), changes in $5\text{-HT}_{1B}$ receptor function downstream of transcription are possible.

### Interaction between Flattened Glucocorticoid Rhythm and Chronic Fluoxetine

A key part of the present study was to examine the interactions between flattened corticosterone rhythm and chronic fluoxetine. We found that the combination of flattened glucocorticoid rhythm and chronic fluoxetine (corticosterone/fluoxetine) caused desensitization of $5\text{-HT}_{1A}$ autoreceptors. Indeed, the combined treatment was, if anything, slightly more effective than either treatment alone. Although we found no statistical evidence for an interaction between the two treatments, this is most likely to be due to the magnitude of the desensitization to either treatment alone being near maximal and does not necessarily suggest that SSRIs and flattened corticosterone rhythm desensitize $5\text{-HT}_{1A}$ receptors via the same molecular mechanism. In this respect, it is perhaps of note that Le Poul *et al* (1997) showed that the mechanism by which fluoxetine causes desensitization of $5\text{-HT}_{1A}$ receptors is independent of its ability to cause acute corticosterone release.

In examining the interaction between flattened corticosterone rhythm and chronic fluoxetine, our most striking finding was that a concurrent flattened glucocorticoid rhythm decreased the ability of chronic fluoxetine to elevate forebrain 5-HT. This was evidenced by the fact that 5-HT levels in the corticosterone/fluoxetine group were lower than those in the sham/fluoxetine group both when 5-HT reuptake was only partially blocked (21 h after the last fluoxetine injection) and when it was maximally blocked (after local perfusion of fluoxetine). It is unclear how corticosterone brings about this effect although, as discussed above, flattened glucocorticoid rhythm may have effects on 5-HT synthesis and/or 5-HT autoreceptors. Whatever the precise molecular effects of glucocorticoids, it is apparent that in animals with flattened glucocorticoid rhythm the ability of chronic fluoxetine to elevate synaptic 5-HT levels is attenuated even though $5\text{-HT}_{1A}$ receptors are effectively desensitized.

### Conclusions

An elevation in synaptic 5-HT levels in the forebrain is proposed to underlie the antidepressant response to SSRIs (see Blier, 2001). Interestingly, it was recently reported that upwards of 75% transporters are occupied at clinically effective doses of SSRIs (Meyer *et al*, 2001). At baseline (ie

21 h after the last fluoxetine injection) it seems probable that reuptake blockade was only partial; however, blockade was probably close to maximal following local perfusion of fluoxetine. Hence the levels of 5-HT attained here following local reuptake blockade may be the most clinically relevant. Corticosterone alone caused an increase in basal 5-HT (which might have some antidepressant effect); however, levels remain much lower than those that may be achieved by (total) reuptake blockade. Importantly in animals with flattened glucocorticoid rhythm, the maximum 5-HT levels achieved following reuptake blockade were significantly reduced. The desensitization of $5-HT_{1A}$ autoreceptors may be crucial in allowing the effect of SSRIs on terminal 5-HT to be manifest; however, the present study provides evidence that concurrent flattened glucocorticoid rhythm compromises the ability of an SSRI to elevate forebrain 5-HT, even in the presence of desensitized $5-HT_{1A}$ autoreceptors.

Flattened glucocorticoid rhythm with raised nadir is seen in a significant proportion of depressed patients as well as in the elderly (Wong et al, 2000; Ferrari et al, 2001), and the present data suggest that in these individuals the anti-depressant efficacy of SSRIs may be severely compromised. Indeed our findings may explain the recent observation of limited efficacy of SSRIs in patients with abnormal HPA axis function (Young et al, 2002). Tricyclic antidepressants unlike SSRIs are reported to alter glucocorticoid receptor function (Pepin et al, 1989). Hence TCAs may be able to normalize glucocorticoid rhythm, a property that may confer greater efficacy in depressed patients with glucocorticoid abnormalities. Further investigations of the interplay between glucocorticoids and 5-HT function in relation to antidepressants are clearly warranted.

## ACKNOWLEDGEMENTS

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