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Effects of Antidepressants on 5-HT_7 Receptor Regulation in the Rat Hypothalamus

U. Lena Mullins, Ph.D., G. Gianutsos, Ph.D., and A. S. Eison, Ph.D.,

Recent evidence suggests that a novel serotonin receptor 5-HT_7 localized in the hypothalamus downregulates in response to treatment with the antidepressant fluoxetine (Sleight et al. 1995). This receptor has also been implicated in the regulation of circadian rhythms (Lovenberg et al. 1993). Here, we show that several agents administered in a profile consistent with activity at the 5-HT_7 receptor produce significant functional Fos immunoreactivity in the suprachiasmatic nucleus (SCN), an effect reduced upon chronic exposure. Furthermore, binding studies demonstrate that chronic administration of Fos-inducing agents produces a neuroadaptive downregulation of the 5-HT_7 receptor in the hypothalamus. The current studies extend the previous observations to include several pharmacologically distinct antidepressants. In addition, these studies provide further evidence to support the role of the 5-HT_7 receptor in the mechanism of antidepressant action and in the regulation of circadian rhythms controlled by the SCN. ***[Neuropsychopharmacology 21:352–367, 1999]*** *© 1999 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.*

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Depression may involve many possible abnormalities corresponding to multiple biological correlates of dysfunction or dysregulation in either one or several brain neurotransmitter systems (Cooper et al. 1996; Nair and Sharma 1989), a property that may contribute to the apparent disparity in symptomology and response to antidepressant therapy. The strict classical notions of neurotransmitter dysregulation hypotheses that associate depression with a deficiency of a vailable neurotransmitter or subresponsivity of mainly noradrenergic and/or serotonergic receptor systems are recently being expanded to include disturbances in biological rhythm regulation. Impairment of the efficiency of rhythm maintenance or rhythm desynchronization has been suggested by many to lead to mental fatigue and depression (Goodwin et al. 1982; Hallonquist et al. 1986; Healy 1987; Partonen 1994; Schwartz 1993; Wirz-Justice and Campbell 1982; Wirz-Justice et al. 1995). Clinically, it has been extensively documented that the timing and structure of rhythms in physiological, behavioral, and endocrinological functions seem to be abnormal in depression (Coiro et al. 1993; Duncan 1996; File 1990; Kupfer 1995; Nair and Sharma 1989; Wehr et al. 1979). Furthermore, studies investigating the patterns of circadian rhythms of patients diagnosed with depression have been undertaken with the premise of disturbed rhythmicity as a central theme underlying the etiology of some affective disorders (Duncan 1996; Goodwin et al. 1982; Healy 1987; Siever and Davis, 1985; Souetre et al. 1988).

Although melatonin is generally thought to be a primary modulator of circadian function through the suprachiasmatic nucleus (SCN) of the hypothalamus (Armstrong and Redman 1993; Binkley 1993; Cassone et al. 1993; Dubocovich 1991; Ibata et al. 1997; Reiter 1993;

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Stankov et al. 1993), the serotonergic system also plays a critical role in circadian modulation. The SCN receives dense projections from the raphe serotonergic neurons originating in the brainstem (Jacobs and Azmitia 1992; Meyer-Bornstein and Morin 1996; Van De Kar and Lorens 1979). Lesions of the median raphe serotonergic system using the neurotoxic agent 5,7-dihydroxytryptamine (5,7-DHT) produce a severe disruption in rodent circadian locomotor (wheel running) activity, resulting in a decrease in amplitude and a 20% longer activity phase (Morin and Blanchard 1991; Smale et al. 1990). Furthermore, it has been demonstrated that animals lacking intact serotonin innervation exhibit "hypernormal" rhythm responses to light (Meijer and Groos 1988). These data suggests that the serotonin system may provide inhibitory modulation of the circadian system to activation of the SCN by light (Van Den Pol and Dudek 1993; Moore 1995; Morin 1994).

Serotonin is further implicated in circadian function by experiments that show that serotonergic agents affect behavioral rhythms by re-establishing disrupted rhythmicity to external cycles in both mammals and humans (Dawson and Armstrong 1996; DeMet and Chicz-DeMet 1987; Duncan 1996). Studies involving circadian locomotor (wheel-running) activity in rats and hamsters (Cutrera et al. 1994) and body temperature rhythms in humans (Brown and Seggie 1988) have shown that several classes of such mechanistically distinct antidepressant agents as imipramine and desipramine (tricyclics), clorgyline and phenelzine (monoamine oxidase inhibitors), fluvoxamine, fluoxetine (selective serotonin reuptake inhibitors), and quipazine (a nonspecific serotonin agonist) shift established rhythmicity and possess the ability to restore experimentally phase-shifted circadian activity patterns (Edgar et al. 1994; Golda 1993; Nagayama and Lu 1996; Wirz-Justice et al. 1995; Wollnick 1992).

Several serotonergic receptor subtypes have been identified in the SCN and other areas implicated in circadian function including type-1A, 1B, 1D, 2A, and 7 receptors (Roca et al. 1993; Sumner et al. 1992; To et al. 1995; Van Den Pol and Dudek 1993). The putative 5-HT_{1A} agonists 5-CT (5-carboxamidotryptamine), 8-OH-DPAT, and 5-HT (5-hydroxytryptamine) in in vitro excised SCN experiments produced a shift in the timing pattern of the endogenous SCN firing rate. Moreover, DOB, a 5-$HT_{2A/2C}$ agonist; CGS-12066B, a 5-HT_{1B} agonist, and ICS205930, a 5-$HT_{3/4}$ receptor agonist all had no effect on established SCN firing rates (Edgar et al. 1993; Lovenberg et al. 1993; Medanic and Gillette 1992; Prosser and Gillette 1989; Prosser et al. 1990, 1993; Shibata et al. 1992; Ying and Rusak 1994). However, the 5-HT_{1A} agonist-induced responses were blocked by the 5-HT_2 antagonists ritanserin and mesulergine, and, surprisingly, were not sensitive to (−)-pindolol, a known 5-HT_{1A} antagonist. Other studies showed that phosphodiesterase inhibitors and nonhydrolyzable cAMP analogs were able to mimic the effects of exogenously applied 5-HT and produce an equivalent phase shift response in the SCN slice (Prosser and Gillette 1989). Still other investigations demonstrated that H8 and Rp-cAMP (a stereoisomer of cAMP) blocked quipazine-induced phase shifts (Lovenberg et al. 1993). Accordingly, in vitro evidence strongly suggests that treatments that increase intracellular cAMP levels modulate endogenous SCN firing rhythms. This is an effect inconsistent with activity at the 5-HT_{1A} receptor, because this receptor is known to couple negatively to adenylate cyclase through an inhibitory G-protein; G_i (Cooper et al. 1996; Saxena 1995).

Subsequent cloning efforts identified a novel serotonin receptor, type 7, which exhibits less than 40% homology with other known serotonin receptors (Bard et al. 1993). Using [3H]-5-CT autoradiography and in situ hybridization (Waeber and Moskowitz 1995), the 5-HT_7 receptor and its mRNA were detected in relatively high densities in limbic areas, particularly the hypothalamus, hippocampus, amygdala, mammillary nuclei, and raphe nuclei, and also diffusely within the cortex, a localization that suggests that this receptor may be involved in the pathophysiology of affective disease (Gutafson et al. 1996; Kupferman 1991; Plassat et al. 1993; Ranson 1934). Functionally, the 5-HT_7 receptor is a G-protein (G_s) linked receptor positively coupled to adenylate cyclase and, thereby, increases cAMP. Pharmacologically, in the context of a lack of selective ligands, the 5-HT_7 receptor is currently defined as agonist activity by the endogenous ligand serotonin in the presence of the 5-HT_{1A} antagonist (−)-pindolol and the 5-HT_{1D} agonist sumatriptan with sensitivity to the antagonists ritanserin, clozapine, and mesulergine. The rank order of potency of 5-HT_7 agonists is: 5-CT > 5-HT > 8-OH-DPAT (Lovenberg et al. 1993; Roth et al. 1994; Ruat et al. 1993; Shen et al. 1993; To et al. 1995; Tsou et al. 1994). Given this, the preponderance of the in vitro data suggest that the intrinsic circadian pattern of electrical activity rhythm of the excised SCN responds to serotonergics in a manner consistent with the pharmacological profile of the $5HT_7$ receptor subtype (Lovenberg et al. 1993).

The AP-1 family of transcription factors, examples of which are Fos, Jun, and CREB, are induced in response to a variety of stimuli. The acute response protein, Fos, and chronic Fos-related antigens (cFRAs) are thought to play important modulatory roles in the transcriptional regulation of regionally specific brain responses to chronic such perturbations as long-term exposure to psychotropics, dopamine receptor stimulation, kainate exposure, electroconvulsive seizures, and lesioning (Chen et al. 1997; Hope et al. 1994; Hyman and Nestler

1996; Morgan and Curran 1998; Pennypacker et al. 1995). Hence, IEG c-Fos induction acts as a putative downstream third messenger that is transiently induced in one form and then is expressed in another more stable form in response to chronic receptor stimulation and prolonged cAMP elevations (Chen et al. 1997; Dragunow and Faull 1989; Hoffman et al. 1993; Lemke 1992; Morgan and Curran 1989, 1995; Sheng and Greenberg 1990).

More importantly to this discussion, neuronal activation as evidenced by the induction of c-Fos in the ventrolateral portion of the SCN (vSCN) is an indicator of stimulation of the intrinsic entrainment mechanisms controlled by this hypothalamic area. Receptor-mediated IEG expression and Fos protein deposition in the vSCN occurs during stimulus-specific circadian times (CT 20–22) in response to such phase-shifting stimuli as light and melatonin receptor stimulation (Ginty et al. 1993; Kilduff et al. 1992; Kornhauser et al. 1992; Mullins et al. 1999; Sutin and Kilduff 1992). It has been shown that in the SCN, CREB is phosphorylated by light exposure only at the circadian times when Fos expression is induced and the SCN has been activated, an effect that corresponds directly with phase shifts of locomotor activity (Ginty et al. 1993). Evidence to support the role of modulation of the cAMP signaling cascade in response to antidepressants has been provided by studies that show that chronic treatment with pharmacologically distinct antidepressants phosphorylates and upregulates brain-derived neurotrophic factor-associated expression of CREB, which, in turn, may activate the expression of its receptor trkB in rat hippocampus, a receptor whose expression level seems to be decreased in depression (Duman et al. 1996; Hyman and Nestler 1996). In addition, other behavioral responses have also been correlated with the expression of c-Fos. It has been demonstrated that foot shock-induced behavioral stress evokes Fos expression in the amygdala and hippocampus in rats and that chronic administration of the antidepressant desipramine significantly reduced not only the stress-induced behavioral responses, but also produced a significant coincident decrease in Fos expression (Beck and Fibiger 1995).

Although it has been previously shown that the 5-HT_7 receptor is downregulated in the hypothalamus in response to chronic fluoxetine treatment (Sleight et al. 1995), it is not clear what role the 5-HT_7 receptor plays in the hypothalamus; more specifically, within the suprachiasmatic nucleus. It is also not known whether antidepressants induce Fos-LI in the vSCN through activation of the 5-HT_7 receptor. To investigate this, activation of the vSCN during the appropriate circadian time known to produce phase shift was measured by using the induction of c-Fos in response to the acute administration of agents in a pharmacology consistent with activity at the 5-HT_7 receptor. Furthermore, to determine whether chronic treatments would qualitatively affect the responsivity of the 5-HT_7 receptor, Fos responses were measured in the vSCN after chronic administration. To assess whether chronic antidepressant treatments quantitatively altered the density of 5HT_7 receptors and to supply a means of mechanistically verifying the postreceptor effects observed in the Fos studies, 5-HT_7 receptor binding analyses were performed in hypothalamic membrane homogenates to investigate receptor expression changes in response to chronic treatment versus control-vehicle tissues.

MATERIALS AND METHODS

Drug Administration

All studies were performed using male Wistar rats weighing 150–250 grams (Harlan Sprague–Dawley, Indianapolis, IN), which were singly housed in the environmentally controlled animal care facilities of Bristol-Myers Squibb Company (Wallingford, CT). Animals were maintained on a 12:12 light/dark cycle, with lights on at 11:0AM (CT 1) and off at 11:00 PM (CT 12) with standard rodent chow and water available *ad libitum.*

For acute studies, all agents were administered through intraperitoneal injection with the exception of 5-carboxamidotryptamine (5-CT). 5-CT was administered at a dose of 5 μg (in saline vehicle at 1 μg/μl) through sterile surgically implanted (under ketamine supplemented with xylazine anesthesia, 70/10 mg/kg, IP) in-dwelling intracerebroventricular polyethelene cannulae located in the lateral ventricle (stereotaxic coordinates from Bregma: AP-0.9, ML ±1.5, DV-3.7). Animals were chronically administered the appropriate vehicle (saline or 20% dimethylsulfoxide/saline) or one of the antidepressants fluoxetine, mianserin, imipramine, desipramine, or clorgyline at a dose of 5 mg/kg/day continuously for 21 days through subcutaneous implantation of a minipump (Alzet osmotic minipumps model 2ML4, Alza Corporation, Palo Alto, CA). Minipumps were implanted under the skin on the back of the animal using isoflurane inhalant anesthesia. Drug treatment was followed by removal of the minipump on day 21 using the same anesthesia. Nefazodone was injected IP mg/kg once per day for 21 days. All animals were then allowed a 2-day drug clearance period before initiation of experimental protocols. For chronic Fos desensitization studies after the clearance period of 48 hours, animals were challenged on the day of the experiment with an acute IP dose of the same drug or with 5-CT at a dose of 5 μg in saline (ICV).

All agents were purchased from either Research Biochemicals Inc., Natick, MA or Sigma Chemical Co., St.

Louis, MO; except for sumatriptan and N-acetyltryptamine, which were synthesized by Bristol-Myers Squibb Company, Wallingford, CT and Fluoxetine, which was a generous gift from Eli Lilly, Indianapolis, IN.

Fos Immunohistochemistry Procedure

To correlate drug-induced changes in Fos expression with possible changes in SCN regulation of circadian parameters, CT 22 was chosen because of its low endogenous Fos-LI expression and, more importantly, because of its previously established status as a specific time window of sensitivity in which certain modulatory treatments produce behavioral phase-shift responses (Kornhauser et al. 1992; Sutin and Kilduff 1992). To detect only drug-induced Fos-like immunoreactivity in the (vSCN) optimally, all animals were intraperitioneally injected in the total absence of room lighting, ICV injections were done in the presence of a dim red light.

After drug treatment and before light exposure, rats were anesthetized by an intramuscular injection of ketamine/xylazine (70/10 mg/kg). Animals were then transcardially perfused with ice-cold saline followed by cold 4% paraformaldehyde (PFA) in 0.1 m phosphate buffered saline (PBS) for approximately 30 min. Brains were removed and postfixed at 4°C overnight in 4% PFA.

Guided by a rat brain stereotaxic atlas (Paxinos and Watson 1986), desired 40 μm serial coronal sections containing the suprachiasmatic nucleus and hippocampus were sliced on a vibratome and collected in 0.05 m PBS. Sections were processed by an indirect immunohistochemistry procedure (Beck and Fibiger 1995) utilizing a sheep polyclonal antibody to pan-Fos oncoproteins (Cambridge Pharmaceuticals, Cambridge, MA), which was subsequently complexed to a secondary biotinylated antisheep IgG antibody (Vector Labs, Burlingame, CA). Fos-like immunoreactivity (Fos-LI) in reacted cells was detected by avidin-biotinylated horseradish peroxidase (ABC kit, Vector Labs) coupled to diaminobenzidine-nickel (Sigma Chemical Co.) precipitation. Sections were mounted on chrom-alum-gelatin coated glass slides and allowed to dry overnight before staining. Slides were counterstained with 0.2% neutral red and glass coverslipped for examination and quantitation under the microscope.

Quantitation was performed by screening each SCN section for the presence of Fos-LI (Fos-positive cells contain a clearly visible black nuclear precipitate) using an Olympus BH-2 microscope 20x objective fitted with a calibrated grid objective of 800 mm^2. Each experimental group contained at least six animals. Comparisons between experimental groups were obtained by analyzing at least 10 200 mm^2 sequential vSCN sections per animal per group. Data represent the mean and standard error of the number of Fos-positive cells per 200 mm^2 per vSCN. Significant differences between control and experimental groups were determined by a one-way analysis of variance (ANOVA) and Student-Neuman-Keuls post hoc test. The chosen minimum level of significance was $p < .05$.

Tissue Membrane Homogenate 5-HT_7 Receptor-Binding Assay

Rats were chronically treated with vehicle or antidepressant and after removal of their subcutaneous minipumps at CT 22, were allowed to remain in their home cages for 48 hours. At CT 22 after the 48-hour drug clearance period, the brains were removed following rapid decapitation without anesthesia. Dissection of the hypothalamus and striatum were done on ice and the desired regions were subsequently placed in 50 μl polypropylene microcentrifuge tubes and then placed on dry ice before storage at −80°C. Upon the day of assay, membrane homogenates were made in polycarbonate tubes in 20 volumes of 50 mm Tris-HCl using a polytron and then centrifuged at 40,000 × *g* for 15 min at 4°C. The pellet was resuspended in the same volume of buffer and centrifuged as before. The pellet produced was resuspended in 10 mls of buffer and incubated for 15 min at 37°C to remove endogenous serotonin. After this incubation, the homogenate was centrifuged as before, and the pellets were collected for assay. 5-HT_7 receptor binding parameters were measured using a modification of the method of Sleight et al. (1995). The final tissue concentration for 5-HT_7 binding was 4 to 5 mg/assay. All assays were carried out in an incubation buffer consisting of: 50 mm Tris-HCl, 0.5 mm sodium EDTA, 10 mm $MgSO_4$, 10 μm pargyline, 2 mm $CaCl_2$, and 0.1% ascorbate (Sigma Chemical Company).

Total binding was determined by using the radioligand [3H]-5-carboxamidotryptamine ([3H]-5-CT; New England Nuclear, specific activity 83 Ci/mmol) in the presence of 1 μM mindolol and 1 μm sumatriptan (to eliminate binding to 5-$HT_{1A/1B}$ and 5-HT_{1D} receptors). Nonspecific binding was determined in the presence of excess cold 1 μm 5-HT. Specific binding was defined as the difference between total and nonspecific binding and represented 60–70% of the total radioactivity bound. All saturation experiments were performed using eight concentrations of [3H]-5-CT (8, 5, 2, 1, 0.5, 0.25, 0.1, and 0.05 nm). Assay tube contents consisted of 0.1 ml of radioligand, 0.1 ml of 1 μM pindolol, 0.1 ml of 1 μm sumatriptan or 0.1 μm 5-HT (to define nonspecific binding), and 0.5 ml of tissue homogenate. The assay tubes were incubated at room temperature for 2 hours, after which they were rapidly filtered under vacuum (using a Brandel cell harvester model M48RP, Brandel, Gaithersburg, MD) with 2 × 5 ml washes of cold buffer through Whatman GF/B filters (Brandel). The radioactivity retained on the filters was measured by liquid

scintillation counting (Model 2500TR, Packard Instrument Company, Sherling, VA) in 4 ml of scintillation fluid (ReadySafe, Packard). Each experimental group contained at least 25 animals per treatment group, and each assay was performed in triplicate. To obtain adequate tissue concentration for assay, four hypothalami were pooled from the same treatment group allowing for at least six separate determinations.

Data were analyzed by the method of Scatchard (1949) using the nonlinear regression program EBDA/Ligand to determine the saturation binding parameters of receptor density (B_{max}) and affinity (K_d) in chronically drug-treated versus control groups. Data represent the mean and standard error of each separate experiment. Scatchard plots obtained were linear and demonstrated correlation coefficients of at least 0.9. Differences in the mean K_d and B_{max} values obtained from hypothalamic and striatal membranes prepared from animals that were chronically treated with antidepressants versus those of vehicle-treated subjects were analyzed using a one-way ANOVA, followed by post hoc analysis (Dunnett's *t*-test). The chosen minimal level of significance was $p < .05$.

RESULTS

Fos-Like Immunoreactivity in the vSCN

Acute intracerebroventricular (ICV) administration of the serotonin 5-$HT_{1A/1D}$ and 5-HT_7 agonist 5-CT potently stimulated Fos-like immunoreactivity (Fos-LI) over vehicle-induced expression in the ventrolateral suprachiasmatic nucleus (vSCN) at circadian time 22 (CT 22) before exposure to room light at CT 1 (Figures 1 and 2, Table 1). 5-CT induction of Fos-LI at CT 22 in the vSCN was dose dependent and not affected by pretreatment with the 5-HT_{1A} antagonist pindolol and the 5-HT_{1D} agonist sumatriptan (Figure 2). Fos-LI stimulated by 5-CT in the vSCN at CT 22 was sensitive to pretreatment with the putative 5-HT_7 antagonist ritanserin (Table 1). Neither pindolol, sumatriptan, nor ritanserin nor their combination acutely produced significant Fos-LI in the vSCN (Table 1). In addition, acute single dosage of rats with the 5-HT_{1A} and 5-HT_7 agonist 8-OH-DPAT or with the antidepressant agents fluoxetine (selective serotonin reuptake inhibitor), imipramine, desipramine (tricyclics, norepinephrine reuptake inhibitor, and 5-HT_2 antagonist), mianserin (atypical,

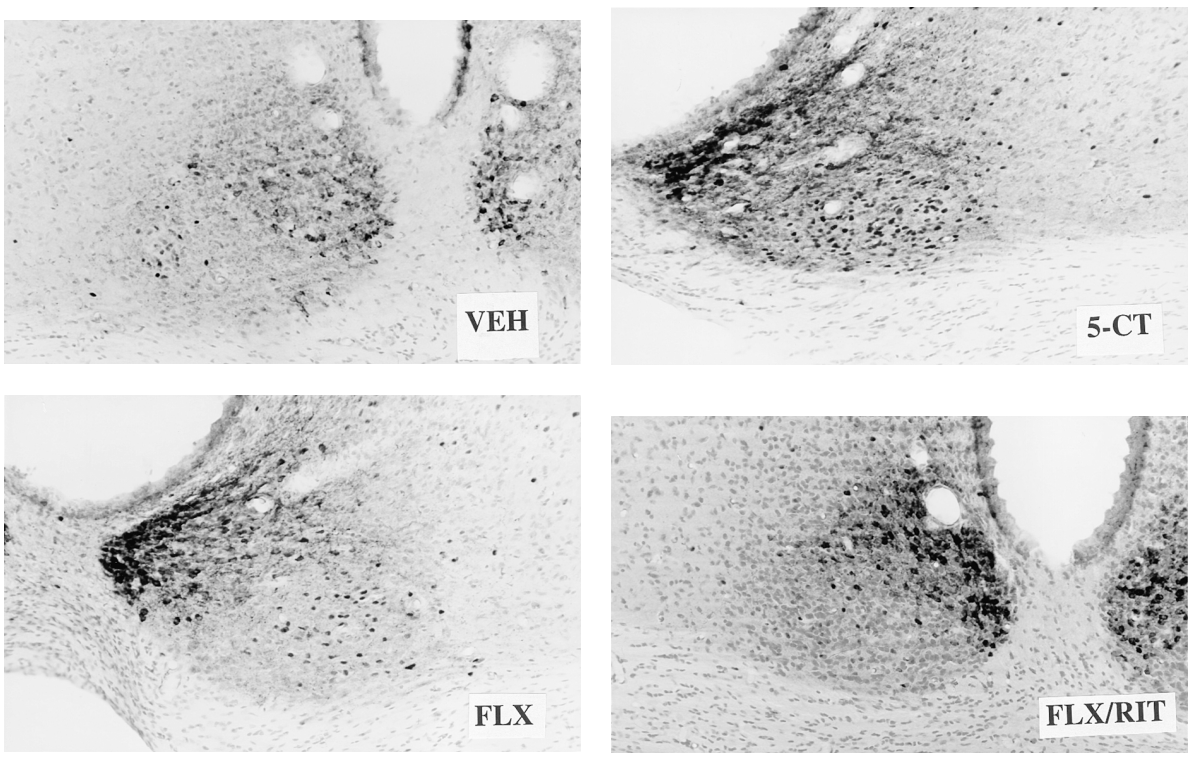

Figure 1. Fos Immunoreactivity in the ventrolateral suprachiasmtic nucleus of the rat. Representative photographs of 40 μm sections of ventrolateral SCN demonstrating the black precipitate of positive neurons for Fos immunoreactivity that was induced in response to vehicle (VEH), 5-carboxamidotyptamine (5-CT), fluoxetine (FLX), in the presence of pindolol and sumatriptan. The final photograph (FLX/RIT) shows the reduction of Fos reactivity to fluoxetine administered in the presence of the 5-HT_7 antagonist, ritanserin.

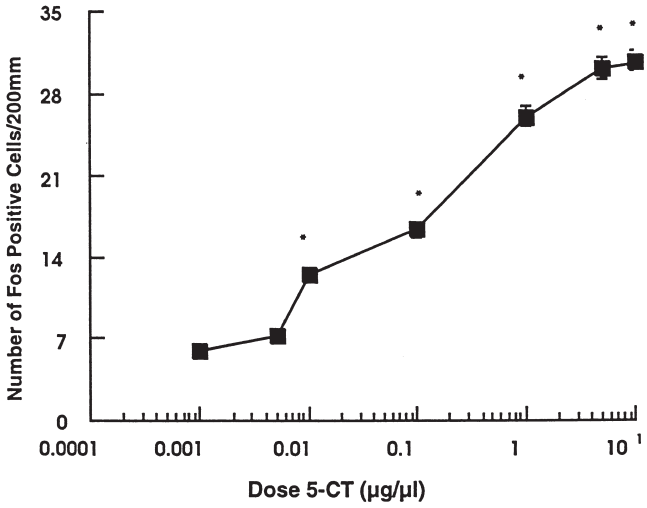

Figure 2. 5-Carboxamidotryptamine (5-CT)-induced acute Fos-like immunoreactivity in the vSCN at CT 22. 5-CT administered intracerebroventricularly in the presence of the 5-HT_{1A} antagonist pindolol (5 mg/kg, IP) and the 5-HT_{1D} agonist sumatriptan (1 mg/kg, IP) dose dependently stimulated Fos immunoreactivity (Fos-LI) at CT 22 in the vSCN. The number of Fos-positive cells in the vSCN induced at CT 22 were determined as described in Materials and Methods. Data represent the mean and standard error of the number of Fos-positive cells per 200 mm^2 of 10 sequential vSCN sections from at least six animals per dose, 1 hour after injection. *$p < .05$, as compared to saline vehicle.

5-$HT_{2A/2C}$ antagonist), clorgyline (monoamine oxidase type A inhibitor), and nefazodone (serotonin reuptake inhibitor and 5-HT_2 antagonist) induced significant Fos-LI at CT 22 as compared to control in the vSCN (Figures 1 and 3, Table 1). Fos-LI in the vSCN mediated by the acute administration of these agents was also pindolol and sumatriptan insensitive. Fos-LI at CT 22 in the vSCN stimulated by the acute administration of 8-OH-DPAT or the antidepressants was significantly reduced by pretreatment with the putative 5-HT_7 antagonist ritanserin (graphically representative by fluoxetine in Figure 4, Table 1). Acute administration of the antidepressant nomifensine (norepinephrine and dopamine reuptake inhibitor) and the 5-$HT_{2A/2C}$ agonists DOI or DOB did not elicit significant Fos reactivity in the vSCN at CT 22 as compared to control levels (Figure 3, Table 1).

These results suggest that acute administration of 5-CT, 8-OH-DPAT or antidepressants induce Fos immunoreactivity in the vSCN at CT 22 by activation of a receptor with a pharmacological profile consistent with the 5-HT_7 receptor.

Fos immunoreactivity in the vSCN at CT 22 stimulated by the agonists 5-CT, 8-OH-DPAT or the antidepressants fluoxetine, imipramine, desipramine, mianserin, clorgyline, or nefazodone was not affected by pretreatment with the melatonin antagonist N-acetyltryptamine (Table 1). These results suggest that Fos-LI is induced in the vSCN in response to agents administered in a pharmacologic profile consistent with the 5-HT_7 receptor and, moreover, that is inconsistent with the profile of 5-HT_{1A} or melatonin receptors.

To investigate further the actions of 8-OH-DPAT and the selected antidepressants on the neuroadaptive functional response of the 5-HT_7 receptor in the vSCN, animals were chronically treated with these agents. Chronic administration of 8-OH-DPAT or the antidepressants fluoxetine, mianserin, imipramine, desipramine, clorgyline, or nefazodone in the presence of pindolol and sumatriptan given chronically produced a significant reduction in the number of Fos-positive cells detected at CT 22 in the vSCN in comparison to the amount of Fos expression previously demonstrated with acute exposure to the same drug (see Table 2). The amount of Fos "desensitization" (defined as the relative percentage change from the former acute induction levels of the Fos response observed after chronic treatment, pump removal, and a subsequent drug challenge) produced in the vSCN at CT 22 stimulated by chronic 8-OH-DPAT and the antidepressants was statistically significant and varied slightly according to the drug administered. Desensitization of Fos immunoreactivity was observed following chronic treatment with fluoxetine (50%), and was also seen following 8-OH-DPAT (48%), nefazodone (48%), imipramine (34%), desipramine (32%), mianserin (26%), or clorgyline (24%) treatment. The desensitization of the Fos response observed following chronic 8-OH-DPAT or antidepressant exposure was also not significantly overcome by an acute challenge with the most effective 5-HT_7 agonist 5-CT. Fos-LI responses in the vSCN after chronic treatment remained significantly decreased in comparison from that amount previously stimulated by 5-CT in the acute administration studies (represented by fluoxetine in Figure 5, Table 2). This reduction in the inducible Fos response suggests that the 5-HT_7 receptor at which 5-CT evoked acute Fos expression in the vSCN had functionally desensitized in response to the long-term presence of 8-OH-DPAT or the antidepressants, thus implicating the 5-HT_7 receptor in the mechanism of antidepressant action.

Desensitization of inducible Fos-LI responses in the vSCN at CT 22 following the long-term administration of 8-OH-DPAT or antidepressants was significantly blocked by the simultaneous chronic administration of the 5-HT_7 antagonist ritanserin (Table 2). Chronic administration of the antagonists (pindolol, sumatriptan, ritanserin) or vehicle alone or in combination did not induce significant Fos expression over vehicle in the vSCN at CT 22. Nor did these agents produce functional neuroadaptation by themselves, because chronic treatment did not affect the response observed to a challenge with ICV 5-CT after pump removal. In response

Table 1. Acute Fos-Like immunoreactivity (Fos-LI) Induction Profile in the vSCN at CT 22

	Number of Fos-Positive Cells in the Presence of Pindolol and Sumatriptan		
Treatment	**No Additional Pretreatment**	**Plus Ritanserin**	**Plus N-acetyltryptamine**
Naive Control	5.1 ± 0.1	4.9 ± 0.2	4.6 ± 0.2
Vehicle	5.2 ± 0.2	7.3 ± 0.3	6.9 ± 0.4
5-CT	27.8 ± 1.2*	11.1 ± 0.1**	28.3 ± 0.9*#
8-OH-DPAT	18.5 ± 0.3*	10.9 ± 0.9**	17.9 ± 1.1*#
Fluoxetine	17.5 ± 0.9*	8.7 ± 0.3**	17.7 ± 0.8*#
Imipramine	16.4 ± 1.1*	10.7 ± 0.9**	16.2 ± 0.9*#
Desipramine	13.9 ± 0.7*	10.1 ± 0.4**	13.8 ± 0.6*#
Clorgyline	13.5 ± 0.5*	9.1 ± 0.2**	12.9 ± 0.5*#
Mianserin	14.6 ± 0.4*	8.8 ± 0.2**	14.9 ± 0.8*#
Nefazodone	14.9 ± 0.7*	9.7 ± 0.2**	15.2 ± 1.1*#
Nomifensine	7.8 ± 0.4	n/d	n/d
DOI	8.7 ± 0.6	n/d	n/d
DOB	7.3 ± 0.6	n/d	n/d
Haloperidol	6.4 ± 0.6	n/d	n/d
Raclopride	7.5 ± 0.3	n/d	n/d

Fos-LI induction was observed in the presence of pindolol (5 mg/kg, IP) and sumatriptan (1 mg/kg, IP). In those groups receiving ritanserin (5 mg/kg, IP) or N-acetyltryptamine (10 mg/kg, IP), these were given 15 minutes prior to pindolol and sumatriptan. The number of Fos positive cells per vSCN section per 200mm^2 were determined as described in the Materials and Methods section. Data represent the mean and standard error of at least six animals per treatment group. See also Figures 2 and 3.

*$p < .05$, compared to vehicle (pindolol/sumatriptan alone).

**$p < .05$, compared to test agent alone; # not significant from test agent alone; Student-Newman-Tukey's; n/d = not done.

to 5-CT, a marked and significant Fos-LI was obtained that was not statistically different from levels of Fos expression previously observed with acute 5-CT (Table 2).

These data provide strong evidence to suggest that chronic administration of agents in a pharmacologic profile that corresponds to the 5-HT$_7$ receptor functionally desensitizes the inducible Fos response in the vSCN at CT 22.

Membrane Homogenate 5-HT$_7$ Receptor Binding

Tissue membrane 5-HT$_7$ receptor binding studies were performed as previously described in the Materials and Methods section to investigate whether the proposed functional desensitization of Fos immunoreactivity demonstrated in the vSCN at CT 22, which reflected a neuroadaptive response of the 5-HT$_7$ receptor, was correlated with a downregulation of 5-HT$_7$ receptor sites. Hypothalamic and striatal membranes were prepared from rats chronically treated with 8-OH-DPAT or the antidepressants fluoxetine, imipramine, desipramine, clorgyline, mianserin, and nefazodone (at the same doses used in the Fos immunoreactivity chronic administration studies) and were assayed for the 5-HT$_7$ binding parameters of receptor affinity (K_d) and receptor density (B_{max}) according to a method modified from Sleight et al. (1995).

Using the radioligand [3H]-5-carboxamidotryptamine (specific activity 80 Ci/mmol) in the presence of the 5-HT$_{1A}$ antagonist (-)-pindolol (1 μm) and 5-HT$_{1D}$ agonist sumatriptan (1 μm), 5-HT$_7$ receptor K_d and B_{max} in animals chronically treated with vehicle or drug were determined in rat hypothalamic tissues. [3H]-5-CT saturation analysis of the binding of rat hypothalamic membranes demonstrated a saturable population of binding sites. Scatchard analysis of the data provided evidence for the existence of a single homogeneous population of binding sites from hypothalami of vehicle-treated rats for [3H]-5-CT with a K_d of 2.03 ± 0.28 nm and a B_{max} of 10.51 ± 1.34 fmol/mg tissue (Figure 5 and Table 3).

These results are consistent with previously published values for rat hypothalamic membranes (Sleight et al. 1995), which demonstrated a K_d of 1.94 nm and a B_{max} of 8.15 fmol/mg tissue, utilizing [3H]-5-HT as the ligand. In the present studies, specific binding was approximately 60% of the total binding. Striatal tissues from treated animals were included as a negative control and showed no specific binding (data not shown).

After the chronic administration of 8-OH-DPAT or antidepressants for 21 days, a significant reduction in the number of 5-HT$_7$ binding sites was detected in the hypothalamus (see Figure 6, Figure 7, and Table 3, analysis graphically represented by fluoxetine isotherms).

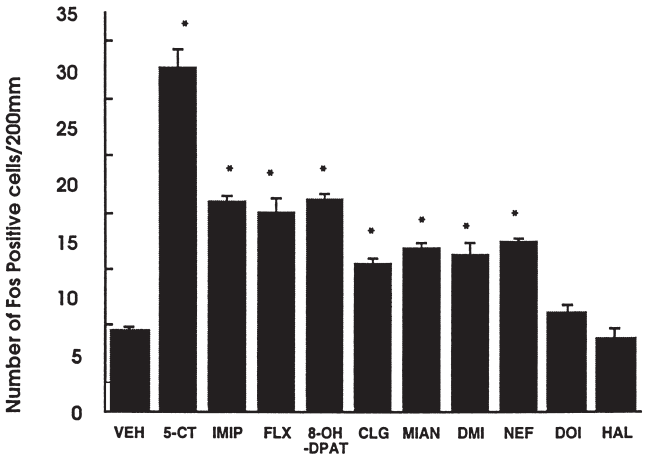

Figure 3. Acute administration of 5-HT_7 agonists and antidepressants in the presence of pindolol and sumatriptan induce Fos-LI at CT 22 in the vSCN. Acute administration of the 5-HT_7 agonists 5-CT (5μg, ICV) and 8-OH-DPAT (10 mg/kg, IP), in the presence of pindolol (5 mg/kg, IP) and sumatriptan (1 mg/kg, IP), induced significant Fos-LI at CT 22 in the vSCN compared to vehicle (see Table 1). Similarly, acute administration of each antidepressant (10 mg/kg, IP; except nefazodone, which was 20 mg/kg, IP) also evoked a significant Fos response as compared to control. The antidepressant nomifensine (dopamine and norepinephrine reuptake inhibitor), the 5-$HT_{2A/2C}$ agonist DOI and the dopamine antagonist haloperidol did not elicit a significant response. Data represent the mean and standard error of the number of Fos positive cells per 200 mm^2, from at least six animals per drug. Vehicle (VEH); 5-CT with pindolol and sumatriptan (5-CT+); imipramine (IMIP); fluoxetine (FLX); clorgyline (CLG); mianserin (MIAN); desipramine (DMI); nefazodone (NEF); nomifensine (NOMF); haloperidol (HAL). *$p < .05$, Student–Newman-Tukey's.

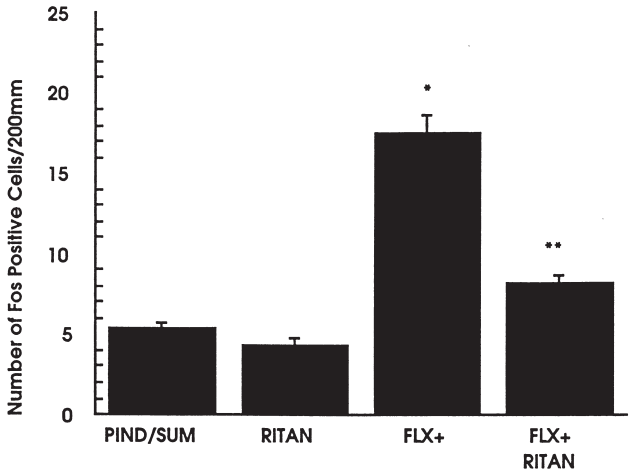

Figure 4. Acute administration of fluoxetine induces Fos-LI at CT 22 in the vSCN and is reduced by pretreatment with ritanserin. This pattern of Fos-LI reactivity is representative of the responses obtained after administration of the numerous pharmacologically distinct agents listed in Table 1. Here, the acute administration of the selective serotonin reuptake inhibitor fluoxetine (10 mg/kg, IP) in the presence of pindolol (5 mg/kg, IP) and sumatriptan (shown as FLX+; 1 mg/kg, IP) induced significant Fos-LI at CT 22 in the vSCN as compared to vehicle (5.2 ± 0.2 Fos-positive cells). Fos-LI stimulated by fluoxetine in the vSCN at CT 22 was reduced by pretreatment with the 5-HT_7 antagonist ritanserin (5 mg/kg, IP). Data represent the mean and standard error of the number of Fos-positive cells per 200 mm^2, from at least six animals per group. Pindolol/sumatriptan (PIND/SUM); ritanserin (RITAN); fluoxetine with pindolol and sumatriptan (FLX+); fluoxetine with pindolol, sumatriptan and ritanserin (FLX+ RITAN). *$p < .05$, as compared to pindolol/sumatriptan; *$p < .05$, as compared to FLX+, Student–Newman-Tukey's.

Reductions in [3H]-5-CT binding sites in either hypothalamic or hippocampal tissues after chronic drug administration was not accompanied by a significant alterations from control in the observed K_d values for the 5-HT_7 receptor.

The reductions in 5-HT_7 receptor density in the hypothalamus after chronic administration of 8-OH-DPAT or the antidepressants are consistent with and may, in part, account for the functional desensitization of the 5-HT_7 receptor reflected in the observed reductions of inducible Fos expression.

DISCUSSION

In response to receptor stimulation, the immediate early gene c-Fos is induced and is indicative of neuronal activation. In these experiments, Fos-like immunoreactivity was significantly induced in the hypothalamic ventrolateral suprachiasmatic nucleus (vSCN) at circadian time 22 (CT 22) in response to the acute administration of the 5-HT_7 agonists 5-CT, 8-OH-DPAT or the antidepressants fluoxetine, mianserin, imipramine, desipramine, clorgyline, or nefazodone. Fos-LI immunoreactivity levels induced by these agents were not significantly affected by the simultaneous presence of the 5-HT_{1A} antagonist pindolol and the 5-HT_{1D} agonist sumatriptan, but were significantly reduced by pretreatment with the 5-HT_7 antagonist ritanserin. The pattern of Fos immunoreactivity observed in the vSCN at CT 22 that is stimulated by the acute administration of 5-CT, 8-OH-DPAT or antidepressants in the presence of pindolol and sumatriptan and blocked by pretreatment with the antagonist ritanserin is highly consistent with the activation of a receptor pharmacologically similar to the in vitro profile established for the 5HT_7 receptor. Acute administration of the norepinephrine and dopamine reuptake inhibitor nomifensine or the 5-HT_2 agonists DOI or DOB did not produce significant elevations in Fos-LI. Interestingly, although the 5-HT_2 agonist DOI has been reported to have nanomolar affinity for the 5-HT_7 receptor, functional studies using the stimula-

Table 2. Chronic Treatment with 8-OH-DPAT and Antidepressants Reduces Fos-Like Immunoreactivity Response in the vSCN at CT 22

	Number of Fos-Positive Cells in the Presence of Pindolol and Sumatriptan		
Chronic Treatment	**No Additional Pretreatment**	**Plus Ritanserin**	**After 5-CT Challenge**
Vehicle	4.1 ± 0.2	3.9 ± 0.4	30.1 ± 1.4
8-OH-DPAT	9.5 ± 0.3* (-48)	19.1 ± 0.5**	10.2 ± 0.4
Fluoxetine	8.2 ± 0.2* (-50)	17.4 ± 0.3**	10.1 ± 0.3
Imipramine	10.9 ± 0.3* (-34)	15.9 ± 0.5**	10.6 ± 0.9
Desipramine	9.5 ± 0.4* (-32)	12.4 ± 0.5**	9.8 ± 0.6
Clorgyline	9.9 ± 0.3* (-24)	12.9 ± 0.6**	9.2 ± 0.3
Mianserin	10.9 ± 0.1* (-26)	13.2 ± 0.4**	9.2 ± 0.3
Nefazodone	7.8 ± 0.4* (-48)	13.7 ± 0.5**	8.2 ± 0.1
Haloperidol	7.2 ± 0.3	n/d	31.9 ± 0.9*
Raclopride	7.5 ± 0.3	n/d	28.2 ± 0.9*

Measurements of Fos-LI in response to acute drug after chronic treatment were performed in the presence of the chronic co-administration of pindolol (1 mg/kg) and sumatriptan (1 mg/kg) and ritanserin (1mg/kg) as specified in the Materials and Methods section. 5-CT challenge (5 μg in saline) was administered ICV 30 min before sacrifice. Data represent the mean and standard error of the number of Fos-positive cells per 200 mm^2 of at least consecutive vSCN sections from at least six animals per treatment group. Data in parentheses represent the percentage change from acute induction levels shown in Table 1. See also Figures 5 and 6.

*$p < .05$, compared to acute induction response from Table 1.

**$p < .05$, compared to chronic response; n/d = not done, Student-Newman-Tukey's.

tion of cAMP in cultured cells expressing the 5-HT_7 receptor have shown that it does not evoke significant elevations of cAMP over basal levels (Lovenberg et al. 1993; Ruat et al. 1993). Similarly, the dopamine antagonists raclopride and haloperidol were not effective inducers of Fos-LI in the vSCN at CT 22 suggesting that these compounds either do not possess affinity for or have functional activity at the 5-HT_7 receptor. Of note, haloperidol has previously been reported to have micromolar affinity for the 5-HT_7 receptor, but like DOI, it also does not induce a functional cAMP response in cultured cells (Lovenberg et al. 1993; Ruat et al. 1993). By demonstrating that agents exhibiting functional activity at the 5-HT_7 receptor are those that induce Fos-LI in the vSCN, the observations add substantial validity and physiologic relevance to the specificity of the Fos-LI response induced in the present studies.

The hypothesis that activation of the 5-HT_7 receptor induces Fos-LI is further supported in these studies by experiments showing that Fos immunoreactivity in the vSCN at CT 22 evoked by the acute administration of 5-CT, 8-OH-DPAT or antidepressants was not affected by pretreatment with the melatonin antagonist N-acetyltryptamine. Melatonin receptors are present in their highest densities in the SCN, and although the pharmacologic agents utilized in these studies do not possess affinity for melatonin receptors, it is important to rule out that the Fos reactivity observed was not caused by melatonin receptor stimulation. In addition, detection of Fos-LI in the vSCN in response to melatonin and light at this particular circadian time window has been previously established as an indicator of a phase shift response (Ginty et al. 1993, Kornhauser et al. 1992). The inability of N-acetyltryptamine to block the Fos response induced by 8-OH-DPAT, 5-CT, or antidepressants obviates the possibility that acute Fos responses at CT 22 in the vSCN induced by these agents were mediated by melatonin receptor stimulation. This lack of sensitivity to N-acetyltryptamine, when considered together with the pharmacologic profile of agents that did induce a significant Fos response in these studies, substantiates the hypothesis that acute activation of the 5-HT_7 receptor is responsible for the observed Fos immunoreactivity in the vSCN.

It is intriguing to note that although desipramine is classically defined as a norepinephrine reuptake inhibitor, it is active in this paradigm, although the Fos response elicited was not as marked as that produced by fluoxetine, a potent selective serotonin reuptake inhibitor. Fluoxetine does not have affinity for the 5-HT_7 receptor; however, chronic administration produces elevated levels of synaptic serotonin, the endogenous ligand of the 5-HT_7 receptor. It has been reported that the serotonergic reuptake property of desipramine is approximately 1000-fold less than its norepinephrine reuptake blockade (Baldessarini 1990; Cooper et al. 1996; Richelson 1990), and, in fact, the receptor binding results reported in the present studies show that it exhibits micromolar affinity for the 5-HT_7 receptor. Therefore, the ability of desipramine to induce Fos expression in the vSCN may be attributable to its, albeit low, affinity for the 5-HT_7 receptor and serotonin reuptake prop-

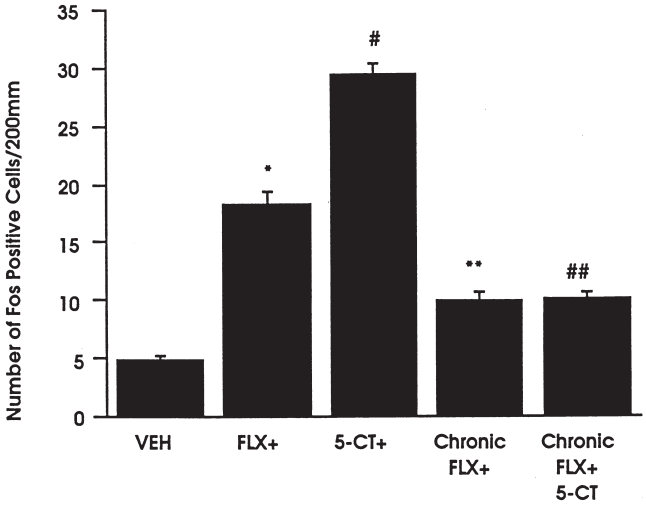

Figure 5. Chronic administration of fluoxetine in the presence of pindolol and sumatriptan desensitizes Fos-LI at CT 22 in the vSCN. This graph is representative of the typical Fos-LI induction response observed in experiments where animals were chronically exposed to the agents listed in Table 2. Chronic administration (subcutaneous osmotic minipump, performed as described in Materials and Methods) of the selective serotonin reuptake inhibitor fluoxetine (Chronic FLX+) in the presence of chronic pindolol (minipump, 1 mg/kg) and sumatriptan (minipump, 1 mg/kg) produced a significant reduction in the number of Fos-positive cells detected at CT 22 in the vSCN after challenge (10 mg/kg, IP) as compared to the response previously evoked by the same dose acutely (acute response designated as FLX+). Similarly, when challenged with the more potent agonist 5-CT, the Fos-LI response was unchanged (5-CT+/Chronic FLX). Data represent the mean and standard error of the number of Fos-positive cells per 200 mm^2, from at least six animals per group. *#$p < .05$, as compared to vehicle (VEH); **$p < .05$ as compared to acute fluoxetine (FLX+); ##$p < .05$ as compared to acute 5-CT (5-CT+); Student–Newman-Tukey's.

erties. The Fos response seen in the vSCN after desipramine suggests that, although the representative density of the 5-HT_7 receptor in the hypothalamus is comparatively lower than other more density predominant receptors, such as melatonin and GABA (Miller et al. 1996), the vSCN may be highly sensitive to serotonergic stimuli during this time window through activation of the 5-HT_7 receptor.

Chronic administration of 8-OH-DPAT or antidepressants (in the presence of pindolol and sumatriptan) produced significant attenuation of the level of Fos expression at CT 22 in the vSCN, as compared to the level produced by acute administration. The Fos-LI desensitization produced in the vSCN by chronic exposure to 8-OH-DPAT or antidepressants was significantly blocked by chronic co-administration of the 5-HT_7 antagonist ritanserin. Chronic administration of the dopamine antagonists haloperidol and raclopride did not cause a

Table 3. Hypothalamic 5-HT_7 Receptor Binding in Rats After Chronic Treatment with 8-OH-DPAT or Antidepressants

Treatment	Kd (nM)	Bmax (fmol/mg tissue)	Percentage Change
Vehicle-Control	2.03 ± 0.28	10.5 ± 1.34	
8-OH-DPAT	1.98 ± 0.31	7.39 ± 1.57*	-29.7
Fluoxetine	1.96 ± 0.21	7.39 ± 1.07*	-30.7
Imipramine	1.98 ± 0.37	7.53 ± 0.42*	-28.4
Desipramine	1.87 ± 0.35	7.16 ± 0.84*	-30.8
Clorgyline	1.87 ± 0.37	7.78 ± 0.89*	-25.9
Mianserin	1.99 ± 0.26	7.44 ± 0.73*	-29.1
Nefazodone	1.89 ± 0.24	7.78 ± 1.29*	-25.9
Nomifensine	1.89 ± 0.41	9.83 ± 1.41	n/c
DOI	2.08 ± 0.78	9.51 ± 1.84	n/c
DOB	2.13 ± 0.29	10.3 ± 1.54	n/c
Haloperidol	1.83 ± 0.32	9.51 ± 1.34	n/c
Raclopride	1.99 ± 0.18	9.81 ± 1.04	n/c

Animals were chronically treated with agents as described in Materials and Methods. Data represents the mean and standard error of three experiments done in triplicate on groups which contained six animals per treatment.

*$p < .05$, compared to vehicle-control; n/c = not significant from vehicle, Dunnett's.

change in Fos expression in the vSCN at CT 22. This lack of desensitization after chronic administration of either haloperidol or raclopride was further validated by experiments showing that acute ICV challenge with the 5-HT_7 agonist 5-CT was able to induce significant Fos expression over control levels in the vSCN of chronically treated animals. This effect suggests that neither chronic haloperidol nor raclopride have a significant impact on the functional response of the 5-HT_7 receptor in the vSCN at CT 22. Accordingly, these chronic studies suggest that a functional adaptation of the inducible Fos response in the vSCN had occurred as a result of prolonged exposure to drugs with a pharmacologic profile consistent with the 5-HT_7 receptor. Furthermore, as an extension, these results support the hypothesis that the 5-HT_7 receptor may be involved in the mechanisms by which antidepressant drugs modulate circadian function controlled by the vSCN.

Autoradiographic studies have shown that the 5-HT_7 receptor is localized in high densities within the hypothalamus; however, mRNA for the 5-HT_7 receptor was not initially detected in the SCN (Gustafson et al. 1996). Subsequent studies performed during CT 18 to CT 20; however, did detect 5-HT_7 receptor mRNA, suggesting that expression of this receptor itself may also be subject to circadian regulation (Miller et al. 1996). In the present studies, the functional pattern of acute and chronic Fos immunoreactivity in the vSCN induced by the agonists 8-OH-DPAT, 5-CT or by antidepressants (in the presence of pindolol and sumatriptan), coupled with the sensitivity of the observed responses to ritanserin, is a pharmacology distinct from other serotonin

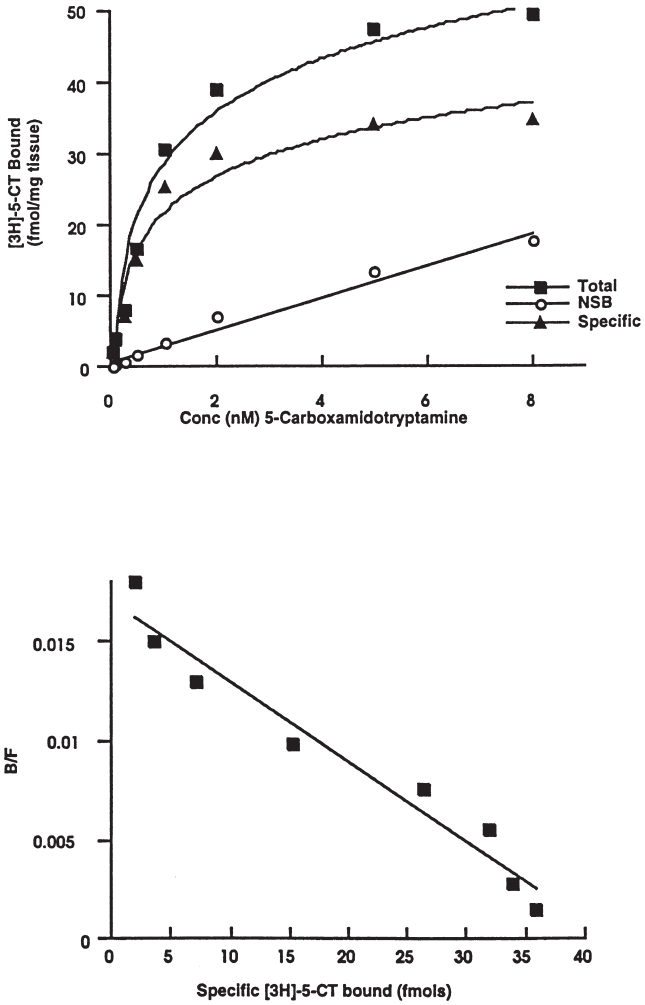

Figure 6. 5-HT_7 receptor binding in hypothalamic membranes. Representative binding data (Scatchard transformation) for [3H]-5-carboxamidotryptamine in the presence of pindolol and sumatriptan (each at 1 μm) to hypothalamic tissues from vehicle-treated rats. Data represent the mean of three separate experiments done in triplicate. Nonspecific binding was defined in the presence of 1 μm 5-HT. Hypothalamic 5-HT_7 receptor binding in vehicle-treated animals represented a saturable, single population of sites with a k_d of 2.03 ± 0.28 nm and a B_{max} of 10.51 ± 1.34 fmol/mg of tissue.

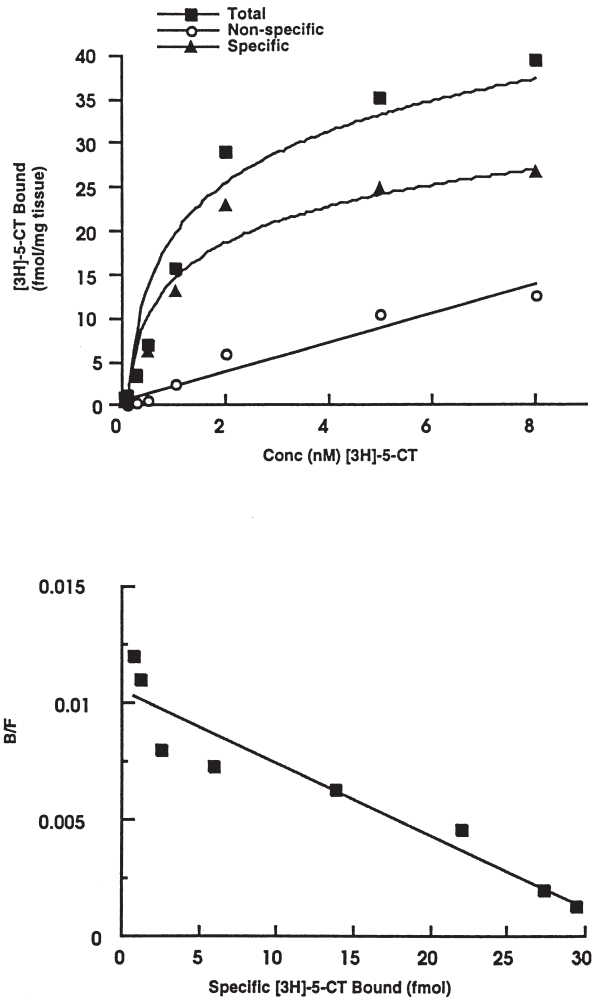

Figure 7. Downregulation of 5-HT_7 receptor binding density in hypothalamic membranes after chronic fluoxetine administration. Representative binding data (Scatchard transformation) for [3H]-5-carboxamidotryptamine in the presence of pindolol and sumatriptan (1 μm) to hypothalamic tissues from rats chronically treated with fluoxetine (5 mg/kg, 21 days). This response was also observed after administration of several agents listed in Table 3. Data represent the mean of three separate experiments done in triplicate. Nonspecific binding was defined in the presence of 1 μm 5-HT. Hypothalamic 5-HT_7 receptor binding in chronic fluoxetine-treated animals demonstrated a decrease in binding sites from control without a significant change in affinity. (k_d =1.96 ± 0.21 nm and a B_{max} of 7.28 ± 0.68 fmol/mg).

receptors known to be localized within the SCN. Although 5-HT_{1A} receptors are present in the SCN, and both 5-CT and 8-OH-DPAT are high-affinity agonists at this receptor, their actual representation within the SCN is disputed (Miller et al. 1996; Roca et al. 1993; Sumner et al. 1992). Nevertheless, 5-HT_{1A} pharmacology is distinguishable from that of the 5-HT_7 receptor, most notably by a sensitivity of the 5-HT_{1A} receptor to the antagonist (−)-pindolol and a sensitivity of the 5-HT_7 receptor to ritanserin. All of the Fos immunoreactivity studies performed here were done in the presence of pindolol and sumatriptan, indicating that the response seen was not attributable to 5-HT_{1A} or 5-HT_{1D} receptor stimulation. Moreover, specific action of these agents at the 5-HT_7 receptor is further substantiated by the significant blockade of the stimulated Fos-LI by the 5-HT_7 antagonist ritanserin.

The functional role and target genes for the AP-1 proteins, such as Fos, are unclear, but it is known that they are expressed in a heterogeneous population of

SCN neurons. Several investigations have demonstrated that Fos protein expression is involved in light-induced entrainment mechanisms (Ginty et al. 1993; Kilduff et al. 1992; Moore 1995). Furthermore, induction of Fos expression is anatomically specific and exclusively occurs in response to phase-shifting stimuli in the ventrolateral portion of the SCN (Ginty et al. 1993; Moore 1995; Miller et al. 1996). c-Fos expression has been directly correlated with photic phase shifts of overt behavioral rhythms, and pharmacologic agents that block behavioral rhythms also inhibit Fos induction (Kornhauser et al. 1992; Miller et al. 1996). Similarly, it has been shown that blocking c-Fos induction with antisense oligonucleotides to c-Fos phase delays emergent rat locomotor rhythms (Miller et al. 1996; Wollnick et al. 1995).

Serotonergic agents have been described as blocking the photic induction of Fos in the vSCN, suggesting a modulatory role of serotonin innervation to the nucleus. In hamsters, light-induced Fos expression at CT 7 was blocked by the nonspecific serotonin agonist quipazine (Selim et al. 1993). In other hamster studies, 8-OH-DPAT, buspirone, the serotonin releaser fenfluramine, or the monoamine oxidase inhibitor harmaline given 7 hours after lights off (equivalent to CT 19 in the studies conducted for this work) inhibits the intense Fos expression induced by light in the vSCN. This blockade effect was attenuated by the 5-HT_{1A} antagonist NAN-190 and the serotonin antagonists metergoline and ritanserin (Glass et al. 1994). These results implicate serotonin in the regulation of the photic signal transduction transmitted by the SCN. It is of particular interest to note that the response was inhibited by the nonspecific serotonin antagonist metergoline and ritanserin, the 5-HT_7 antagonist. Unfortunately, 8-OH-DPAT, buspirone, fenfluramine, and harmaline were not administered alone at this circadian period to determine if they themselves induced Fos expression in the vSCN. In fact, only one report in the literature provides evidence that nonphotic serotonergic activation induces Fos expression in the vSCN at the appropriate circadian time window to produce a phase shift. In that investigation, the nonspecific 5-HT agonist quipazine administered to rats at CT 18 induced significant Fos expression in the vSCN in a comparable regional and quantitative fashion to other animals that were given an appropriate light stimulus within the circadian cycle (Kennaway et al. 1996). Accordingly, the results presented here are novel in that they demonstrate that pharmacologically distinct antidepressants induce Fos immunoreactivity in the vSCN at CT 22 by activation of a serotonin receptor, the 5-HT_7 receptor.

Chronic adminstration of 8-OH-DPAT or the antidepressants fluoxetine, imipramine, desipramine, mianserin, clorgyline, or nefazodone produced reductions in 5-HT_7 receptor density (B_{max}) in hypothalamic tissues but did not alter receptor affinity (K_d). These data provide evidence to support that the desensitized Fos response in the vSCN induced by chronic treatment with these agents reflected 5-HT_7 receptor downregulation and validate the Fos studies in which chronic administration of agents resulted in a significant reduction in the inducible Fos response in the vSCN. Decreases in the number of hypothalamic 5-HT_7 receptors indicate that the observed decrease in the Fos response is consistent with and attributable to a proposed mechanism of action by 8-OH-DPAT or antidepressants on 5-HT_7 receptors localized in the vSCN.

Chronic administration of the norepinephrine and dopamine reuptake inhibitor nomifensine did not produce a decrease in 5-HT_7 receptor number in the hypothalamus. There also were no adaptive changes (e.g., decreases in B_{max} or K_d) detected in 5-HT_7 receptor binding in animals chronically treated with the 5-HT_2 agonists DOI or DOB, or the dopamine antagonists haloperidol or raclopride. This lack of effect on 5-HT_7 receptor regulation by these agents correlated with their inability to induce acutely or desensitize chronically chronically the 5-HT_7-mediated Fos response.

Neurotransmitter and receptor abnormalities have been previously noted in the serotonergic system in depressed patients and implicated in the pathophysiology of depression. Neuroadaptive effects have also been observed in other serotonergic systems (5-HT_{1A} or 5-HT_2 receptor systems) in response to chronic antidepressant treatment (Cooper et al. 1996; Hyman and Nestler 1996; Nair and Sharma 1989; Schwaninger et al. 1997). In the postmortem brains of depressed subjects and suicides most investigators report an increase in 5-HT_2 receptor binding (Arango et al. 1990; Arora and Meltzer 1989) as compared to nondepressed patients matched for age, sex, and time to autopsy. Furthermore, winter reductions in serotonin levels have been reported in postmortem hypothalamic tissues of patients diagnosed with seasonal affective disorder (Oren and Rosenthal 1992; Carlsson et al. 1980). In addition, 5-HT_2 receptors are also expressed on human platelets and have been reported to be increased in depressed and suicidal patients (Arora and Meltzer 1989; Biegon et al. 1990a; Delgado et al. 1992; Pandey et al. 1990). Furthermore, the 5-HT_2 binding on platelets of depressed patients was reported to normalize after successful antidepressant treatment (Biegon et al. 1990b; Delgado et al. 1992). The results presented here suggest that the number of postsynaptic 5-HT_7 receptors in the hypothalamus are decreased in response to chronic antidepressant treatment. This is an effect consistent with previous investigations in the hypothalamus in response to chronic fluoxetine (Sleight et al. 1995). Currently, it is not known and would be of interest to determine whether 5-HT_7 receptors are increased in patients exhibiting dysregulation of circadian rhythms or in depressive states.

Desensitization of receptor-mediated responses may also be achieved by drug-induced internalization of receptor, uncoupling of the transducer system to the receptor, or through latency of receptor degradation (Hamon et al. 1990; Hyman and Nestler 1996; Zelman and Garver 1990). Therefore, theoretically, it is not critical to observe a decrease in the number of binding sites to achieve a desensitized functional response. Nevertheless, the results obtained in the present studies provide strong evidence supporting the hypothesis that the 5-HT_7 receptor in the hypothalamus adapts to the chronic drug exposure in a manner consistent with a downregulatory response (reduced receptor density). The data suggest that the antidepressants used that possess the ability to modulate serotonergic tone, albeit through a variety of mechanisms, act, ultimately, at the 5-HT_7 receptor to produce neuroadaptation represented by a reduction in receptor number within the hypothalamus. Although in these studies the observed Fos desensitization response was approximately twofold greater than the observed decrease in 5-HT_7 receptor number, the results are, nonetheless, positively correlated. However, the difference between the observed Fos desensitization and the relative amount of receptor downregulation may, in part, be caused by the occurrence of any of the other regulatory mechanisms described above, which would not be detected in the receptor binding studies performed here. Nevertheless, the results presented here strongly suggest that a functional desensitization of the Fos response occurred with a downregulation of the receptor responsible for the initial acute response.

In conclusion, the results provide strong evidence to suggest that the 5-HT_7 receptor is implicated in the mechanism of action of antidepressants. The evidence suggests that antidepressants produce functional effects in a hypothalamic region associated with phase-shift responses, during the appropriate circadian time, and in a pharmacologic manner consistent with activation of the 5-HT_7 receptor. Moreover, the adaptive desensitization and downregulation of the 5-HT_7 receptor in response to chronic treatment occurred in a time course consistent with the proposed mechanism of antidepressant action theoretically required to produce a therapeutic response. Desensitization of 5-HT_7 receptor-induced Fos-LI in the hypothalamus suggests that antidepressants may participate in the modulation of SCN-controlled rhythmic circadian function through action at the 5-HT_7 receptor. When considered with other published reports that suggest that serotonin is capable of modulating the light-induced phase responses by the SCN (Glass et al. 1994), the Fos induction and desensitization responses in the vSCN at CT 22 presented may also suggest that serotonin is involved in the direct initiation and regulation of phase-shift responses. Further studies are needed to assess whether antidepressants produce similar neuroadaptive effects in 5-HT_7 receptor regulation in other limbic areas relevant to affective disease and whether chronic activation of the 5-HT_7 receptor alters in vivo circadian responses.

By demonstrating overt serotonergic activity in the vSCN, the work accomplished in the present investigation expands the functional importance of serotonin in the circadian system to include response modulation through a specific serotonin receptor and furthermore, strongly implies that the serotonin receptor responsible for such SCN regulation is not of the 1A-subtype, but is the serotonin subtype-7. Just as important, these results provide further evidence to support the theory that one of the consequent mechanisms of antidepressant treatment is a modulation of possibly dysrhythmic circadian function in depression (Duncan 1996; Healy 1987; Hallonquist et al. 1986). Clinically, the therapeutic relevance of circadian modulatory activity by antidepressants enhances our knowledge of the interactive, and perhaps, interdependent nature of circadian function and affective disorders.

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