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5-HT_{2A} Receptor-Mediated Regulation of Brain-Derived Neurotrophic Factor mRNA in the Hippocampus and the Neocortex

Vidita A. Vaidya, Gerard J. Marek, George K. Aghajanian, and Ronald S. Duman

Laboratory of Molecular Psychiatry, Departments of Psychiatry and Pharmacology, Yale University School of Medicine, Connecticut Mental Health Center, New Haven, Connecticut 06508

The influence of 5-HT receptor agonists on the expression of BDNF in brain was determined. Administration of a hallucinogenic 5-$HT_{2A/2C}$ receptor agonist, but not a 5-HT_{1A} receptor agonist, resulted in a significant but differential regulation of BDNF mRNA levels in hippocampus and neocortex. In the hippocampus, the 5-$HT_{2A/2C}$ receptor agonist significantly decreased BDNF mRNA expression in the dentate gyrus granule cell layer but did not influence expression of the neurotrophin in the CA subfields. In parietal cortex and other neocortical areas, but not piriform cortex, the 5-$HT_{2A/2C}$ receptor agonist dramatically increased the expression of BDNF mRNA. The effect of the 5-$HT_{2A/2C}$ receptor agonist on BDNF mRNA in both the hippocampus and the neocortex was blocked by pretreatment with a selective 5-HT_{2A}, but not 5-HT_{2C}, receptor antagonist. The expression of BDNF mRNA in the hippocampus is reported to be decreased by stress, raising the possibility that the 5-HT_{2A} receptor mediates this effect. Pretreatment with ketanserin, a 5-$HT_{2A/2C}$ receptor antagonist, significantly blocked the stress-induced downregulation of BDNF mRNA in hippocampus, in support of this hypothesis. The results of this study raise the possibility that regulation of BDNF expression by hallucinogenic 5-HT_{2A} receptor agonists leads to adaptations of synaptic strength in the hippocampus and the neocortex that may mediate some of the acute and long-term behavioral effects of these agents.

Key words: BDNF; 5-HT_{2A}; 5-HT_{2C}; hippocampus; cortex; DOI; stress

The neurotrophin family, which consists of NGF (Levi-Montalcini and Angeletti, 1968), BDNF (Leibrock et al., 1989), neurotrophin-3 (Ernfors et al., 1990; Hohn et al., 1990; Maisonpierre et al., 1990; Rosenthal et al., 1990), and neurotrophin-4/5 (Hallbook et al., 1991; Ip et al., 1992), plays an important role in the development, differentiation, maintenance, and survival of distinct and overlapping neuronal populations within the central and peripheral nervous system (Levi-Montalcini, 1987; Barde, 1989; Barde, 1994; Davies, 1994). BDNF has the widest distribution of the neurotrophins in the CNS, and is expressed at highest levels in the hippocampus and the cerebral cortex (Ernfors et al., 1990; Hofer et al., 1990; Wetmore et al., 1990, Phillips et al., 1990). In the hippocampus and/or cerebral cortex, BDNF regulates survival, differentiation, synaptic strength, and neuronal morphology (Ghosh et al., 1994; Korte et al., 1995; McCallister et al., 1995; Thoenen, 1995). BDNF also influences the expression of phenotypic markers such as neurotransmitter synthesizing enzymes, neuropeptides, and calcium-binding proteins (Alderson et al., 1990; Ip et al., 1993; Croll et al., 1994; Jones et al., 1994; Nawa et al., 1994).

Expression of BDNF mRNA is activity dependent, undergoes regulation during development, and also shows marked and transient changes in response to a number of neuronal insults, including ischemia, hypoglycemia, epileptic activity, immobilization stress, and trauma (Maisonpierre et al., 1990; Ernfors et al., 1991; Lindvall et al., 1994; Smith et al., 1995). Regulation of BDNF mRNA levels under basal conditions, as well as in response to insult, involves a complex interplay between different neurotransmitter systems (Thoenen et al., 1991). For example, glutamate plays a role in upregulation of BDNF mRNA, whereas GABAergic pathways are involved in downregulation of BDNF expression in the hippocampus (Zafra et al., 1991; Berninger et al., 1995). Both NMDA and non-NMDA glutamate receptors mediate the effects of glutamate on BDNF mRNA expression (Zafra et al., 1990; Gwag et al., 1993; Wetmore et al., 1994). In addition, cholinergic and noradrenergic pathways also modulate levels of BDNF mRNA *in vivo* (Zafra et al., 1992; da Penha Berzaghi et al., 1993; Lapchak et al., 1993; Knipper et al., 1994; Hutter et al., 1996). Basal expression of BDNF mRNA in the hippocampus and cerebral cortex is stimulated by glucocorticoids, which also influence the regulation of BDNF in response to a neuronal insult (Barbany and Persson, 1993).

The role of 5-HT in the *in vivo* regulation of BDNF mRNA has not been examined in detail. Recent work has shown that chronic administration of 5-HT-selective reuptake inhibitors, used clinically as antidepressants, leads to an upregulation of BDNF mRNA in the hippocampus (Nibuya et al., 1995, Nibuya et al., 1996). 5-HT exerts its actions through a large family of receptors expressed in the periphery and throughout the CNS (Martin and Humphrey, 1994; Boess and Martin, 1994). This family of receptors serves as targets for the treatment of a number of disorders, including anxiety, depression, schizophrenia, obsessive–compulsive disorder, migraine, eating disorders, and panic disorders (Roth, 1994). Two of the best characterized 5-HT receptor subtypes are the 5-HT_{1A} and 5-HT_{2A} receptors. Both the 5-HT_{1A} and 5-HT_{2A} receptors have been of interest in the etiology and treat-

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Correspondence should be addressed to Dr. Ronald S. Duman, Division of Molecular Psychiatry, Yale University School of Medicine, CMHC, 34 Park Street, New Haven, CT 06508.

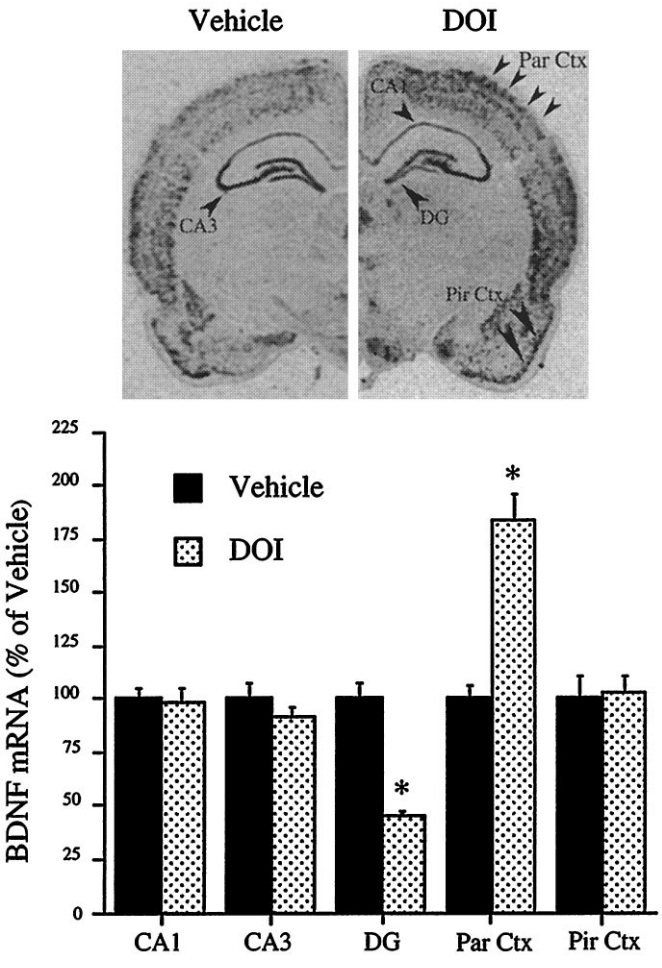

Figure 1. Regulation of BDNF mRNA in hippocampus and neocortex by DOI. The influence of an acute injection of vehicle or DOI (8 mg/kg) on levels of BDNF mRNA was determined by *in situ* hybridization as described in Materials and Methods. Representative autoradiographs from the vehicle- and DOI-treated groups are shown. Levels of BDNF mRNA in the CA1, CA3, dentate gyrus (*DG*), parietal cortex (*Par Ctx*) and piriform cortex (*Pir Ctx*) were determined by quantitative densitometry. Results are expressed as percent of vehicle and are the mean ± SEM (n = 6). $^*p < 0.05$ compared with vehicle (Student's t test).

ment of depression and other affective disorders (Schreiber and De Vry, 1993; Berendsen, 1995). In addition, 5-HT$_{2A}$ receptors are also involved in the actions of hallucinogens and atypical antipsychotics and have been implicated in the pathogenesis of schizophrenia (Titeler et al., 1988; Levy and Van de Kar, 1992; Sorensen et al., 1993; Gellman and Aghajanian, 1994; Marek and Aghajanian, 1994; Fiorella et al., 1995). The closely related 5-HT$_{2C}$ receptor may also play a role in some of these actions (Sanders-Bush and Breeding, 1991; Baxter et al., 1995).

The present study examines the influence of 5-HT$_{1A}$ and 5-HT$_{2A/2C}$ receptor stimulation on the expression of BDNF mRNA in the brain. The results demonstrate that 5-HT$_{2A}$, but not 5-HT$_{1A}$ or 5-HT$_{2C}$, receptors mediate a dramatic, differential regulation of BDNF mRNA expression in neocortex and hippocampus. The results also demonstrate that 5-HT$_{2A/2C}$ receptors mediate, at least in part, the stress-induced downregulation of BDNF mRNA expression in hippocampus. The potential cellular mechanisms mediating the differential regulation of BDNF expression in these brain regions are discussed.

MATERIALS AND METHODS

Animal treatment paradigms. Male Sprague Dawley rats (170–210 gm; CAMM, Wayne, NJ) were group housed and maintained on a 12 hr light/dark cycle with access to food and water *ad libitum*. For treatment with 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), animals were administered 1 mg/kg of 8-OH-DPAT or vehicle (0.9% saline) via an i.p. injection and sacrificed 2 hr after treatment (n = 4). This dose of 8-OH-DPAT was selected so as to activate postsynaptic 5-HT$_{1A}$ receptors (Tricklebank et al., 1984). For treatment with 4-iodo-2,5-dimethoxyphenylisopropylamine (DOI), animals were administered 8 mg/kg of DOI or vehicle (0.9% saline) via an i.p. injection and sacrificed 3 hr after treatment (n = 6). The dose–response experiment had the following treatment groups: vehicle (n = 4), 0.1 mg/kg DOI (n = 3), 0.5 mg/kg DOI (n = 3), and 2 mg/kg of DOI (n = 3). All groups were administered drug or vehicle via an i.p. injection, and animals were sacrificed 2 hr later. For the time course experiment, each time point had a separate vehicle group (n = 3–4) that was sacrificed along with the DOI (2 mg/kg) group. The groups were as follows: 30 min DOI (n = 4), 60 min DOI (n = 4), 120 min DOI (n = 3), and 180 min DOI (n = 4). To examine the effects of chronic treatment with DOI, the vehicle group (n = 6) received 0.9% saline, and the DOI group (n = 6) was administered 2 mg/kg DOI for 7 d and sacrificed 2 hr after the last treatment.

For the receptor antagonists experiments, the drugs were administered (i.p.) 30 min before a 2 mg/kg injection of DOI, and the vehicle group received injections of saline at both time points. The 5-HT$_{2C}$ receptor antagonist 5-methyl-1-(3-pyridylcarbamoyl)−1,2,3,5-tetrahydropyrrolo[2,3-*f*]indole (SB 206553) (Kennett et al., 1996) was administered (15 mg/kg, s.c.) as a suspension in 0.1% methyl cellulose; the 5-HT$_{2A}$ receptor antagonist R-(+)-α-(2,3-dimethoxyphenyl)−1-[2-(4-flurophenylethyl)]−4-piperidine-methanol (MDL 100,907) (Schreiber et al., 1994) was administered (1 mg/kg, i.p.) in saline that was titrated into solution with citric acid. The treatment groups were as follows: experiment 1: vehicle/vehicle (n = 4), vehicle/DOI (n = 4), 5 mg/kg ketanserin/DOI (n = 4); experiment 2: vehicle/vehicle (n = 4), vehicle/DOI (n = 4), 1 mg/kg MDL 100,907/DOI (n = 4), 15 mg/kg SB 206553/DOI (n = 4). All groups were decapitated 2 hr after the last injection. The effects of the antagonists alone were examined by treating animals with either drug or vehicle and decapitation 2 hr later. The treatment groups were as follows: vehicle (0.9% saline, n = 4); 5 mg/kg of ketanserin (n = 4), 1 mg/kg of MDL 100,907 (n = 4), and 15 mg/kg of SB 206553 (n = 4). To study the effect of DOI in the absence of circulating glucocorticoids, animals were subjected to bilateral adrenalectomy (ADX) under sodium pentobarbital anesthesia (50 mg/kg). Sham animals were exposed to the same anesthesia and surgical procedure but without removal of adrenal glands. All animals (sham = 5, ADX = 5, ADX + DOI = 5) were returned to home cages for 1 week. They were then administered vehicle or DOI (8 mg/kg) and decapitated 2 hr later. For the stress experiments, the treatment groups were as follows: vehicle/sham (n = 12), vehicle/stress (n = 12), ketanserin/stress (n = 12). Sprague Dawley rats (250–280 gm) were administered vehicle or ketanserin 30 min before administration of stress; rats were then subjected to immobilization stress in plastic restraint cone bags (Harvard Apparatus) for 2 hr.

After decapitation, the brains were removed and frozen on dry ice and stored at −80° C for *in situ* hybridization analysis. All animal use procedures were in strict accordance with the guidelines of the National Institutes for the Care and Use of Laboratory Animals and were ap-

Table 1. Influence of the 5-HT$_{1A}$ agonist 8-OH-DPAT on BDNF mRNA levels in hippocampus and neocortex

	BDNF mRNA (% of vehicle ± SEM)	
Region	Sham	8-OH-DPAT
Parietal cortex	100 ± 7	101 ± 4
CA1	100 ± 2	97± 5
CA3	100 ± 3	98 ± 4
Dentate gyrus	100 ± 3	97 ± 3

Rats were administered vehicle or 8-OH-DPAT (1 mg/kg), and levels of BDNF mRNA were determined 2 hr later by *in situ* hybridization analysis. Levels of BDNF mRNA were quantified by densitometry. The results are expressed as percent of vehicle and are the mean ± SEM (n = 4).

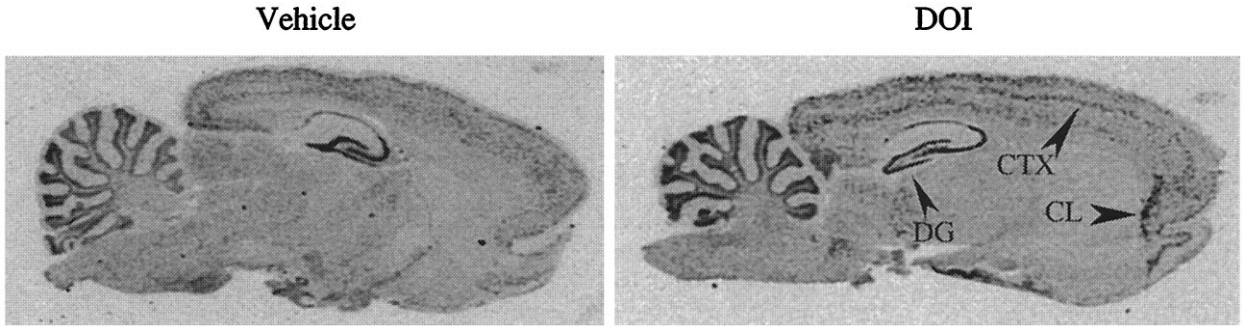

Figure 2. Regulation of BDNF mRNA by DOI. Rats were administered vehicle or DOI (8 mg/kg), and levels of BDNF mRNA were determined by *in situ* hybridization. Representative autoradiographs of sagittal brain sections from vehicle- and DOI-treated groups are shown. *Arrowheads* indicate the decrease in BDNF expression in the dentate gyrus (*DG*) and the induction in neocortex (*CTX*) and claustrum (*CL*). The induction in BDNF mRNA levels in cortex is widespread and seems to be in specific layers.

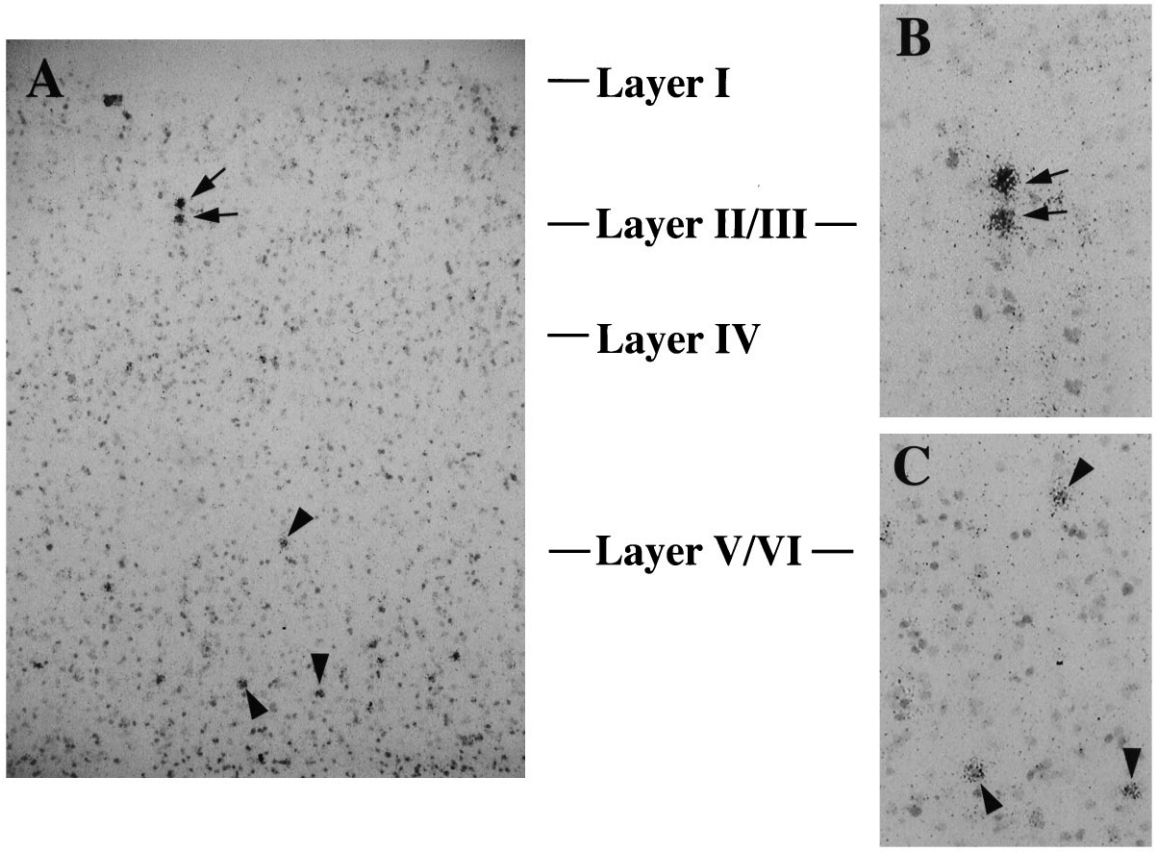

Figure 3. Cellular localization of DOI induction of BDNF mRNA in neocortex. Brain sections from DOI-treated (8 mg/kg) rats were subjected to emulsion autoradiography and then counterstained with cresyl violet. *A*, Note cells within layers II/III and layers V/VI with black silver grains indicating hybridization to BDNF mRNA. Cells specified by *arrows* in layers II/III (*B*) and *arrowheads* in layers V/VI (*C*) are shown at higher magnification.

proved by the Yale Animal Care and Use Committee. The drugs utilized in this study were obtained as follows: DOI and ketanserin tartarate were purchased from Research Biochemicals, Inc. (Natick, MA); MDL 100,907 was a gift from Hoechst Marion Roussel, Inc. (Cincinnati, OH); and SB 206553 was a gift from SmithKline Beecham (Essex, England).

In situ *hybridization. In situ* hybridization for BDNF and trkB mRNA was carried out as described previously (see Nibuya et al., 1995). In brief, coronal sections of 14 μm thickness were cut on the cryostat and thaw mounted onto RNase free Probe-on (+) slides (Fisher). Tissue sections were fixed in 4% formaldehyde, acetylated, and dried. Levels of BDNF and trkB mRNA were examined by probing with ^{35}S-labeled riboprobes (see Nibuya et al., 1995). Rat BDNF and mouse trkB cDNA clones were obtained from Regeneron (Tarrytown, NY) and the National Cancer Institute (Frederick, MD). The sections were hybridized with 2×10^6 cpm/section for 18 hr at 55°C in hybridization buffer (50% formamide, 0.6 M NaCl, 10 mM Tris, 1× Denhardt's solution, 2 mM EDTA, 10 mM DTT, 10% dextran sulfate, 50 μg/ml salmon sperm DNA, 250 mg/ml tRNA). After hybridization, sections were washed in 2× SSC (0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) at 25°C and then treated with 20 μg/ml RNase A for 30 min in RNase buffer (0.5 M NaCl, 10 mM Tris, 1 mM EDTA). The sections were then washed for 10 min in 2× SSC at room temperature and twice for 20 min in 0.2× SSC at 55°C. The sections were then rinsed in 0.2× SSC, dried, and exposed to Hyperfilm (Amersham) for 7–14 d. ^{35}S-labeled sense riboprobes for BDNF and trkB did not yield any significant hybridization (not shown), indicating that the signal observed with BDNF and trkB antisense riboprobes are specific.

Quantitation and data analysis. Levels of BDNF and trkB mRNA were analyzed using the Macintosh-based National Institutes of Health Image

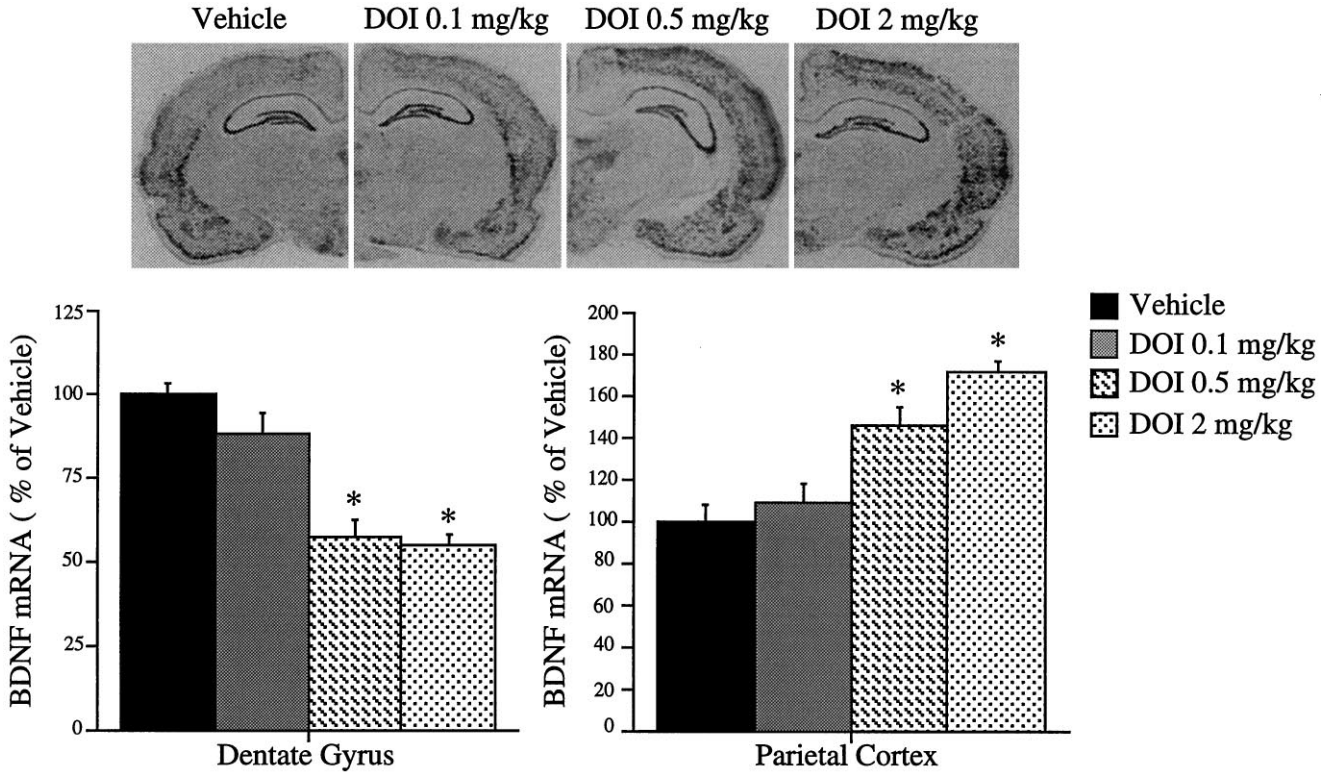

Figure 4. Regulation of BDNF mRNA by different doses of DOI. Rats were administered vehicle or different doses of DOI from 0.1 to 2.0 mg/kg, and levels of BDNF mRNA were determined by *in situ* hybridization. Representative autoradiographs from each treatment group are shown. The results are expressed as percent of vehicle and are the mean ± SEM (n = 3–4). *$p < 0.05$ compared with vehicle (ANOVA; Newmann–Keuls *post hoc* test).

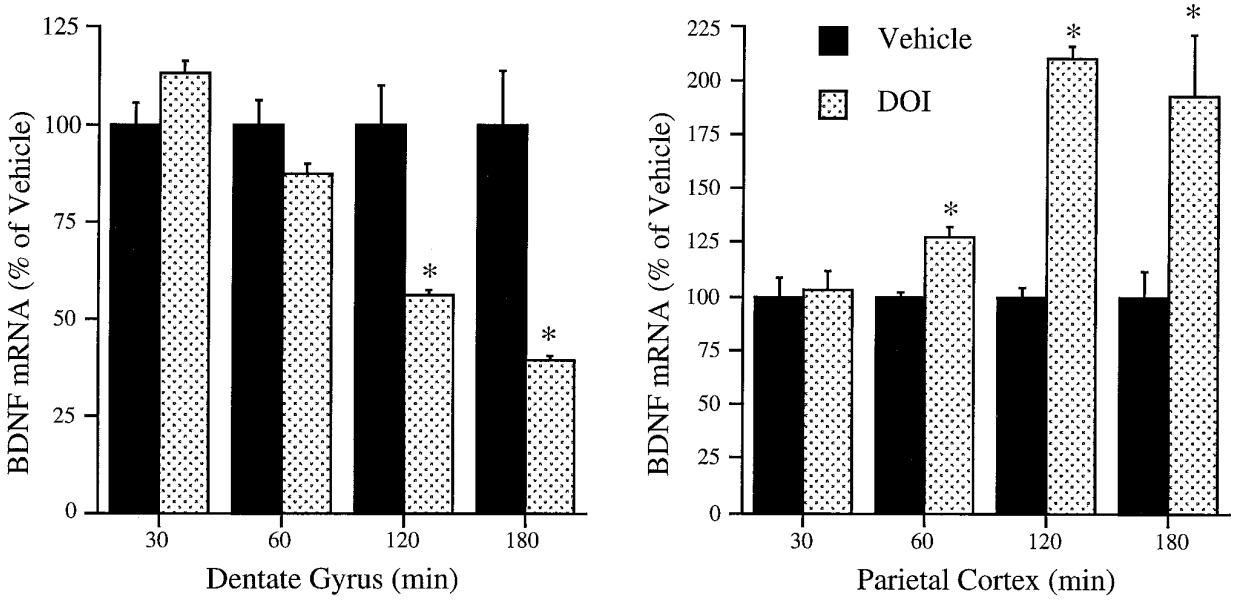

Figure 5. Time-dependent regulation of BDNF mRNA by DOI. Rats were administered vehicle or DOI (2 mg/kg), and levels of BDNF mRNA were determined at different times as indicated by *in situ* hybridization. Each time point had a separate vehicle treatment group. The results are expressed as percent of vehicle and are the mean ± SEM (n = 4). *$p < 0.05$ compared with vehicle (Student's t test).

program, version 1.57. The regions that were analyzed for *in situ* hybridization were parietal cortex, piriform cortex, dentate gyrus granule cell layer, and CA1 and CA3 pyramidal cell layers. These regions were analyzed by outlining the area of interest; an equivalent area was outlined for each sample. For each animal, the optical density measurements from both sides of 3–4 individual sections were analyzed, yielding 6–8 determinations, from which the mean was calculated. To correct for nonlinearity, ^{14}C step standards were used for calibration. To determine cellular localization, some sections were dipped in emulsion (Kodak, NTB-2) and developed with D-19 developer after 3–4 weeks. These sections were stained with cresyl violet to visualize location of the silver grains.

Results were then subjected to statistical analysis. Experiments with two groups were analyzed for differences using the unpaired Student's t test, with significance determined at $p < 0.05$. Experiments with three or

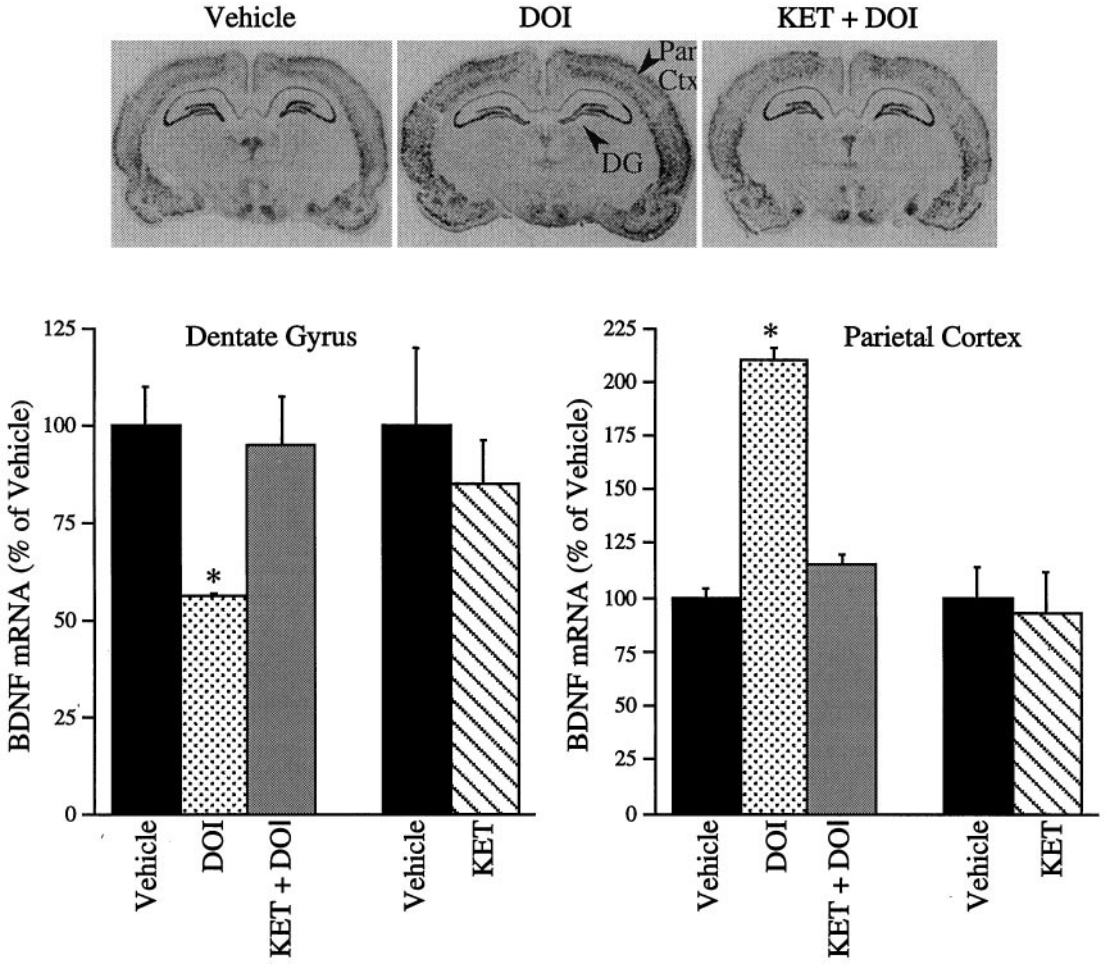

Figure 6. Influence of ketanserin (*KET*) pretreatment on the DOI regulation of BDNF mRNA. Ketanserin (5 mg/kg) was administered 30 min before DOI (2 mg/kg), and levels of BDNF mRNA were determined 2 hr later by *in situ* hybridization. In a separate experiment, the influence of ketanserin (5 mg/kg) alone was examined. Representative autoradiographs from the vehicle-, DOI-, and KET + DOI-treated groups are shown. Results are expressed as percent of vehicle and are the mean ± SEM ($n = 4$). $^*p < 0.05$ compared with vehicle (ANOVA; Newmann–Keuls *post hoc* test).

more groups were subjected to ANOVA, followed by the *post hoc* Newmann–Keuls test with a significance level of $p < 0.05$.

RESULTS

Influence of 5-HT$_{1A}$ and 5-HT$_{2A/2C}$ receptor agonists on BDNF mRNA expression in the hippocampus and neocortex

The effect of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT on the expression of BDNF mRNA was examined by *in situ* hybridization analysis. Treatment with 8-OH-DPAT did not significantly regulate BDNF mRNA levels in the brain regions examined, including the hippocampus and the neocortex (Table 1). The hallucinogenic phenylalkylamine DOI, a 5-HT$_{2A/2C}$ receptor agonist (Shannon et al., 1984), was utilized to characterize the role of 5-HT$_2$ receptors in the regulation of BDNF expression. Administration of DOI led to a dramatic, differential regulation of BDNF mRNA in hippocampus and neocortex. DOI significantly decreased BDNF mRNA levels within the dentate gyrus region of the hippocampus (Fig. 1). Levels of BDNF mRNA expression within the CA1 and CA3 regions were not influenced by DOI. In contrast, in parietal cortex DOI significantly increased levels of BDNF mRNA (Fig. 1). The upregulation in BDNF mRNA was also seen within other regions of cerebral cortex, including frontal and temporal cortex, and in a subcortical area, the claustrum (Fig. 2). The induction of BDNF mRNA by DOI in parietal cortex was observed in layers II/III and layers V/VI (Fig. 3). Levels of BDNF mRNA in piriform cortex were not influenced significantly. The effect of DOI on trkB, the receptor for BDNF, was also examined. There was no regulation of trkB mRNA in either neocortex or hippocampus after acute administration of DOI (data not shown).

The dose- and time-dependent effects of DOI on the expression of BDNF were examined next. The downregulation of BDNF mRNA within the dentate gyrus of the hippocampus reached a near maximal response with a DOI dose of 0.5 mg/kg, whereas the same dose resulted in a half maximal induction of BDNF mRNA in neocortex (Fig. 4). The regulation of BDNF mRNA by DOI was time dependent and reached a maximal or near maximal response at 2 hr in both the parietal cortex and dentate gyrus granule cell layer (Fig. 5). The influence of repeated DOI (seven daily treatments) on the expression of BDNF mRNA was also examined. Although there was a tendency for levels of BDNF mRNA to be decreased in hippocampus and increased in parietal cortex, these effects were not significant after repeated treatment [86 ± 4, 112 ± 4% of control hippocampus and neocortex, respectively (mean ± SEM); $n = 6$]. This could result from the downregulation of 5-HT$_2$ receptors in response to chronic agonist treatment.

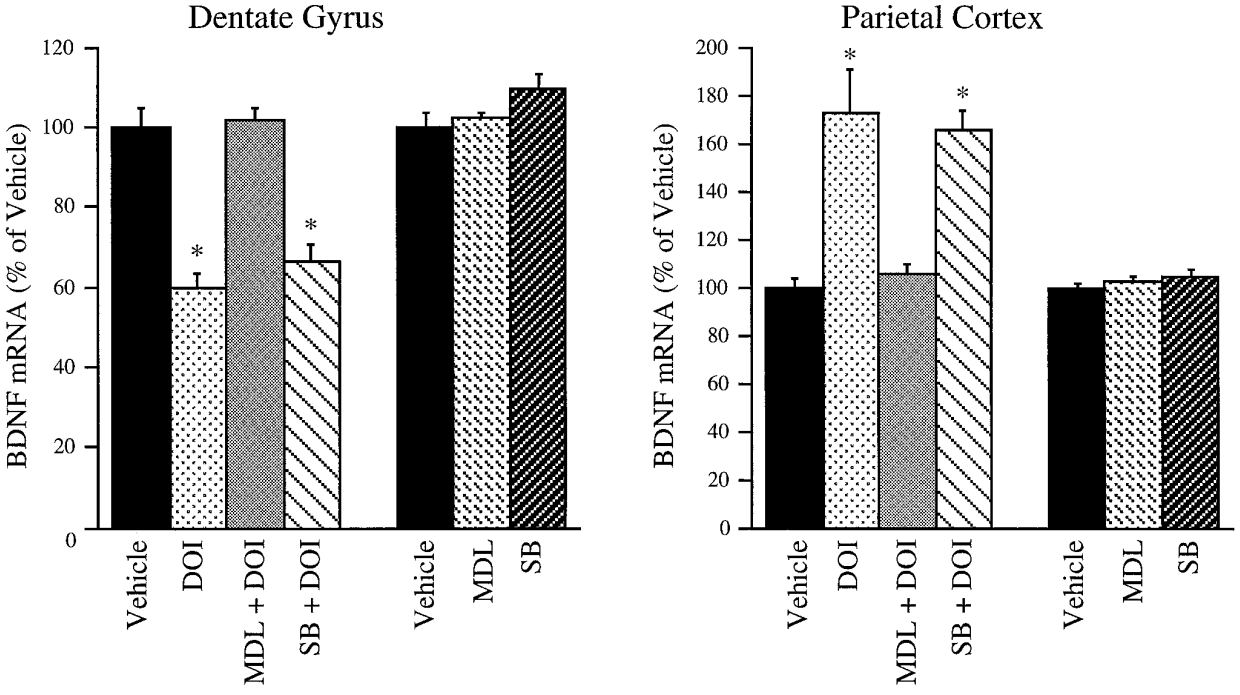

Figure 7. Influence of MDL 100,907 (*MDL*) or SB 206553 (*SB*) pretreatment on DOI regulation of BDNF mRNA. Rats were pretreated (30 min) with vehicle, MDL 100,907 (1 mg/kg), or SB 206553 (15 mg/kg) before administration of DOI (2 mg/kg), and levels of BDNF mRNA were determined 2 hr later by *in situ* hybridization. In a separate experiment, rats were administered vehicle, MDL 100,907, and SB 206553, and levels of BDNF mRNA were determined 2 hr later. Results are expressed as percent of vehicle and are the mean ± SEM ($n = 4$). $^*p < 0.05$ compared with vehicle (ANOVA; Newmann–Keuls *post hoc* test).

Regulation of BDNF mRNA by DOI is mediated via the 5-HT$_{2A}$ receptor subtype

To study the pharmacological specificity of DOI regulation of BDNF mRNA in hippocampus and neocortex, selective 5-HT receptor antagonists were examined. Pretreatment with the 5-HT$_{2A/2C}$ antagonist ketanserin, which has a 30-fold selectivity for the 5-HT$_{2A}$ receptor over the 5-HT$_{2C}$ receptor (Sanders-Bush and Breeding, 1991), before administration of DOI completely blocked DOI regulation of BDNF mRNA in both the hippocampus and parietal cortex (Fig. 6). Administration of ketanserin alone did not influence basal expression of BDNF mRNA in either region (Fig. 6). Selective 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor antagonists were also examined. MDL 100,907 has a 100-fold greater affinity for the 5-HT$_{2A}$ than the 5-HT$_{2C}$ receptor subtype (Kehne et al., 1996). Pretreatment with MDL 100,907 completely blocked DOI regulation of BDNF mRNA in the dentate gyrus and the parietal cortex (Fig. 7). SB 206553 has a >100-fold selectivity for the 5-HT$_{2C}$ than the 5-HT$_{2A}$ receptor (Forbes et al., 1995, Kennett et al., 1996). Pretreatment with SB 206553 did not block DOI regulation of BDNF mRNA expression in either brain region (Fig. 7). BDNF mRNA levels were not regulated by administration of MDL 100,907 or SB 206553 alone (Fig. 7). These findings indicate that DOI regulation of BDNF mRNA in the hippocampus and the neocortex is mediated via activation of the 5-HT$_{2A}$ receptor subtype.

Regulation of BDNF mRNA expression by DOI does not involve the hypothalamic-pituitary-adrenocortical (HPA) axis

DOI is reported to cause a dose-dependent activation of the HPA axis, including elevated levels of circulating glucocorticoids (Rittenhouse et al., 1994; Welch and Saphier, 1994). To examine the possibility that DOI regulation of BDNF mRNA is mediated via activation of the HPA axis, the influence of adrenalectomy was examined. Adrenalectomy did not influence DOI regulation of BDNF mRNA in either the dentate gyrus or the parietal cortex (Fig. 8). In this experiment, DOI regulation of BDNF mRNA in the dentate gyrus, and parietal cortex was similar to that observed in intact animals. ADX alone did not significantly regulate the expression of BDNF mRNA in either brain region.

5-HT$_{2A/2C}$ receptors are involved in the downregulation of BDNF mRNA induced by immobilization stress

Immobilization stress has been shown to downregulate BDNF mRNA expression in the hippocampus (Smith et al., 1995), an effect similar to the DOI regulation of BDNF mRNA in hippocampus observed in the present study. In addition, previous studies have demonstrated that immobilization stress causes an increase in 5-HT release and turnover in the hippocampus (Joseph and Kennett, 1983; Tanaka et al., 1983; Richardson, 1984; Chauloff, 1993; Vahabzadeh and Fillenz, 1994). To examine the possibility that 5-HT$_{2A/2C}$ receptors mediate the stress-induced downregulation of BDNF mRNA levels, the influence of ketanserin on this effect was examined. Pretreatment with ketanserin lead to a partial but highly significant blockade of the stress-induced downregulation of BDNF mRNA in the dentate gyrus granule cell layer (Fig. 9). In the CA3 pyramidal cell layer, stress resulted in a small, nonsignificant downregulation of BDNF mRNA, although the level of BDNF mRNA in the stress group was significantly different from that in the ketanserin plus stress group (Fig. 9). The results indicate that the 5-HT$_{2A/2C}$ receptor mediates, at least in part, the effects of stress on BDNF mRNA levels in the hippocampus. Although the combined results indicate a significant blockade of the stress-induced downregulation

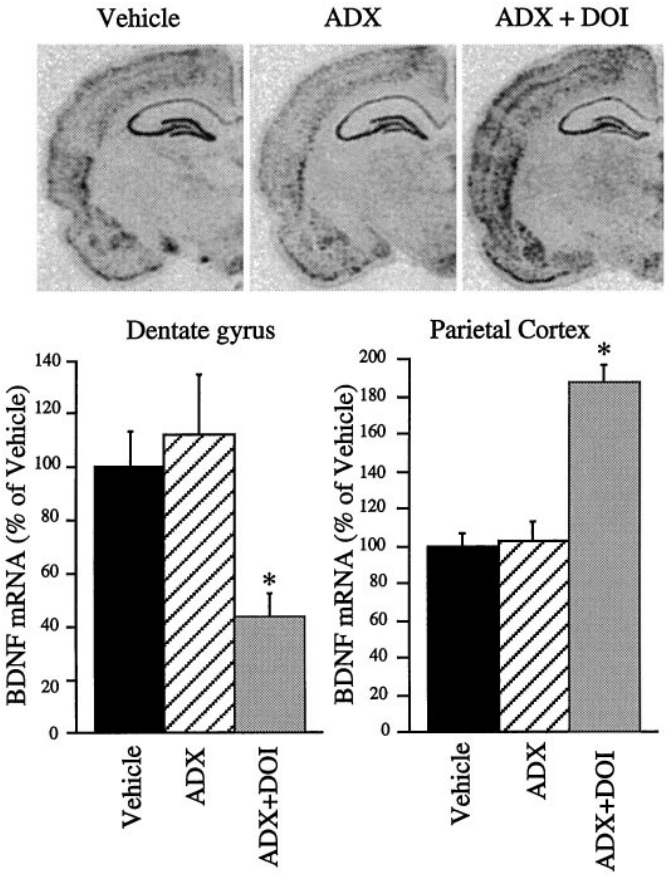

Figure 8. Influence of adrenalectomy on DOI regulation of BDNF mRNA. Rats underwent sham surgery or bilateral adrenalectomy (*ADX*). Seven days later, animals received vehicle or DOI (8 mg/kg), and levels of BDNF mRNA were determined by *in situ* hybridization. Representative autoradiographs for the different treatment groups are shown. Results are expressed as percent of vehicle and are the mean ± SEM ($n = 5$). $^{*}p < 0.05$ compared with vehicle (ANOVA; Newmann–Keuls *post hoc* test).

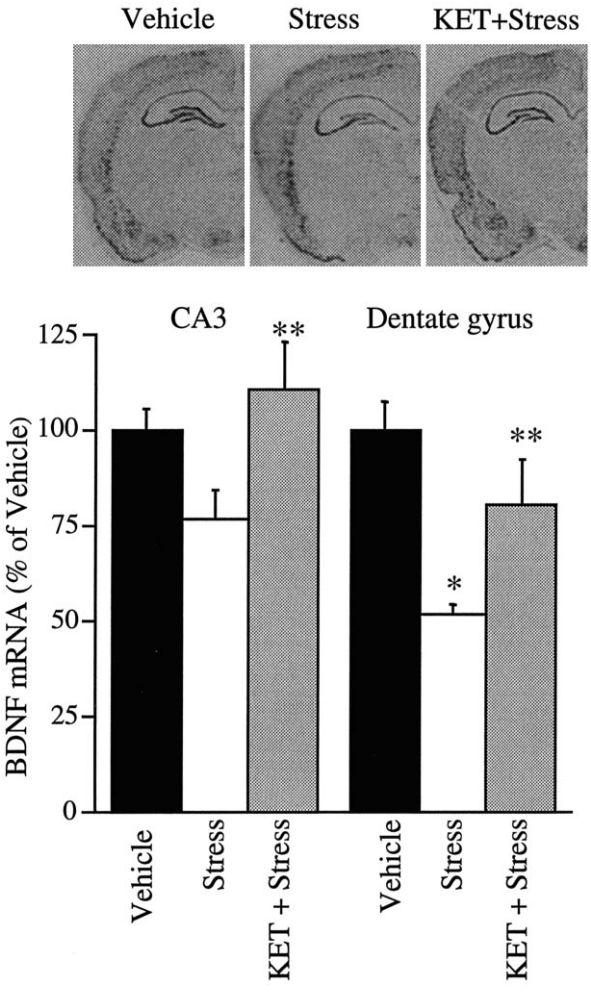

Figure 9. Influence of ketanserin (*KET*) on the stress-induced downregulation of BDNF mRNA. Rats were pretreated with vehicle or ketanserin (5 mg/kg) 30 min before being subjected to immobilization stress (2 hr). Levels of BDNF mRNA were determined immediately after stress by *in situ* hybridization. Representative autoradiographs from the different treatment groups are shown. Results are expressed as percent of vehicle and are the mean ± SEM ($n = 12$). $^{*}p < 0.05$ compared with vehicle; $^{**}p < 0.05$ compared with stress (ANOVA; Newmann–Keuls *post hoc* test).

of BDNF mRNA, interexperimental variability was observed. Ketanserin was found to completely or partially block the stress-induced decrease in BDNF mRNA levels in the different experiments conducted. The reason for this variability is not known but may be related to the previous unknown exposure of animals to stress, or other factors that influence the stress response. Because of this variability, a further characterization of the 5-HT$_2$ receptor subtypes that mediate the stress effect will require an extended series of experiments.

DISCUSSION

The results of this study demonstrate that administration of 8-OH-DPAT, a 5-HT$_{1A}$ receptor agonist, did not influence the expression of BDNF in any of the brain regions examined. However, 8-OH-DPAT is also an agonist of the 5-HT$_7$ receptor (Lovenberg et al., 1993) and could thereby oppose the actions of the 5-HT$_{1A}$ receptor via its opposing effects on the cAMP system. Additional studies with more selective 5-HT$_{1A}$ and 5-HT$_7$ receptor drugs are needed to further characterize the role of these receptors in regulation of BDNF expression. In contrast, administration of DOI, a 5-HT$_{2A/2C}$ receptor agonist, differentially regulated the expression of BDNF mRNA in cerebral cortex and hippocampus. The induction of BDNF mRNA by DOI was observed in different regions of neocortex, including the frontal, parietal, and temporal cortex, but was absent in the piriform cortex, a part of paleocortex. Within the hippocampus, the DOI-induced downregulation of BDNF mRNA was observed in the granule cell layer of the dentate gyrus, but not in the CA1 or CA3 pyramidal cell layers. The effects of DOI in both regions were dose and time dependent, and repeated administration of the agonist resulted in desensitization of the BDNF mRNA response in both hippocampus and neocortex. This probably results from downregulation of 5-HT$_2$ receptors after chronic agonist treatment (Buckholtz et al., 1988; Eison et al., 1989; Leysen et al., 1989).

The neocortical localization of DOI-induced upregulation of BDNF mRNA, which is observed in layers II/III and layers V/VI, is similar to the distribution of the 5-HT$_{2A}$ receptor subtype (Mengod et al., 1990; Pompeiano et al., 1994; Wright et al., 1995). In addition, the 5-HT$_{2A}$ receptor subtype is the most abundant 5-HT receptor in neocortex, whereas expression of the 5-HT$_{2C}$ receptor subtype is relatively low in neocortical areas (Wright et al., 1995). The possibility that the 5-HT$_{2A}$ receptor subtype mediates the DOI induction of BDNF mRNA is supported by the

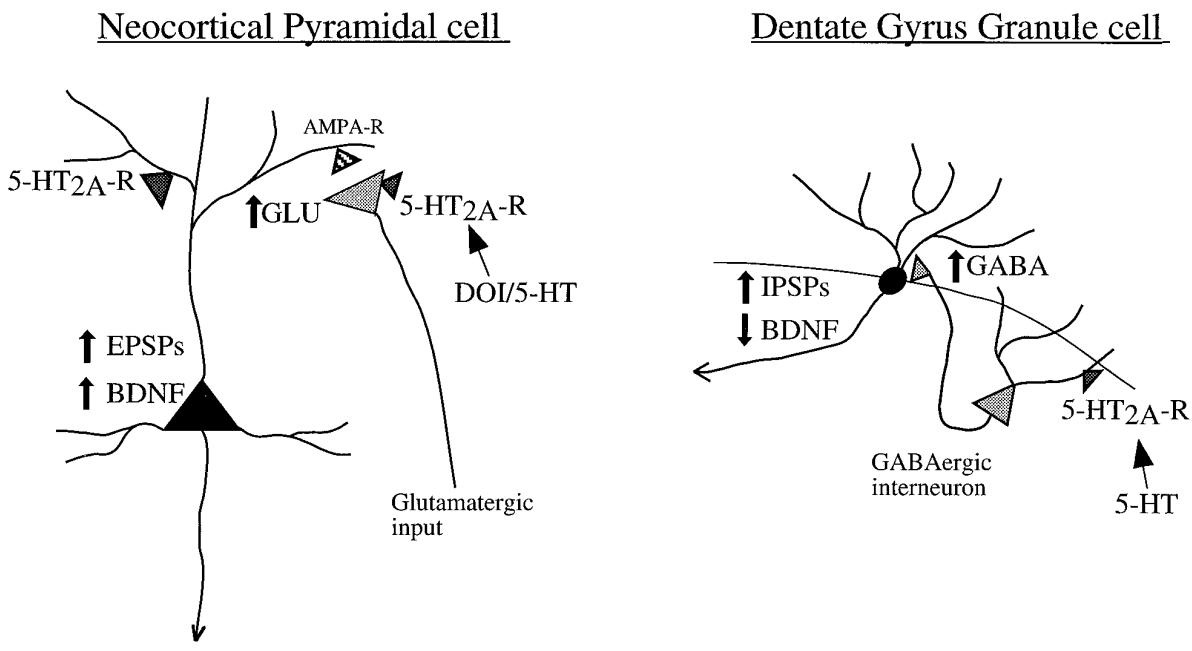

Figure 10. Cellular models for 5-HT$_{2A}$ receptor regulation of BDNF mRNA in neocortex and hippocampus. Within the neocortex, application of 5-HT/DOI has been shown to induce EPSPs in layer V pyramidal neurons via activation of 5-HT$_{2A}$ receptors (*5-HT$_{2A}$-R*). The increase in EPSPs is a result of increased release of glutamate (*GLU*) and activation of AMPA receptors (*AMPA-R*) and can be blocked by the selective AMPA receptor antagonist LY 293558. This suggests that the upregulation of BDNF mRNA by DOI may be mediated via a release of glutamate and an increase in neuronal activity. In contrast, DOI treatment leads to a decrease in levels of BDNF mRNA in the dentate gyrus granule cell layer of the hippocampus. This could be mediated by activation of 5-HT$_{2A}$ receptors located on GABAergic interneurons. Activation of 5-HT$_{2A}$ receptors increases the firing rate of GABAergic interneurons and thereby increases IPSPs in the granule cells. The increased inhibitory control of dentate gyrus granule cells could lead to downregulation of BDNF mRNA.

results of the receptor antagonist studies. Pretreatment with ketanserin, a 5-HT$_{2A/2C}$ receptor antagonist, or MDL 100,907, a selective 5-HT$_{2A}$ receptor antagonist, completely blocked DOI regulation of BDNF mRNA in both the neocortex and hippocampus. In contrast, pretreatment with SB 206553, a selective 5-HT$_{2C}$ receptor antagonist, did not significantly influence DOI regulation of BDNF mRNA in either brain region.

The mechanism by which activation of the 5-HT$_{2A}$ receptors increases the expression of BDNF mRNA in neocortex may be explained by electrophysiological studies. Bath application of 5-HT to cerebral cortical slices has been shown to increase EPSPs in pyramidal neurons of the neocortex (Marek and Aghajanian, 1996), and this effect is mimicked by application of DOI (G. J. Marek and G. K. Aghajanian, personal communication). In addition, the EPSPs induced by 5-HT are blocked by MDL 100,907 and are observed in the same population of pyramidal neurons (layer V) as the elevation of BDNF mRNA in response to DOI. The increase in EPSPs has been shown to be a result of the release of glutamate and can be blocked by an AMPA receptor antagonist. This is consistent with previous reports that the activity-dependent induction of BDNF mRNA in cerebral cortex is mediated by glutamate (Zafra et al., 1990; Ernfors et al., 1991; Lindholm et al., 1994). Taken together, the results suggest that the upregulation of BDNF mRNA by DOI is mediated via the presynaptic release of glutamate and the activation of AMPA receptors (Fig. 10). This hypothesis is currently being tested by examining the influence of an AMPA receptor antagonist on DOI induction of BDNF mRNA.

DOI also upregulates BDNF mRNA in other areas of neocortex in addition to parietal cortex, such as frontal and temporal cortex. In contrast, levels of BDNF mRNA in piriform cortex, a part of paleocortex, are not influenced by DOI. In piriform cortex, DOI activation of 5-HT$_{2A}$ receptors has been shown to induce IPSCs in the pyramidal neurons through the excitation of GABAergic interneurons (Sheldon et al., 1990, 1991; Marek et al., 1994). This inhibitory effect on GABAergic interneurons seems to balance any stimulatory effect of DOI on 5-HT$_{2A/2C}$ receptors located postsynaptically on the pyramidal cells and may explain the absence of regulation of BDNF mRNA by DOI in this brain region.

The mechanism responsible for the downregulation of BDNF mRNA in the dentate gyrus granule cell layer by DOI may also be explained by electrophysiological and receptor distribution studies. Serotonergic inputs from the dorsal raphe are thought to exert a global control over the hippocampus via modulation of local inhibitory interneurons (Freund et al., 1990). The 5-HT$_{2A}$ receptors are expressed at relatively high levels on the GABAergic interneurons and hilar cells, but are present at lower levels in the granule cell layer of hippocampus (Pompeiano et al., 1994; Wright et al., 1995). The 5-HT$_{2C}$ receptor is distributed lightly through the principal layers, with dense distribution in the temporal hippocampus, CA1, and subiculum (Pompeiano et al., 1994; Wright et al., 1995). Activation of the 5-HT$_{2A}$ receptors on GABAergic interneurons is thought to increase spontaneous GABA release and lead to an increased inhibitory control of dentate granule cells (Piguet and Galvan, 1994). Because stimulation of the GABAergic system leads to a decrease in levels of BDNF mRNA, the effects of DOI might be mediated via its activation of 5-HT$_{2A}$ receptors on GABAergic interneurons (Fig. 10).

Levels of 5-HT in the hippocampus are increased in response to several different types of stress, including immobilization (Joseph and Kennett, 1983; Richardson, 1984; Chauloff, 1993). Recent

studies have demonstrated that stress decreases the expression of BDNF in hippocampus, particularly in the dentate gyrus granule cell layer (Smith et al., 1995). The downregulation of BDNF by stress is not blocked by adrenalectomy, indicating that factors other than adrenal glucocorticoids mediate this effect. Considering our findings with DOI, we hypothesized that the influence of stress on BDNF expression is mediated by 5-HT_{2A} receptor activation of GABAergic interneurons. This possibility is supported by the results of the receptor antagonist studies, which demonstrate that pretreatment with ketanserin significantly blocks the downregulation of BDNF mRNA in response to stress. However, ketanserin did not completely reverse the stress effect, and the response to ketanserin varied between experiments. Therefore, additional studies are required to further characterize the 5-HT_2 receptor subtype that mediates this effect, and to examine the potential role of other neurotransmitter receptors in the stress response.

BDNF is known to influence the survival and function of neurons in the brain, including 5-HT neurons (Mamounas et al., 1995; Celada et al., 1996). In addition, BDNF has recently been demonstrated to acutely influence the synaptic efficacy of neurons. Several electrophysiological studies have demonstrated that application of BDNF to hippocampal slices results in increased synaptic strength, and endogenous BDNF has been implicated in formation of long-term potentiation, a cellular model of learning and memory (Kang and Schuman, 1995; Korte et al., 1995, Levine et al., 1995; Figurov et al., 1996; Patterson et al., 1996). Thus, it is conceivable that the effects of BDNF on neuronal survival and function, as well as synaptic efficacy, could contribute to the actions of drugs known to influence 5-HT_{2A} receptors. Upregulation of BDNF in neocortex by certain antidepressant treatments could be mediated, at least in part, by 5-HT_{2A} receptors (Nibuya et al., 1995), although the induction observed in hippocampus seems to involve other 5-HT and monoamine receptor subtype(s) (Nibuya et al., 1995, 1996). Induction of BDNF, through its positive effects on target neurons in neocortex, as well as on serotonergic neurons, could play a role in the therapeutic actions of antidepressants. In cerebral cortex, the enhanced sensory perception that is observed in response to hallucinogenic compounds could also be mediated, in part, by induction of BDNF (Abraham et al., 1996). Further studies will be required to determine the acute and long-term cellular and behavioral effects of antidepressants and hallucinogenic drugs that are related to the regulation of BDNF in cerebral cortex, as well as hippocampus.

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