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A MAJOR ROLE FOR THALAMOCORTICAL AFFERENTS IN SEROTONERGIC HALLUCINOGEN RECEPTOR FUNCTION IN THE RAT NEOCORTEX

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Abstract—Activation of 5-hydroxytryptamine$_{2A}$ (5-HT$_{2A}$) receptors by hallucinogenic drugs is thought to mediate many psychotomimetic effects including changes in affect, cognition and perception. Conversely, blockade of 5-HT$_{2A}$ receptors may mediate therapeutic effects of many atypical antidepressant and antipsychotic drugs. The purpose of the present study was to determine the source of subcortical glutamatergic afferents, which would project widely throughout the anterior–posterior axis of the rat brain to the apical dendrites of layer V pyramidal cells of the medial prefrontal cortex, from which serotonin induces transmitter release via activation of 5-HT$_{2A}$ receptors. Fiber-sparing chemical lesions of the medial thalamus selectively decreased the frequency of serotonin-induced excitatory postsynaptic currents recorded from layer V pyramidal cells in the prelimbic region of the medial prefrontal cortex by 60%. In contrast, large bilateral lesions of the amygdala did not alter the serotonin response. These thalamic lesions significantly decreased the amount of binding to either μ-opioid or metabotropic glutamate 2/3 receptors in the prelimbic region of the medial prefrontal cortex as expected from previous evidence that these agonists for these receptors suppress serotonin-induced excitatory postsynaptic currents by a presynaptic mechanism. Surprisingly, the amount of specific binding to cortical 5-HT$_{2A}$ receptors was significantly increased by the medial thalamic lesions.

Thus, these experiments demonstrate that activation of cortical 5-HT$_{2A}$ receptors modulates transmitter release from thalamocortical terminals. Unexpectedly, lesioning the thalamocortical terminals also alters 5-HT$_{2A}$ receptor binding in the prefrontal cortex. These findings are of interest with respect to understanding therapeutic effects of antidepressant/antipsychotic drugs and the known behavioral effects of thalamic lesions in humans.

Key words: 5-HT$_{2A}$ receptors, intralaminar and midline thalamic nuclei, central medial thalamic nucleus, thalamocortical terminals, prefrontal cortex, metabotropic glutamate receptors.

Activation of 5-hydroxytryptamine$_{2A}$ (5-HT$_{2A}$) receptors by psychedelic hallucinogens appears to mediate the psychotomimetic effects of these drugs (Glennon, 1990; Vollenweider et al., 1998), which include alterations in affect, cognition and perception. Conversely, blockade of 5-HT$_{2A}$ receptors by certain antidepressants (e.g., mirtazepine, mianserin, nefazodone, and trazodone) and atypical antipsychotic drugs (e.g., clozapine, risperidone and olanzepine) may contribute to their therapeutic efficacy. The neocortex, which expresses one of the highest densities of 5-HT$_{2A}$ receptors in the brain, is certainly involved in mediating the psychotropic effects of drugs acting on 5-HT$_{2A}$ receptors. The clear laminar distribution of cortical 5-HT$_{2A}$ receptors also suggests a modulation of specific cellular elements (Blue et al., 1988). At a cellular level, 5-HT$_{2A}$ receptors are richly expressed in pyramidal cells and subpopulations of GABAergic (Jakab and Goldman-Rakic, 1998, 2000; Willins et al., 1997). 5-HT$_{2A}$ receptors are heavily expressed on the proximal apical dendrites of layer V pyramidal cells and chronic treatment with atypical antipsychotic and antidepressant drugs results in a redistribution of 5-HT$_{2A}$ receptors from the apical dendrite to the soma intracellular store (Willins et al., 1999).

Recent work has suggested an additional presynaptic influence of 5-HT$_{2A}$ receptors in the neocortex. We have found a dramatic increase in the frequency of impulse-independent (e.g., spontaneous) 5-HT-induced excitatory postsynaptic currents (EPSCs) on the proximal apical dendrite of layer V pyramidal cells throughout the neocortex (Aghajanian and Marek, 1997). While tetrodotoxin blocked the 5-HT-induced EPSCs, microiontophoretic application of 5-HT to the apical dendrites induced EPSCs within seconds, a time course that is inconsistent

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate; ANOVA, analysis of variance; DAMGO, [D-Ala^2,*N*-Me-Phe^4,Gly-ol]enkephalin; Cg, cingulate cortex; DOI, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride; EPSCs, excitatory postsynaptic currents; 5-HT, 5-hydroxytryptamine; IPSCs, inhibitory postsynaptic currents; LY354740, (1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid; mPFC, medial prefrontal cortex; mGlu, metabotropic glutamate; n., nucleus; NMDA, *N*-methyl-D-aspartate.

with the induction of impulse activity in cortical neurons by 5-HT. In addition, the layer V pyramidal cells do not appear to be a candidate for the source of glutamate release through an increase in impulse activity since these layer V pyramidal cells do not fire action potentials in response to bath application of 5-HT in slices from adult rats. Furthermore, extracellular recording from the microiontophoretic electrode in these experiments failed to detect nearby neurons firing as a result of the focal 5-HT application. Thus, these EPSCs appear to be due to a focal effect on glutamatergic axons/terminals that requires the function of sodium channels in the presynaptic and/or the postsynaptic membrane. These EPSCs also appear to directly reflect the activation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors on the pyramidal cell by excitatory amino acids released subsequent to activation of 5-HT_{2A} receptors (Marek and Aghajanian, 1999; Zhang and Marek, 2000). Anatomical evidence has also suggested that 5-HT_{2A} receptors are present in axons (Cornea-Hebert et al., 1999) and terminals (Jakab and Goldman-Rakic, 1998) making asymmetric synapses in the neocortex. Furthermore, activation of presynaptic metabotropic glutamate (mGlu)2/3 and μ-opioid receptors suppresses the 5-HT-induced EPSCs (Marek and Aghajanian, 1998; Marek et al., 2000). These observations are consistent with the model that 5-HT induces excitatory amino acid release from a subset of afferents to the neocortical pyramidal cells.

Given that μ-receptor mRNA expression within intrinsic glutamatergic cells is extremely low in the neocortex (Mansour et al., 1994b), activation of μ-receptors may suppress 5-HT-induced EPSCs in terminals originating from a subcortical site. The thalamus and amygdala, which contain significant expression of μ-receptor mRNA, are the primary source of subcortical glutamatergic afferents innervating the entire neocortex. We made lesions of the medial thalamus and amygdala, and examined the frequency of 5-HT-induced EPSCs from intracellular recordings of layer V pyramidal cells in the medial prefrontal cortex (mPFC). In addition, since there is a laminar overlap in the peak density of 5-HT_{2A}, mGlu2 and μ-opioid receptors in layers I and Va (Blue et al., 1988; Marek et al., 2000; Ohishi et al., 1997; Tempel and Zukin, 1987), we also examined radioligand binding in this cortical region following lesions that effectively decreased the frequency of 5-HT-induced EPSCs. Therefore, the aim of the present study was to provide evidence for modulation by 5-HT_{2A} receptors of a subcortical–cortical pathway.

EXPERIMENTAL PROCEDURES

The animals for both the electrophysiological studies and the autoradiographical studies were treated in adherence to protocols approved by the Yale University Animal Care and Use Committee and in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised, 1996). All efforts were made to minimize both the number of animals used and their suffering.

Electrophysiology

Brain slices were prepared from male Sprague–Dawley rats (200–250 g, Harlan, Indianapolis, IN, USA) as described previously (Aghajanian and Rasmussen, 1989). The rats had all been exposed to intracranial surgery, and differed only according to the placement of a thalamic or amygdaloid lesion. Briefly, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and decapitated. Coronal slices (500 μm) were cut with a oscillating-blade tissue slicer at a level corresponding to approximately 2.5 mm anterior to bregma (Paxinos and Watson, 1986). A slice containing the mPFC was then transferred to the stage of a fluid–gas interface chamber which had a constant flow of humidified 95% O_2, 5% CO_2. The slices were perfused in a chamber heated to 34°C with normal artificial cerebrospinal fluid which consisted of (in mM) NaCl 126; KCl 3; $CaCl_2$ 2; $MgSO_4$ 2; $NaHCO_3$ 26; NaH_2PO_4 1.25; D-glucose 10.

Intracellular recording and single-electrode voltage clamping were conducted in layer V pyramidal cells using an Axoclamp-2A (Axon Instruments, Burlingame, CA, USA) as previously described (Aghajanian and Marek, 1997). Stubby electrodes (~8 mm, shank to tip) with relatively low capacitance and resistance (30–60 MΩ) were filled with 1 M potassium acetate. The cells were voltage-clamped at −70 mV. The EPSCs recorded under these conditions do not appear to be contaminated by reversed inhibitory postsynaptic currents (IPSCs) for the following reasons. Only a small fraction of 5-HT-induced EPSCs (~15%) are blocked by bicuculline during intracellular recordings using KCl-containing electrodes suggesting the presence of some reverse IPSCs. In contrast, the 5-HT-induced EPSCs recorded with non-chloride-containing electrodes (i.e., potassium acetate or gluconate) at holding potentials near E_{Cl}, are completely blocked by the AMPA/kainate antagonist LY293558 (Aghajanian and Marek, 1997). The voltage-clamp signals were low-pass filtered (1000 Hz) and data were acquired with a pCLAMP/Digidata 1200 system (Axon Instruments). EPSC frequencies were obtained from 10 successive episodes (1 s duration) during the baseline and drug treatment periods. We have previously obtained essentially identical results in determining the effects of drugs on the frequency of EPSCs obtained by intracellular recording with sharp electrodes compared to whole cell recordings (Aghajanian and Marek, 1997, 1999; Marek and Aghajanian, 1998, 1999).

EPSC frequency and amplitude were determined with a 'Mini Analysis Program' (Synaptosoft, www.synaptosoft.com), using thresholds of 10 pA and an area of ~150 fC/s for synaptic currents. This analysis program includes an algorithm for detecting multiple and complex peaks that occur with high frequency events. The algorithm adjusts the baseline of closely occurring peaks by the method of exponential extrapolation of decay. The mean (±S.E.M.) EPSC frequency and amplitude under basal conditions, 5-HT, and AMPA were calculated for each animal ($n = 2$–5 cells). Slices were obtained on successive days from animals in the two treatment conditions to minimize variability. However, potential seasonal variation was not controlled with respect to thalamic- vs. amygdala-lesioned rats. The data for the two different lesion groups were analyzed with a two-tailed Student's *t*-test.

Autoradiography

Whole brains were obtained from adult male Sprague–Dawley rats (200–250 g), rapidly frozen in powdered dry ice and mounted on cryostat chucks. The rats had all been exposed to intracranial surgery, and differed only according to the placement of a thalamic lesion with *N*-methyl-D-aspartate (NMDA). Coronal sections (20 μm) were cut from the prefrontal cortex and thaw-mounted on gelatin-coated slides. Sections were stored at −20°C prior to use. In the brain from each subject, we examined the binding of radioligands to all three receptors (5-HT_{2A}, mGlu2/3, and μ-opioid) using consecutively cut sections. For tritium-labeled (1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid ([^{3}H]LY354740) binding, tissue sections were preincubated in ice cold 10 mM potassium phosphate buffer

with 100 mM potassium bromide (phosphate/bromide buffer), pH 7.6, for 30 min to remove endogenous receptor ligands, then rapidly dried under a stream of cool air. The sections were then incubated for 60 min at room temperature in phosphate/bromide buffer with 50 nM [^{3}H]LY354740 (custom prepared by Amersham, Buckinghamshire, UK). Non-specific binding was determined using adjacent sections with 1 mM L-glutamate in the buffer solution. Following incubation, each slide was rapidly rinsed three times with 2 ml ice cold phosphate/bromide buffer, followed by a final rinse with 2 ml of ice cold glutaraldehyde (49%)/acetone mixture (1:19, v/v), then quickly dried under a stream of warm air. Rinse time was <10 s per slide. Sections were opposed, with tritium microscales, to tritium-sensitive film (Hyperfilm®-^{3}H, Amersham, Piscataway, NJ, USA) for 10 days.

For measurement of 5-HT_{2A} binding sites, tissue sections from the same rats were preincubated in a pH 7.4 buffer (50 mM Tris–HCl, 4 mM $CaCl_2$, 0.1% ascorbate and 0.1% bovine serum albumin) for 30 min at room temperature and were then dried under a stream of cool air. The tissue sections were then incubated for 90 min at room temperature in the same Tris buffer with 0.5 nM of (±)-1-(2,5-dimethoxy-4-[^{125}I]iodophenyl)-2-aminopropane ([^{125}I](±)DOI, NEN™ Life Science Products, Boston, MA, USA). Non-specific binding was defined by incubation of adjacent sections in buffer and radioligand with 1 μM MDL 100,907 added. Following incubation, the slides were rinsed twice for 10 min in ice cold assay buffer, followed by a brief immersion in ice cold purified water (Milli-Q), then dried under a stream of warm air. Tissue sections were opposed, with ^{125}I microscales, to ^{125}I-sensitive film (Amersham, Piscataway, NJ, USA) for 30 h.

For measurement of μ-opioid binding sites, methods were modified from Unterwald et al. (1998). Tissue sections from the same rats were preincubated at room temperature in a pH 7.4 Tris–HCl (50 mM) buffer. They were then incubated for 60 min at 4°C in the same Tris buffer with 4 nM [^{3}H][D-Ala^2, *N*-MePhe^4,Gly-ol^5]enkephalin (DAMGO, Amersham, Buckinghamshire, UK). Non-specific binding was defined by incubation of adjacent sections in buffer, radioligand and a 1 μM endomorphin-1 (Tocris, Ballwin, MO, USA). Following incubation, each slide was rapidly rinsed three times with ice cold Tris–HCl buffer, followed by a brief immersion in ice cold purified water. Sections were opposed, with tritium microscales, to tritium-sensitive film (Hyperfilm®-^{3}H, Amersham) for 11–12 weeks.

Quantitative autoradiogram analysis was performed using a computer-assisted image analyzer (MCID, Imaging Research, St. Catherines, ON, Canada). Optical density values were converted to fmol/mg protein using a computer-generated regression analysis. Values displayed in Fig. 8 represent data averaged from eight sham-operated and 13 lesioned rats with two sections per rat.

Lesions

Male Sprague–Dawley rats (150–180 g) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and burr holes were drilled for stereotaxic cannula or electrode placement. All sham-operated animals were similarly anesthetized and the cannula or electrode was lowered 1 mm short of the desired target. In these animals, no infusion or stimulation occurred. Unilateral thalamic radiofrequency lesions were made with a 60-s, 10-mA stimulation using insulated insect pins (size 1) which had 1.0 mm of the tip exposed. The electrode placement with respect to bregma was −2.5 mm anterioposterior (AP), 1.0 mm mediolateral (ML), and 6.5 mm dorsoventral (DV) to the skull. Animals were allowed a 6–14-day recovery period. The bilateral amygdala lesion placement was 3.5 mm AP, 5.5 mm ML, and 8.5 mm DV. These animals were allowed a 6–8-day recovery period. The chemical thalamic lesions using 100 mM NMDA (an infusion rate of 0.1 μl/min, and an infusion duration of 3 min/site) were made using multiple cannula placements in the AP plane. These animals were allowed a 12–18-day recovery period. The longer recovery periods were used for the thalamic lesions compared to the amygdala lesions since there is no guide in the literature as to the time period required for the degeneration of thalamocortical afferents originating specifically from the midline and intralaminar thalamic nuclei. In contrast, clear behavioral effects consistent with degeneration of amygdala projection cells occur with 6–8-day recovery periods. The three cannula placements for the NMDA lesions were all 0.7 mm ML and (1) −1.8 mm AP; −5.7 DV; (2) −2.7 mm AP and −6.3 mm DV; and (3) −3.6 mm AP and −6.5 mm DV. The animals used for autoradiography were allowed 15–18 days before obtaining their brains for autoradiography and histology.

Histology

After obtaining the frontal cortex for electrophysiological or autoradiographic binding studies, the remainder of the brain was placed in 30% sucrose in 10% formalin solution. Coronal sections (40 μm) were cut through the thalamus or amygdala and every third section was mounted onto gelatin-coated slides. The sections were stained with Cresyl Violet.

Data analysis

Multiple cells (two to five) were recorded from each slice and the data were analyzed by comparing the mean value for the EPSC frequency for each slice. An unpaired *t*-test ($P<0.05$) was used to compare the sham-operated vs. the lesion group. The autoradiographical data were normalized to percent of the 'sham' condition for each given region or layer within a region. The data for each radioligand in each area were tested using an analysis of variance (ANOVA). Significant main effects and interaction effects were tested using the Newman–Keuls test ($P<0.05$).

Compounds

The drugs used in this study were obtained from the following suppliers: Sigma (St. Louis, MO, USA: 5-HT creatine sulfate); RBI (Natick, MA, USA: NMDA, AMPA). LY354740 was prepared by Dr. James A. Monn at the Lilly Research Laboratories (Indianapolis, IN, USA).

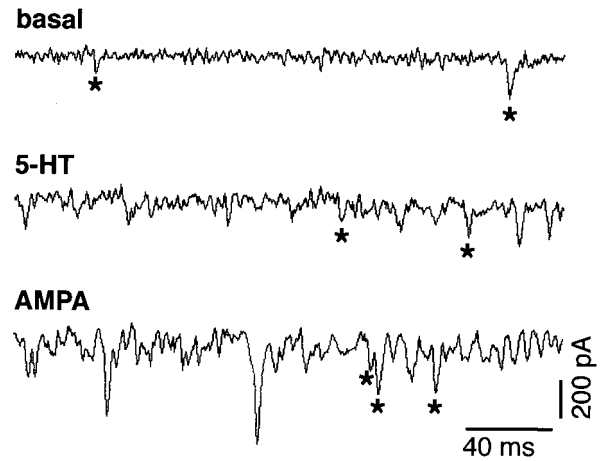

Fig. 1. 5-HT- and AMPA-induced EPSCs from a layer V pyramidal cell of the mPFC. The top tracing shows excitatory synaptic currents under basal conditions where the asterisks denote the EPSCs which were detected by the 'Mini Analysis Program' during the period shown. The middle trace demonstrates the increase in both the frequency and amplitude of EPSCs during bath application of 5-HT (100 μM). The bottom trace shows the increase in the frequency and amplitude of EPSCs during bath application of AMPA (5 μM). The asterisks denote examples of EPSCs that were detected with the peak detection software. The EPSC frequencies (number/s) in this cell were: basal (5.8); 5-HT (69.1); AMPA (145).

Table 1. EPSC frequency comparisons for lesioned and sham-operated rats

Group	Basal	5-HT (100 μM)	AMPA (5 μM)
Experiment 1			
Sham-operated	4.3 ± 0.83 (5, 15)	33.6 ± 5.4 (5, 15)	92.4 ± 41.6 (2, 8)
RF thalamic lesion	4.3 ± 1.7 (6, 20)	11.3 ± 3.8** (6, 20)	72.7 ± 11.2 (4, 9)
Experiment 2			
Sham-operated	5.8 ± 0.5 (5, 20)	33.1 ± 4.5 (5, 20)	86.1 ± 17.3(5, 20)
NMDA thalamic lesion	4.1 ± 1.0 (5, 17)	13.2 ± 2.6** (5, 17)	89.8 ± 11.8(5, 17)
Experiment 3			
Sham-operated	4.8 ± 1.2 (4, 11)	18.1 ± 3.0 (4, 11)	106 ± 14.1 (4, 10)
RF amygdala lesion	4.2 ± 0.8 (5, 14)	22.7 ± 4.9 (5, 14)	79.7 ± 8.4 (5, 11)

RF, radiofrequency. **Significantly different from sham-operated group, $P < 0.01$. Values are expressed as mean ± S.E.M. (number of subjects, number of total cells tested).

RESULTS

Electrophysiological characteristics of cortical layer V pyramidal cells

Layer V pyramidal cells were recorded in a zone ca. one half to two thirds of the distance between the pial surface and the subcortical white matter. The pyramidal cells in the present study had the following characteristics: resting potential, −70.5 ± 0.7 mV; action potential amplitude, 79.6 ± 0.5 mV; action potential duration (at half amplitude), 0.87 ± 0.01 ms; input resistance (−0.4 nA test pulse), 33.2 ± 2.1 MΩ ($n = 98$). All of the cells in the present series, except a single cell with an intrinsically bursting firing pattern to a constant depolarizing pulse, had the previously reported characteristics of regularly spiking pyramidal cells (Connors and Gutnick, 1990; McCormick et al., 1985). We have previously observed the relative lack of intrinsically bursting pyramidal cells in layer V in the mPFC compared to motor cortex or somatosensory cortex.

Relatively few EPSCs were observed when recording from layer V pyramidal cells of the mPFC under basal conditions (Fig. 1). As reported previously (Aghajanian and Marek, 1997), 5-HT (100 μM) increased the EPSC frequency and amplitude in these same layer V pyramidal cells. The ionotropic glutamate agonist AMPA (5 μM) also increased the EPSC frequency and amplitude (Fig. 1). The AMPA-induced EPSCs measured using the 'Mini Analysis Program' did not appear to be a reflection of increased baseline noise as the sodium channel blocker tetrodotoxin (2 μM) suppressed the AMPA-induced EPSCs by 97.1% (initial frequency = 92.1 ± 19.8 EPSCs/s, $n = 6$, not shown) while suppressing the AMPA-induced steady-state inward current by only 45.6% (initial amplitude = 1982 ± 320 pA). In addition, the AMPA-induced EPSC frequency in the cells from the present study ranged from 17.5 to 163.9 EPSCs/s;

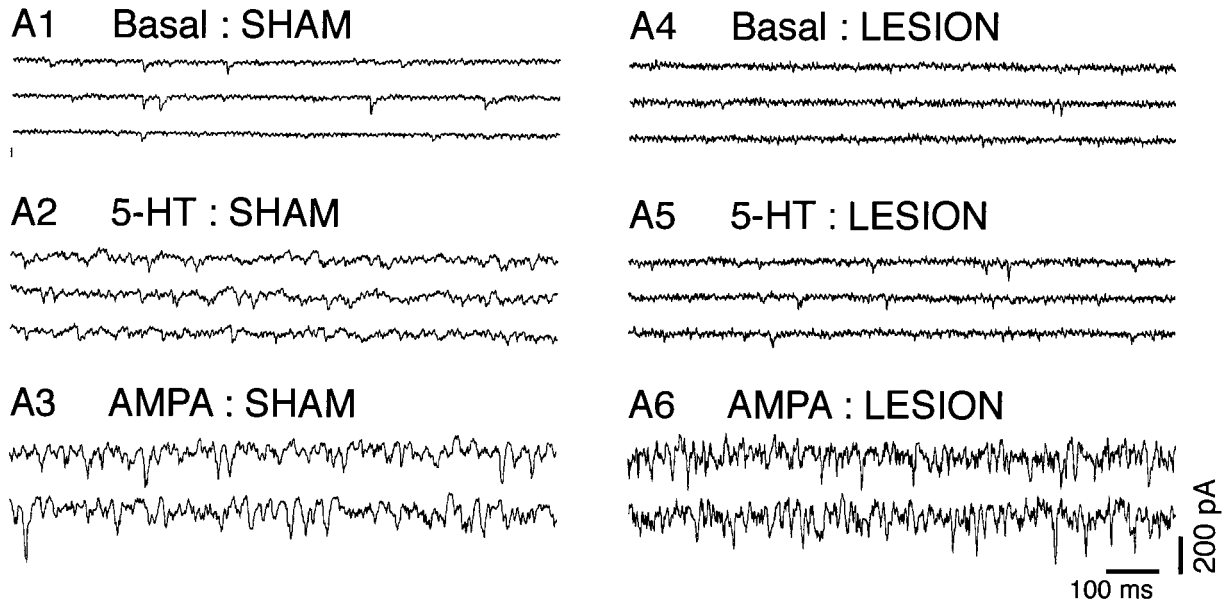

Fig. 2. Effect of chemical thalamic lesions (NMDA) on basal, 5-HT-, and AMPA-induced EPSCs. A2 shows the effect of 5-HT (100 μM) while recording from a layer V pyramidal cell of the mPFC from a rat with 'sham' surgery while A5 shows the relative failure of 5-HT to increase the EPSC frequency in a layer V pyramidal cell. A3 and A6 demonstrate the similar AMPA (5 μM)-induced EPSCs in these same rats.

the AMPA-induced EPSC frequency within a given cell was found to be a reliable measure (data not shown).

Experiment 1: radiofrequency thalamic lesions and electrophysiology

In initial pilot experiments, unilateral radiofrequency lesions of the thalamus were made in an attempt to destroy the central medial and paraventricular thalamic nuclei, which project most heavily to the same layers (I and Va; Berendse and Groenewegen, 1991) in the prelimbic region of the medial prefrontal cortex that are enriched in 5-HT_{2A}, mGlu2 and μ-opioid receptors. These lesions would also destroy fibers of passage. The thalamic lesions did not alter the frequency of 'spontaneous' basal EPSCs which were present in the unstimulated slice [Table 1; lesion group, $n=6$ (20 cells); sham group, $n=5$ (15 cells)] during recordings from layer V pyramidal cells of the mPFC (prelimbic region; Cg3). Similarly, we have previously found that mGlu2/3 and μ-opioid agonists do not suppress the basal EPSC frequency or amplitude (Marek and Aghajanian, 1998; Marek et al., 2000). These presynaptic agonists do, however, decrease the frequency of 5-HT-induced EPSCs. Accordingly, the frequency of EPSCs induced by a near maximal concentration of 5-HT (100 μM) was decreased by 68% in the slices from thalamic-lesioned rats compared to the sham-operated group ($P<0.01$, Table 1). In a subset of these animals in which AMPA was tested [lesion, $n=4$ (9 cells); sham, $n=2$ (8 cells)], the frequency of AMPA-induced EPSCs was not altered (Table 1).

Histological verification of the lesions (not shown) revealed that a number of the midline and intralaminar thalamic nuclei (e.g., central medial n., paracentral n., centrolateral n., paraventricular n., and the rhomboid n.), which project to layers I and Va of the frontal cortex with the highest density of 5-HT_{2A} receptors, were extensively destroyed. However, the reuniens n. was largely spared in most of the lesioned animals. The medial dorsal thalamic n. was almost completely destroyed. Another thalamic nucleus with significant projections to the frontal cortex, the anteromedial n., was completely destroyed. Several thalamic nuclei generally were spared by the lesions: ventroposterolateral, ventral posteromedial, ventral lateral geniculate, dorsal lateral geniculate and the reticular thalamic nucleus.

Experiment 2: NMDA-induced thalamic lesions and electrophysiology

Chemical lesions of the medial thalamus were made with NMDA in order to spare fibers of passage. Like the radiofrequency lesions, the NMDA-induced thalamic lesions did not alter the frequency of 'spontaneous' (non-electrically evoked) basal EPSCs [Fig. 2, Table 1; lesion group, $n=5$ (17 cells); sham group, $n=5$ (20 cells)] recorded from layer V pyramidal cells of the mPFC (prelimbic region; Cg3). Again, the frequency of spontaneous EPSCs induced by 5-HT (100 μM) was decreased by 60% in the slices from thalamic-lesioned rats compared

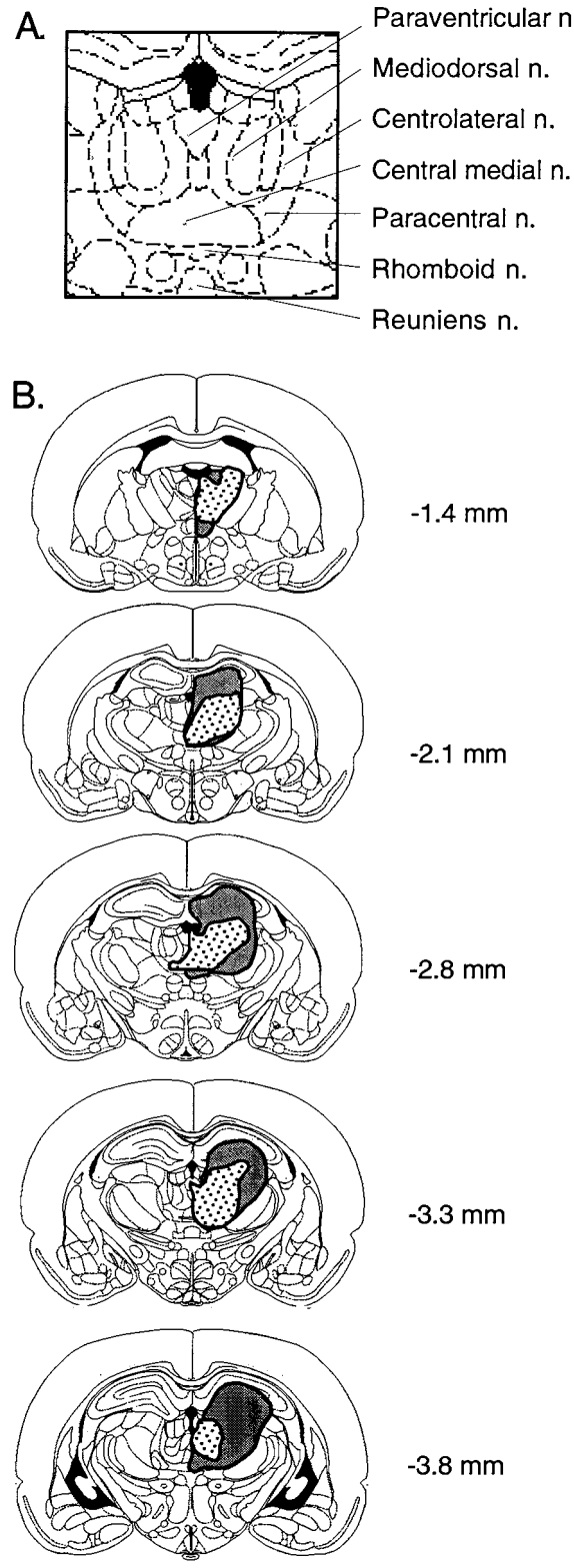

Fig. 3. Histological verification of chemical thalamic lesions induced by NMDA. The tracings show the smallest (stippled area) and the largest (shaded area) radiofrequency lesions that were directed at the thalamus. Note that the ventral midline thalamic nuclei (reuniens n. and rhomboid n.), unlike the dorsal midline thalamic nuclei (central medial n. and paraventricular n.) were largely spared.

to the sham-operated group ($P < 0.01$, Fig. 2, Table 1). In these same cells, the frequency of AMPA-induced EPSCs was not altered by the thalamic lesion (Fig. 2, Table 1). AMPA was used as a control since this ionotropic agonist would be expected to non-specifically release glutamate throughout the slice by activating postsynaptic receptors to depolarize cells past their firing threshold and/or activating presynaptic receptors. Consistent with this idea, our previous study of recordings from layer V pyramidal cells of the mPFC showed that μ-opioid agonists also do not suppress the frequency of AMPA-induced EPSCs, unlike the virtually complete suppression of 5-HT-induced EPSCs (Marek and Aghajanian, 1998).

Histological verification of the lesions (Fig. 3) revealed that a number of the midline and intralaminar thalamic nuclei (e.g., central medial n., paracentral n., centrolateral n., and the paraventricular n.) which project to layers I and Va of the frontal cortex with the highest density of 5-HT_{2A} receptors, were extensively, though not completely, destroyed. Two other midline thalamic nuclei, the rhomboid and the reuniens n., were largely spared. The medial dorsal thalamic n. was almost completely destroyed. Another thalamic nucleus with significant projections to the frontal cortex, the anteromedial n., was completely destroyed. Several thalamic nuclei generally were spared by the lesions: the ventral posterolateral n., ventral posteromedial n., ventral lateral geniculate n., dorsal lateral geniculate n. and the reticular thalamic n. Two of the five lesions extended into the hippocampus. In comparison to the previously described thalamic radiofrequency lesions, these chemical-induced lesions appeared to spare the ventral midline thalamic nuclei to a greater degree. In addition, none of the radiofrequency lesions extended into the hippocampus.

Experiment 3: amygdala lesions and electrophysiology

Bilateral radiofrequency lesions of the amygdala did not alter the frequency of 'spontaneous' basal EPSCs [Fig. 4; Table 1; lesion group, $n = 5$ (14 cells); sham group, $n = 4$ (11 cells)] recorded from layer V pyramidal cells of the medial prefrontal cortex (prelimbic region; Cg3) in animals allowed a 6–8-day recovery following surgery. The frequency of spontaneous 5-HT-induced EPSCs was not altered in the amygdala-lesioned rats compared to the sham-operated group (Fig. 4, Table 1). Similarly, the frequency of AMPA-induced EPSCs was not significantly altered in the amygdala-lesioned rats compared to the sham-operated group (Fig. 4, Table 1).

Histological verification of the lesions (Fig. 5) showed that the basolateral nucleus of the amygdala was completely destroyed bilaterally by the lesions. The basolateral amygdala n. is known to project to the deep part of layer I, layer II, and the entire width of layer V of the prefrontal cortex (Krettek and Price, 1977). This nucleus is the principal source of afferents to the cortex from the amygdala (Krettek and Price, 1977). Much of the central nucleus of the amygdala as well as regions adjacent to the amygdala were also destroyed.

Experiment 4: thalamic lesions and autoradiography

Although questions have been raised regarding the ability of current antibodies to label 5-HT_{2A} receptors associated with the plasma membrane due to epitope masking or steric hindrance, the presence of 5-HT_{2A} receptors has nevertheless been demonstrated in every myelinated pathway known to arise from immunoreactive cell body groups (Cornea-Hebert et al., 1999). While the vast majority of 5-HT_{2A} receptors in the prefrontal

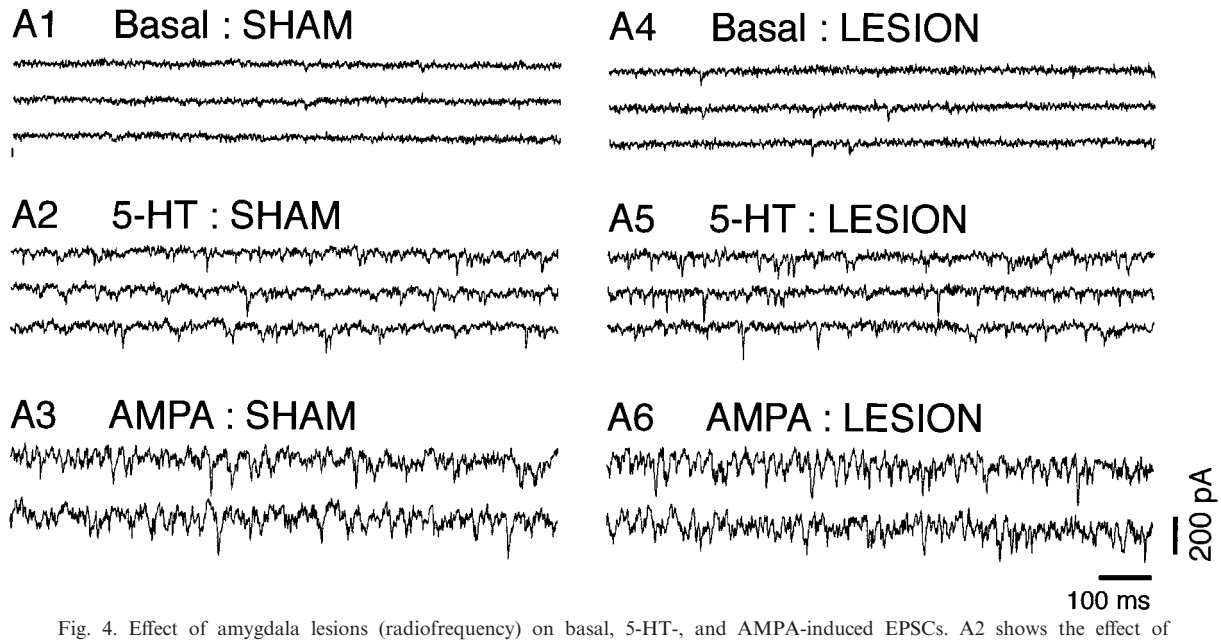

Fig. 4. Effect of amygdala lesions (radiofrequency) on basal, 5-HT-, and AMPA-induced EPSCs. A2 shows the effect of 5-HT (100 μM) while recording from a layer V pyramidal cell of the mPFC from a rat with 'sham' surgery while A5 shows the relative sparing of the 5-HT response to increase the EPSC frequency in a layer V pyramidal cell. A3 and A6 demonstrate the similar AMPA (5 μM)-induced EPSCs in these same rats.

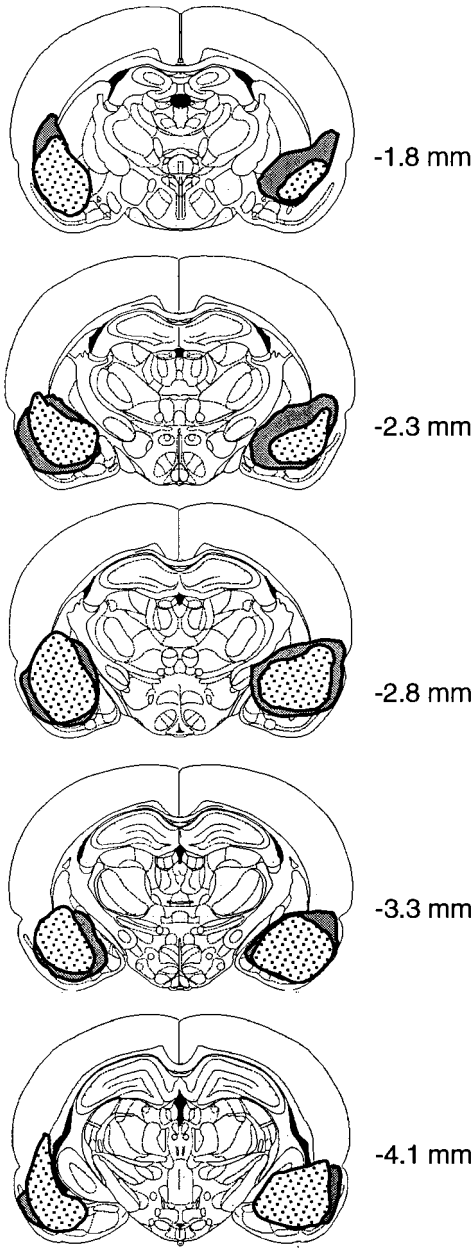

Fig. 5. Histological verification of amygdala radiofrequency lesions. The tracings show the smallest (stippled shading) and the largest (shaded area) radiofrequency lesions that were directed at the amygdala.

cortex are probably present on pyramidal cell dendrites and some parvalbumin/calbindin-containing interneurons (Jakab and Goldman-Rakic, 1998; Willins et al., 1997), thalamocortical terminals may contribute a fraction of the 5-HT$_{2A}$ receptors present in the mPFC since the midline thalamic nuclei appear to express 5-HT$_{2A}$ receptor mRNA (Cyr et al., 2000). Therefore, we made NMDA-induced thalamic lesions in an attempt to demonstrate a small decrease in [^{125}I]DOI binding following the lesion. DOI was used as the radioligand in order to quantify high affinity agonist binding sites that would presumably be associated with the plasma membrane.

A critical assumption in this experiment is that postsynaptic 5-HT$_{2A}$ receptors are not directly modulated by this lesion of excitatory amino acid-containing afferents. The majority of postsynaptic 5-HT$_{2A}$ receptors would be expected to be in pyramidal cells since it has been reported that almost all cortical pyramidal cells express 5-HT$_{2A}$ receptors (Jakab and Goldman-Rakic, 1998; Willins et al., 1997). GABAergic interneurons make up only approximately 21% of the cells in the prelimbic region of the mPFC (Gabbott et al., 1997). 5-HT$_{2A}$ receptor-expressing interneurons appear to be restricted to certain subpopulations of parvalbumin- and calbindin-containing interneurons (Jakab and Goldman-Rakic, 1998, 2000; Willins et al., 1997), and would make up less than 15% of the cells in layer V of the mPFC (Gabbott et al., 1997).

In addition, we also measured [^{3}H]LY354740 and [^{3}H]DAMGO binding following the thalamic lesions as our previous work had suggested that activation of either μ-opioid or mGlu2/3 receptors suppresses 5-HT-induced EPSCs by a presynaptic mechanism. An example of the sampling from layers I, II/III, and Va of the prelimbic region of the mPFC, the dorsal agranular insular cortex, and the nucleus accumbens is shown in Fig. 6 from autoradiography of [^{125}I]DOI binding.

Surprisingly, the thalamic lesions increased [^{125}I]DOI binding in the prelimbic region of the medial prefrontal

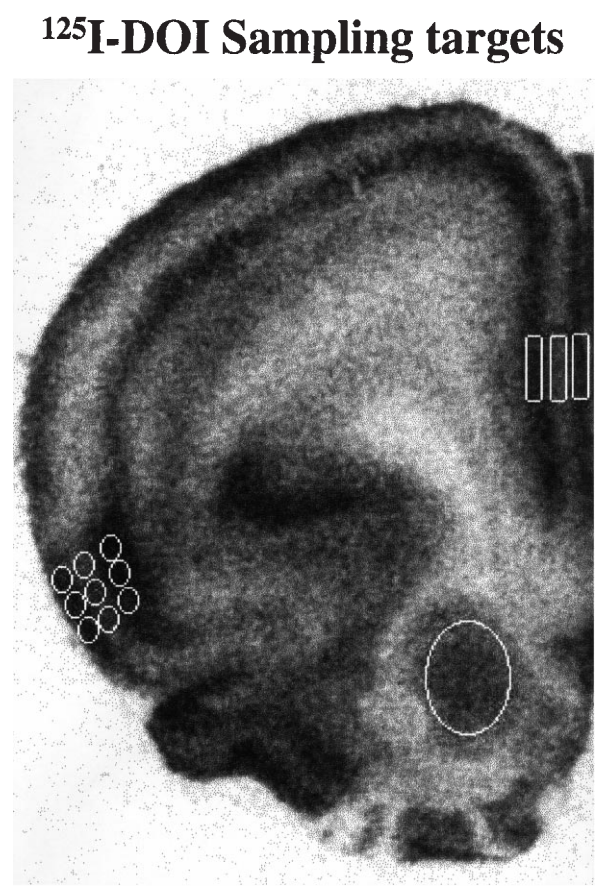

Fig. 6. Autoradiography of [^{125}I]DOI binding with representative sampling from layers I, II/III, and Va of the prelimbic region of the mPFC (right); layers I, II/III, and Va of the dorsal agranular insular cortex (left); and the nucleus accumbens.

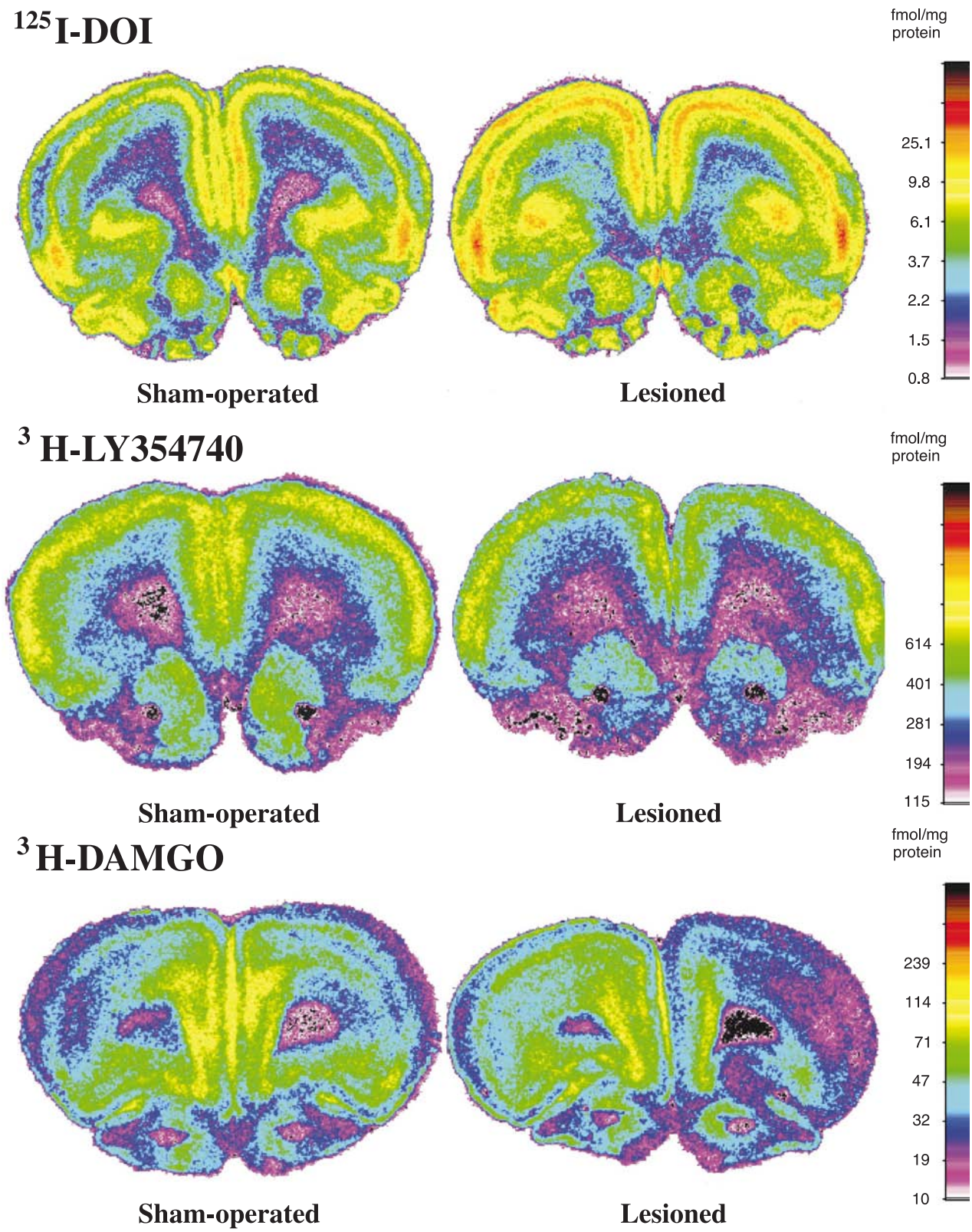

Fig. 7. Autoradiography of [^{125}I]DOI binding (top), [^{3}H]LY354740 binding (middle), and [^{3}H]DAMGO binding (bottom) in the prelimbic region of the mPFC, the dorsal agranular insular cortex, and the shell of the nucleus accumbens of the same sham-operated control (left) and lesioned (right) rats. This anterior–posterior level corresponds to the physiological studies. The binding values are expressed in fmol/mg protein, with violet representing the lowest binding values obtainable.

cortex (Figs. 7 and 8). The ANOVA revealed a significant main lesion group effect ($F(1,38) = 3.98$, $P < 0.05$), a significant effect of cortical layers ($F(2,76) = 3.41$, $P < 0.05$), and a significant interaction of the lesion group × the cortical layers ($F(2,76) = 3.57$, $P < 0.05$). The [^{125}I]DOI binding was significantly increased to 117 ± 10 and $129 \pm 10\%$ of sham-operated controls ($P < 0.05$, Newman–Keuls test) in layers I and Va, respectively, on the right side of the thalamic-lesioned animals. On the non-lesioned left side, [^{125}I]DOI binding was significantly increased to $120 \pm 7\%$ of sham-operated controls in layer Va. This is consistent with spread of the lesion to the contralateral midline thalamic nuclei in a number of animals as discussed below.

As expected, the thalamic lesions decreased the [^{3}H]LY354740 binding in the prelimbic region of the medial prefrontal cortex (Figs. 7 and 8). The ANOVA demonstrated significant main effects of the lesion group ($F(1,38) = 61.24$, $P < 0.001$) and the side of the brain ($F(1,38) = 4.11$, $P < 0.05$) in addition to a significant lesion group × side interaction ($F(1,38) = 4.73$, $P < 0.05$). When comparing the lesion group to the sham-operated group, the [^{3}H]LY354740 binding was significantly decreased by 19–22% in each of the layers on the lesioned right side of the brain in addition to a 11–12% decrease in each of the layers on the 'non-lesioned' left side ($P < 0.001$, Newman–Keuls test). Within the group of lesioned rats, each layer on the lesioned right side was significantly lower than on the non-lesioned left side ($P < 0.001$). Again, the decrease in LY354740 binding on the 'non-lesioned' side is consistent with spread of the lesion to the contralateral midline thalamic nuclei in a number of animals as discussed below. It should be noted that binding for each of the radioligands was determined from consecutively cut sections for each subject. In addition, the changes in [^{3}H]LY354740 binding were negatively and significantly correlated to the changes in [^{125}I]DOI binding in layers I ($r_s = -0.32$), II/III ($r_s = -0.36$) and Va ($r_s = -0.40$) for the prelimbic region (Cg3) of the medial prefrontal cortex.

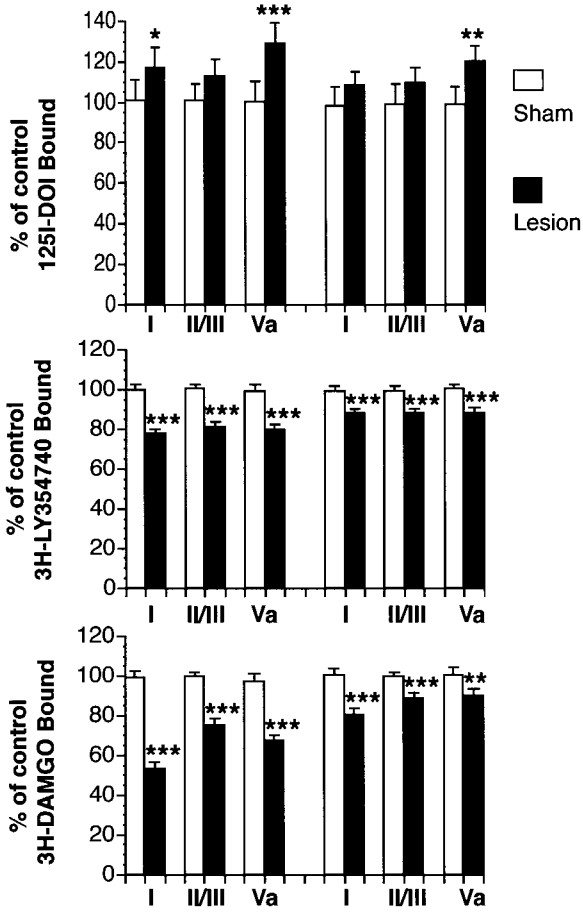

Fig. 8. Histogram of [^{125}I]DOI binding (top), [^{3}H]LY354740 binding (middle), and [^{3}H]DAMGO binding (bottom) in the prelimbic (Cg3) region of the rat mPFC in layers I, II/III, and Va of sham-operated ($n = 8$) and lesioned ($n = 13$) rats. The data are presented in % of the control binding of the respective ligand for a given cortical layer. The autoradiography was performed on two different cohorts with $n = 4$ for the sham group in each cohort. The same pattern of changes for each of the radioligands was present for the two sets of brains and the data were normalized as % of control. Significantly different from the similar layer of the sham group, *$P < 0.05$ (Newman–Keuls test); **$P < 0.01$; ***$P < 0.001$.

Again, as expected and consistent with the literature, the thalamic lesions decreased the [^{3}H]DAMGO binding in the prelimbic region of the medial prefrontal cortex (Figs. 7 and 8). The ANOVA resulted in significant main effects of lesion group ($F(1,38) = 72.62$, $P < 0.001$), side of the brain ($F(1,38) = 17.24$, $P < 0.001$), and the cortical layers ($F(2,76) = 18.48$, $P < 0.001$). Significant interactions were observed for lesion group × side ($F(1,38) = 12.26$, $P < 0.001$), lesion group × cortical layers ($F(2,76) = 19.44$, $P < 0.001$), and side × layers ($F(2,76) = 4.35$, $P < 0.05$). The largest decreases in DAMGO binding were observed on the lesioned right side in layer I ($46 \pm 3\%$) and layer Va ($32 \pm 3\%$, $P < 0.001$, Newman–Keuls). Layer II/III on the right side was reduced by a slightly smaller amount ($24 \pm 3\%$, $P < 0.001$). On the 'non-lesioned' left side, the greatest decrease was observed in layer I ($20 \pm 3\%$, $P < 0.001$). Layers II/III and Va were significantly reduced by 9 and 11%, respectively ($P < 0.001$). Within the group of lesioned rats, each layer on the lesioned right side was significantly lower than on the non-lesioned left side ($P < 0.001$). Again, the decreases in [^{3}H]DAMGO binding on the 'non-lesioned' side is consistent with spread of the lesion to the contralateral midline thalamic nuclei in a number of animals as discussed below. Although the changes in [^{3}H]DAMGO binding on the 'non-lesioned' left side were significantly correlated with changes in [^{3}H]LY354740 binding in layers I ($r_s = 0.81$), II/III ($r_s = 0.61$), and Va ($r_s = 0.61$), the changes in [^{3}H]DAMGO binding were not significantly correlated with the changes in [^{125}I]DOI binding ($r < -0.2$).

Histological verification of the lesions (Fig. 9) revealed that a number of the midline and intralaminar thalamic nuclei (e.g., central medial n., paracentral n., centrolateral n., and the paraventricular n., rhomboid n. and reuniens n.) which project to layer I and Va of the frontal cortex with the highest density of 5-HT$_{2A}$ receptors were extensively, though not completely, destroyed on the right side. The medial dorsal thalamic n. was almost completely destroyed on the right side. The medial aspect

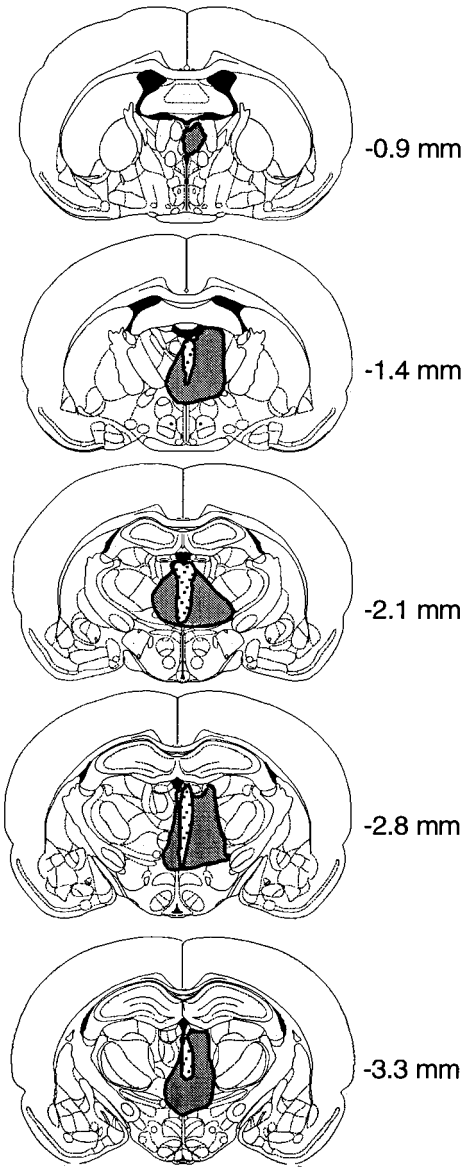

Fig. 9. Histological verification of chemical thalamic lesions induced by NMDA for autoradiography. The tracings show the smallest (stippled area) and the largest (shaded area) radiofrequency lesions that were directed at the thalamus. The lesioned animals not shown more closely resembled the largest lesion shown.

of another thalamic nucleus with significant projections to the frontal cortex, the anteromedial n., was completely destroyed. Several thalamic nuclei generally were spared by the lesions: the ventral posterolateral n., ventral posteromedial n., ventral lateral geniculate n., dorsal lateral geniculate n. and the reticular thalamic n. A number of the lesions extended into the left side of the brain with the most extensive destruction of the central medial n., the interanteromedial thalamic n., and the rhomboid n. The medial division of the dorsomedial n. was generally spared on the left side. The central division of the dorsomedial n. was completely spared on the left side.

In order to assess the generality and specificity of the increased 5-HT_{2A} receptor binding in the prelimbic region of the medial prefrontal cortex, we also examined the dorsal agranular insular cortex and the nucleus accumbens, which are both known to receive afferents from the midline and intralaminar thalamic nuclei (Berendse and Groenewegen, 1990, 1991). The generality of the increased DOI binding in the mPFC was supported by the observation that the thalamic lesions increased [^{125}I]DOI binding in layer Va of the dorsal agranular insular cortex (Fig. 7). The ANOVA revealed a significant effect of insular cortical layers ($F(2,76) = 15.63$, $P < 0.001$) and a significant interaction between the lesion condition and cortical layers ($F(2,76) = 15.57$, $P < 0.001$). The [^{125}I]DOI binding was significantly increased to 114 ± 5.0 and $113 \pm 5.6\%$ of sham-operated controls, ($P < 0.01$ and 0.05, respectively, Newman–Keuls test) in layer Va of the right and left sides for the lesioned subjects.

The effect of the thalamic lesions on prefrontal cortical DOI binding was not due to a non-specific stress effect. This effect was specific to the cortex. The [^{125}I]DOI binding was non-significantly decreased to 96 ± 7 and $93 \pm 5\%$ of sham-operated controls in the right and left nucleus accumbens from the lesioned subjects. In accordance with the known innervation of the nucleus accumbens by the midline and intralaminar thalamic nuclei, the medial thalamic lesions did decrease [^{3}H]LY354740 binding. The ANOVA revealed a significant effect of the lesion ($F(1,26) = 27.59$, $P < 0.001$) and an interaction between the lesion and side ($F(1,26) = 3.95$, $P < 0.05$). The thalamic lesion significantly decreased the [^{3}H]LY354740 binding by 28.3 ± 5 (right side) and $16.5 \pm 5.7\%$ (left side). The binding was also significantly lower on the right side of the lesioned subjects compared to the left side ($P < 0.05$, Newman–Keuls test). It should be noted that, unlike in the prefrontal cortex, the primary postsynaptic localization of 5-HT_{2A} receptors in the striatum would not be on glutamatergic neurons. Instead, the primary localization of this receptor appears to be in GABAergic medium spiny projection cells (Rodriguez et al., 1999).

DISCUSSION

The present results provide the first direct anatomical evidence that activation of 5-HT_{2A} receptors in the medial prefrontal cortex results in transmitter release from thalamocortical terminals. The most intense expression of 5-HT_{2A} receptor mRNA in the thalamus is within the midline and intralaminar nuclei. These also are the thalamic nuclei whose termination in layers I and Va of the prefrontal cortex best match the same laminar distribution of peak 5-HT_{2A} receptor binding (Berendse and Groenewegen, 1991; Blue et al., 1988; Marek et al., 2000). A partial destruction of presynaptic afferents regulated by 5-HT_{2A} receptors would be expected to decrease the frequency of EPSCs. Fiber-sparing lesions of the medial thalamus decreased the frequency of 5-HT-induced EPSCs recorded from layer V pyramidal cells of the medial prefrontal cortex (e.g., prelimbic area or Cg3). It should be noted that this *in vitro* slice prepara-

tion does not contain the thalamus proper. Thus, these results support previous evidence that 5-HT induces glutamate release from afferent fibers independent of impulse flow originating from cells intrinsic to the cortex (Aghajanian and Marek, 1997). More specifically, 5-HT is inducing glutamate release from thalamocortical afferents. However, it is unknown at the present time whether activation of 5-HT$_{2A}$ receptor induces glutamate release from thalamocortical terminals by acting upon (1) thalamic axons, (2) thalamic axon terminals, or (3) cortical pyramidal apical dendrites with a subsequent 'retrograde' influence on the thalamocortical terminals.

The NMDA-induced thalamic lesions also decreased the binding of [^{3}H]LY354740 and [^{3}H]DAMGO in the medial prefrontal cortex in a manner to be expected from previous electrophysiological evidence that mGlu2/3 and μ-opioid agonists suppress 5-HT-induced EPSCs through a presynaptic action (Marek and Aghajanian, 1998; Marek et al., 2000). The radioligand binding was decreased in a proportional manner on both sides in the lesioned animals, consistent with the expected, and observed, spread of the lesion to the contralateral side. This represents the first demonstration that a portion of the mGlu2/3 receptors in the neocortex are present on thalamocortical afferents. The results with [^{3}H]DAMGO confirm previous reports that a considerable fraction of cortical μ-opioid receptor binding is also present on subcortical afferents (Sahin et al., 1992; Vogt et al., 1995).

This autoradiography also suggests that the midline and intralaminar thalamic nuclei are the most likely source of subcortical afferents from which 5-HT induces EPSCs onto the apical dendrites of layer V pyramidal cells. There is a striking laminar overlap between the highest density of 5-HT$_{2A}$ (Blue et al., 1988), μ-opioid (Tempel and Zukin, 1987), and mGlu2 (Marek et al., 2000; Ohishi et al., 1997) receptors and the axon terminals of neurons originating from these same nuclei in layers I and Va of the prefrontal cortex (Berendse and Groenewegen, 1991). The mRNA for all three receptors is present within these thalamic nuclei (Cyr et al., 2000; Mansour et al., 1994b; Ohishi et al., 1993; Wright et al., 1995). We have previously pointed out that 'hot spots' for the induction of EPSCs by microiontophoresis of 5-HT appear to be present in layers I and Va (Aghajanian and Marek, 1997). In contrast, the mediodorsal nucleus of the thalamus primarily projects to layer III rather than Va of the prefrontal cortex and there is relatively little mGlu2 mRNA in this thalamic nucleus, especially compared to the midline and intralaminar thalamic nuclei. The relatively weak expression of mGlu2 mRNA in the medial dorsal nucleus appears restricted to the lateral segment (Ohishi et al., 1993), which does not project to the prelimbic region (Groenewegen, 1988). Furthermore, the most intense expression of μ-opioid receptor mRNA in the thalamus is also within the midline and intralaminar thalamic nuclei (Mansour et al., 1994a). The anterior tier nuclei do not terminate sharply within layer Va as do the midline and intralaminar thalamic nuclei. These nuclei also do not contain appreciable μ-opioid or mGlu2 mRNA expression (Mansour et al., 1994b; Ohishi et al., 1993). Therefore, although the lesions also damaged adjacent thalamic nuclei, it is likely that the changes seen in the medial prefrontal cortex were primarily due to damage to cell bodies in the midline and intralaminar nuclei. The participation of thalamocortical pathways, 5-HT$_{2A}$ heteroreceptor activation and glutamate release in mediating the *in vivo* effects of hallucinogenic drugs in the neocortex is corroborated by a recent report that lesions of the ventrobasal thalamus suppressed the DOI-induced increase in Fos immunopositive cells extending from deep layer III to superficial V in the somatosensory cortex (Scruggs et al., 2000). This DOI-induced c-*Fos* response in the cortex, similar to 5-HT-induced EPSCs in the prefrontal cortex (Aghajanian and Marek, 1997; Zhang and Marek, 2000), is blocked both by non-NMDA receptor antagonists and by mGlu2/3 agonists (George et al., 2000; Scruggs et al., 2000).

Another subcortical region with widespread afferents

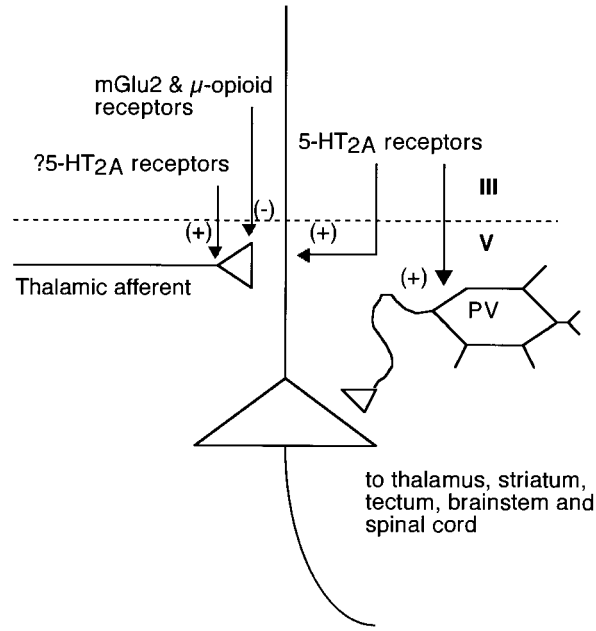

Fig. 10. Proposed model to account for relationships between thalamocortical terminals and 5-HT$_{2A}$ receptors in layer V of the mPFC with respect to glutamate release and 5-HT$_{2A}$ receptor binding. The midline and intralaminar thalamic nuclei terminate in the superficial aspect of layer V where the main neuronal compartment is the ascending apical dendrites. These thalamocortical terminals contain mGlu2 and μ-opioid receptors which function as autoreceptors and heteroreceptors, respectively. Activation of 5-HT$_{2A}$ receptors in either the thalamocortical axons/terminals or postsynaptic pyramidal apical dendrites (with a 'retrograde' influence on the thalamocortical terminals) induces glutamate release from these thalamocortical terminals. This accounts for the substantial reduction in 5-HT-induced EPSCs recorded from layer V pyramidal cells in rats with lesions of the midline thalamus that result in significant, though not complete, lesions of the intralaminar and midline thalamic nuclei. Shown also is a parvalbumin-containing interneuron in layer V. 5-HT$_{2A}$-immunopositive GABAergic interneurons account for less than 15% of the total cells found within layer V in the prelimbic region of the mPFC (Gabbott et al., 1997; Jakab and Goldman-Rakic, 2000). Thus, 5-HT$_{2A}$ receptors in the apical dendrites of the layer V pyramidal cells are major candidates for the site(s) where DOI binding is altered after chemical lesions of the midline thalamus. PV, parvalbumin-containing interneuron.

to the prefrontal cortex, the basolateral nucleus of the amygdala (Bacon et al., 1996; Krettek and Price, 1977), does not appear to provide afferents to the prefrontal cortex upon which 5-HT_{2A} receptor activation induces glutamate release. Complete bilateral destruction of the basolateral nucleus did not alter the frequency of 5-HT-induced EPSCs either. 5-HT_{2A} and mGlu2 mRNA is present within the basolateral nucleus of the amygdala, although relatively low expression of μ-opioid receptor mRNA is present. Nevertheless, the termination of these afferents from the amygdala to the prefrontal cortex is relatively widespread within layer V (Bacon et al., 1996; Krettek and Price, 1977), unlike the relatively restricted expression of 5-HT_{2A} receptors in the superficial aspect of layer V (Blue et al., 1988).

The most surprising result of the present study is that thalamic lesions, while decreasing binding to μ-opioid and mGlu2/3 receptors, actually increased binding of [^{125}I]DOI to 5-HT_{2A} receptors in the prefrontal cortex. As discussed previously, a critical assumption underlying the attempt to observe presynaptic 5-HT_{2A} receptors on thalamocortical afferents is that the remaining 5-HT_{2A} receptors in the cortex would not be altered by lesioning of a glutamatergic source of afferents. The strong expression of both 5-HT_{2A} receptor mRNA and protein within the prefrontal cortex would lead one to expect that most of the 5-HT_{2A} receptor is expressed on cellular elements intrinsic to the cortex (Cornea-Hebert et al., 1999; Mengod et al., 1990; Willins et al., 1997; Wright et al., 1995). Further work will be required to identify the nature of the change in [^{125}I]DOI binding following the thalamic lesions, particularly if this represents increased labeling of postsynaptic 5-HT_{2A} receptors. The principal population of postsynaptic 5-HT_{2A} receptors would appear to be present in pyramidal cells (Jakab and Goldman-Rakic, 1998; Willins et al., 1997). GABAergic interneurons represent only ~21% of the neurons present in the prelimbic region of the mPFC (Gabbott et al., 1997). The subpopulations of parvalbumin- and calbindin-containing interneurons, which also express 5-HT_{2A} receptors, would be less than 15% of the total cells found within layer V of the mPFC (Gabbott et al., 1997; Jakab and Goldman-Rakic, 1998, 2000; Willins et al., 1997). Thus, the most parsimonious interpretation of the present results is that (1) activation of 5-HT_{2A} receptors induces glutamate release from thalamocortical terminals which also contain inhibitory mGlu2 autoreceptors and μ-opioid heteroreceptors (see Fig. 10) and (2) these same thalamocortical terminals may also regulate postsynaptic 5-HT_{2A} receptor binding in the cortex.

It should be noted that the autoradiographic experiments included both within-subject (left vs. right hemisphere) and between-subjects (lesion vs. sham) controls. This aspect of the experimental design and the absence of changes in 5-HT_{2A} receptor binding in the nucleus accumbens make it unlikely that the changes observed were due to a non-specific effect of the lesion such as stress or a general deterioration in the health of the cortical tissue after the thalamic lesions. The same conclusion is supported by the specificity of the effects of the lesions on electrophysiological responses of cells in the medial prefrontal cortex. Thus, this represents the first evidence that subcortical glutamatergic afferents may regulate cortical 5-HT_{2A} receptors.

Clinical implications

A number of striking parallels exist between the modulation of affect, attention and cognition by (1) hallucinogenic drugs that activate 5-HT_{2A} receptors, (2) thalamic lesions in humans, and (3) antidepressant/antipsychotic drugs which either are 5-HT_{2A} antagonists and/or down-regulate cortical 5-HT_{2A} receptors. The similarities in the affective changes, loosened associations and perceptual alterations induced by either psilocybine or acute schizophrenia have been noted by others and these psychotomimetic effects of psilocybine have recently been demonstrated to result from activation of 5-HT_{2A} receptors in healthy human volunteers (Vollenweider et al., 1998). Echoing the clinical phenomenology of schizophrenia, lesions of the intralaminar thalamic nuclei in humans result in apathy, a loss of initiative, somnolence and hallucinations (e.g., peduncular hallucinosis), disordered thought processes and disruptions of executive cognitive function (Chatterjee et al., 1997; Noda et al., 1993; Van der Werf et al., 2000). Atypical antipsychotic drugs that are potent 5-HT_{2A} antagonists such as clozapine, risperidone and olanzepine treat the withdrawal, social isolation and apathy that characterize the negative symptoms of schizophrenia. These atypical antipsychotic drugs also appear to enhance cognitive function in schizophrenic patients beyond that observed with typical antipsychotic drugs (Honey et al., 1999; Meltzer and McGurk, 1999). Interestingly, the single common pharmacological effect shared by a number of atypical antidepressants (which do not appreciably block monoamine uptake or monoamine oxidase; e.g., mirtazepine, mianserin, trazodone and nefazodone) is blockade of 5-HT_{2A} receptors. The selective 5-HT_{2A}/dopamine D_2 antagonist risperidone also appears to augment antidepressant effects in depressed patients at low doses (0.5–1 mg/day) that are relatively selective for blockade of 5-HT_{2A} receptors (Ostroff and Nelson, 1999).

Summary

In summary, 5-HT_{2A} receptors appear to play a key role in the treatment of the major neuropsychiatric disorders. Previous research has demonstrated that activation of 5-HT_{2A} receptors induces glutamate release onto layer V pyramidal cells in the medial prefrontal cortex, an effect that is suppressed by activation of μ-opioid or mGlu2 receptor activation. Understanding the origin of these glutamatergic afferents is necessary to integrate these findings within known cortical circuitry. The present studies suggest that these effects are mediated by thalamocortical afferents arising in the midline and/or intralaminar thalamic nuclei. The importance of multiple thalamocortical pathways in neuropsychiatric disor-

ders ranging from depression to neurogenic pain has been recently postulated (Llinas et al., 1999). Recent work has also suggested alterations in the circuitry between the thalamus and prefrontal cortex as a substrate for the disrupted affect and cognition of schizophrenia (Bunney and Bunney, 2000; Lewis, 2000). Thus, by implicating thalamocortical transmission in the effects of 5-HT_{2A} receptor stimulation in the mPFC, the present findings suggest a role for thalamocortical circuitry in the mechanism by which potent 5-HT_{2A} antagonists alleviate the symptoms of mood disorders and schizophrenia.

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