Behavioral/Systems/Cognitive

Serotonergic Regulation of Membrane Potential in Developing Rat Prefrontal Cortex: Coordinated Expression of 5-Hydroxytryptamine (5-HT)$_{1A}$, 5-HT$_{2A}$, and 5-HT$_7$ Receptors

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The developing prefrontal cortex receives a dense serotonergic innervation, yet little is known about the actions of serotonin [5-Hydroxytryptamine (5-HT)] in this region during development. Here, we examined the developmental regulation of 5-HT receptors controlling the excitability of pyramidal neurons of this region. Using whole-cell recordings in *in vitro* brain slices, we identified a dramatic shift in the effects of 5-HT on membrane potential during the postnatal developmental period. In slices derived from young animals [postnatal day (P) 6 to P19], administration of 5-HT elicits a robust depolarization of layer V pyramidal neurons, which gradually shifts to a hyperpolarization commencing during the third postnatal week. This progression is the result of coordinated changes in the function of 5-HT$_7$ and 5-HT$_{2A}$ receptors, which mediate different aspects of the depolarization, and of 5-HT$_{1A}$ receptors, which signal the late developing hyperpolarization. The loss of the 5-HT$_7$ receptor-mediated depolarization and the appearance of the 5-HT$_{1A}$ receptor-mediated hyperpolarization appears to reflect changes in receptor expression. In contrast, the decline in the 5-HT$_{2A}$ receptor depolarization with increasing age was associated with changes in the effectiveness with which these receptors could elicit a membrane depolarization, rather than loss of the receptors per se. Together, these results outline coordinated changes in the serotonergic regulation of cortical excitability at a time of extensive synaptic development and thus suggest a key role for these receptor subtypes in the postnatal development of the prefrontal cortex.

Key words: **5-HT; serotonin; *in situ* hybridization; postnatal development; prefrontal cortex; *in vitro* electrophysiology; single-cell RT-PCR**

Introduction

Since the initial report by Harlow (1848), detailing the behavioral sequelae of prefrontal cortex damage, converging work from a variety of disciplines, has identified this region as key to the integration of behavior (Frith and Frith, 1999; Duncan, 2001; Fuster, 2001; Miller and Cohen, 2001) and has implicated it in the pathophysiology of neuropsychiatric disorders (Davidson, 2001; Frith, 2001; Weinberger et al., 2001; Harrison, 2002). In an effort to understand the cellular basis of prefrontal cortical function, numerous studies have focused on the physiology of prefrontal cortical neurons, their function during behavioral tasks (Durstewitz et al., 2000; Fuster, 2001; Miller and Cohen, 2001), and their regulation by neurotransmitters and neuromodulators (Araneda and Andrade, 1991; Tanaka and North, 1993; Otani et al., 1998; Aghajanian and Marek, 1999; Yang et al., 1999; Gao et al., 2001; Seamans et al., 2001). Among the latter, the effects of serotonin [5-Hydroxytryptamine (5-HT)] are of particular interest given its hypothesized role in a variety of neuropsychiatric disorders involving prefrontal cortex.

There is now abundant evidence suggesting a key role for 5-HT in regulating prefrontal cortex function. 5-HT-synthesizing cells of the dorsal and median raphe innervate the prefrontal cortex (Conrad et al., 1974; Azmitia and Segal, 1978; Wilson and Molliver, 1991; Vertes and Kocsis, 1994; Vertes et al., 1999), and neurons of this region express a variety of 5-HT receptors (Pompeiano et al., 1992, 1994; Bruinvels et al., 1994; Gustafson et al., 1996; Waeber et al., 1996; Lopez-Gimenez et al., 1997). In particular, pyramidal cells of the adult prefrontal cortex have been shown to coexpress 5-HT receptors of the 5-HT$_{1A}$ and 5-HT$_{2A}$ subtypes (Martin-Ruiz et al., 2001), and parallel electrophysiological studies have identified that 5-HT$_{1A}$ receptors mediated hyperpolarizing–inhibitory and 5-HT$_{2A}$ receptors mediated depolarizing–excitatory responses in these cells (Davies et al., 1987; Araneda and Andrade, 1991; Tanaka and North, 1993). As such, 5-HT, by acting on multiple 5-HT receptor subtypes,

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may regulate how pyramidal cells function within prefrontal neuronal circuits.

These studies are beginning to lay the groundwork for an understanding, at the cellular level, of the effects of 5-HT in prefrontal cortex. However, most of these studies have focused on adult animals. This leaves an important gap in our knowledge, because perinatal factors are thought to play an important role in the normal development of cortex and also in the etiological processes leading to mental disorders involving prefrontal cortex, most notably schizophrenia and anxiety (Goldman-Rakic and Selemon, 1997; Raedler et al., 1998). To address this gap, we now investigate the cellular electrophysiological effects of 5-HT in developing prefrontal cortex.

Materials and Methods

Male Sprague Dawley rats (6–19, 25, and 35–45 d of age) were anesthetized with halothane and killed by decapitation. The brain was quickly removed and cooled in ice-cold Ringer solution of the following composition (in mM): 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 1 NaH_2PO_4, 26.2 $NaHCO_3$, and 11 glucose, bubbled to saturation with 95% O_2–5% CO_2. The anterior pole of the brain was then isolated and affixed to a stage with cyanoacrylate glue, and coronal slices (300 μm thick) were cut using a vibratome (Lancer series 1000; Ted Pella, Irvine, CA). The slices were then transferred to a holding chamber (Sakmann and Stuart, 1995) where they were allowed to recover for at least 1 hr in Ringer solution with the addition of 30 mM sucrose.

Electrophysiological recordings. Whole-cell patch-clamp recordings were obtained from pyramidal neurons of layer V of the prelimbic or anterior cingulate regions of the medial prefrontal cortex (Krettek and Price, 1977). For recording, slices were transferred one at a time to a recording chamber of standard design (Sakmann and Stuart, 1995) where they were perfused with Ringer (30–33°C) bubbled to saturation with 95% O_2–5% CO_2. Neurons were targeted using differential interference contrast (DIC) imaging on a fixed-stage upright microscope (Olympus BX50WI; Olympus Optical, Tokyo, Japan).

All current-clamp recordings were performed with the use of an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA), whereas those in voltage-clamp were performed with the use of either an Axoclamp 2A, Axopatch 200, or 200B or with an EPC 10 (Heka Elektronik, Lambrecht/Pfalz, Germany). Electrical signals were recorded on-line with the use of a paper chart recorder (model 3400; Gould Instruments, Valley View, OH) and digitized and stored in an Intel (Santa Clara, CA) processor-based personal computer. The recording pipettes were pulled from borosilicate glass (outer diameter, 1.2 mm) using a Flaming-Brown horizontal puller (model P80; Sutter Instruments, Novato, CA) to give resistance ranging from 4 to 10 MΩ when filled with an internal solution of the following composition (in mM): 115 potassium gluconate, 20 KCl, 2 $MgCl_2$, 10 HEPES, 4 Na_2ATP, 0.3 GTP, and 10 Na_2 phoshocreatine. The pH was adjusted to 7.3–7.4. The voltages reported in this study were not compensated for liquid junction potentials (~5–6 mV). The series resistance ranged from 8 to 25 MΩ. Neuron input resistance was determined by applying short-duration (140 msec) hyperpolarizing pulses (40–50 pA). In most voltage-clamp experiments, series resistance was corrected by ~70%. Experiments in which access resistance increased by more than ~30% were discarded. Some experiments involved simultaneous recordings from close neighboring cells; the maximal distance between these cells was ~300 μm.

Agonists [5-HT, (±)-2,5-dimethoxy-4-bromoamphetamine hydrochloride (DOB), α-methyl-5-HT and 5-CT] were applied to the slices either by drop applications (5–20 μl from a 1 mM solution) or by bath administration at known concentrations as indicated. Unless otherwise indicated, agonists were administered only once to each slice to minimize any potential impact of receptor desensitization. All antagonists were applied to the slice by bath administration at known concentration.

Histology. To positively identify pyramidal cells, some electrodes were filled with Alexa Fluor 488-hydrazide (250–500 μM) dissolved in the intracellular solution (Panchuk-Voloshina et al., 1999). Whole-cell recordings were obtained from visually identified putative pyramidal neurons as usual, and recording was maintained for at least 20 min to allow for adequate dye filling. After recording, slices were fixed in 4% paraformaldehyde, rinsed three times in PBS, and whole mounted in aqueous media (Polysciences, Warrington, PA). Filled neurons were imaged using an Olympus Fluoview laser scanning confocal microscope equipped with an argon laser.

Table 1. Primer sequences for RT-PCR

5-HT_{2A} receptor	Sequence	Position	Length of fragment
Primer 1			
Sense	5′ AGC CGCTTCAACTCCAGA A	609–627	410
Antisense	5′ TTTTGCTCATTGCTGATGGA	1018–999	
Primer 2			
Sense	5′ TGTGCGATCTGGATTTAACTGG	501–522	571
Antisense	5′ GGCACCACATTACAACAAACAGG	1071–1049	
5-HT_7 receptor			
Primer 1			
Sense	5′ TTACCTCCTCTCTTCGGATG	767–786	660
Antisense	5′ GTCTTACAGCACAAACTCGG	1426–1407	

Reverse transcriptase-PCR. In the present study, mRNA was amplified either from pooled RNA extracted from medial prefrontal cortex (anterior cingulate and prelimbic subdivisions) or from the cellular content of individual prefrontal cortex pyramidal neurons. Pooled RNA was isolated with the Micro RNA Isolation kit (Stratagene, La Jolla, CA) per the instruction of the manufacturer. For single-cell reverse transcriptase (RT)-PCR, the cytoplasmic content of visually identified pyramidal neurons was harvested essentially as described by Monyer and Jonas (1995). Briefly, recordings were obtained using nominally RNase-free recording solution. Pipette glass was made RNase free by heating at 200°C for at least 1 hr, and the electrode holder and silver chloride wire were treated with RNaseZap (Ambion, Austin, TX). The electrode contents (~7 μl) were expelled into an Eppendorf tube (Eppendorf Scientific, Westbury, NY) containing 20 U of Super RNasin (20,000 U/ml; Ambion) and sterile water for a final volume of 10 ml. Complete expulsion of the electrode content was obtained by gently breaking the tip on the tube wall while applying positive pressure. All subsequent procedures were the same for mRNA obtained from single cell and whole tissue. The cDNA was synthesized with the use of the SUPERSCRIPT first strand synthesis system (Invitrogen, Grand Island, NY) using 50 ng of random hexamers as per the instruction of the manufacturer. The cDNA generated from the reverse transcription step was subjected to conventional PCR using a programmable thermal cycler (PTC 200; MJ Research, Watertown, MA). Two rounds of PCR amplification were performed, each of 35 cycles. PCR primers were developed using PCGene software (Intelligenetics, Mountain View, CA). Two sets of primers were used to amplify the cDNA obtained from the RT reaction for the 5-HT_{2A} and one set for 5-HT_7 receptors. Primer sequences are given in Table 1. The amplification was performed in 10 mM Tris/HCl, 50 mM KCl, 1.5–2.5 mM MgCl, 200 mM dNTP mix, and 200 nmol of each primer, pH 8.3, with 5 U of *Taq*DNA polymerase (HotStarTaq Master Mix Kit; Qiagen, Chatsworth, CA). Restriction digest used *Xho*I for the 5-HT_{2A} receptor and *Taq*I for the 5-HT_7 receptor (Promega, Madison, WI). Parallel control experiments in which the cellular template for the RT-PCR reaction was replaced by water were invariably negative. We also conducted additional control experiments involving mock recordings followed by amplification of 5-HT_{2A} and 5-HT_7 receptor mRNA from the electrode contents. These control experiments were also generally negative (one of seven experiments resulted in amplification of 5-HT_{2A} receptor message).

In situ *hybridization.* Postnatal day (P) 3, P7, P14, P21, and P28 rats were decapitated and the brains postfixed in 4% paraformaldehyde in 0.1 M PBS for 1 week. Coronal brain sections containing tissue from the prefrontal cortex were obtained and processed for free-floating *in situ* hybridization as described previously (Basura and Walker, 1999; Walker et al., 2000). 5-HT_{2A} receptor cRNA probes were transcribed from a 900 bp *Pst*I fragment subcloned into a bluescript KS+ plasmid (Basura and Walker, 1999). 5-HT_7 cRNA probes were generated from BamH1 linear-

ized plasmid BE379 (Heidmann et al., 1998). The resulting ^{35}S-UTP-labeled antisense probes were subjected to alkaline hydrolysis to produce <200 nt probe fragments, which were phenol:chloroform extracted and ethanol precipitated. Before hybridization, tissue slices were partially digested by 10 mg/ml proteinase K and acetylated with 0.25% acetic anhydride. The sections were then incubated in hybridization buffer (50% deionized formamide, 5× saline sodium phosphate EDTA, 10% dextran sulfate, 5× Denhardt's solution, 100 mg/ml denatured ssDNA, 100 mg/ml *E. coli* tRNA, and 10 mM dithiothreitol) without radio-labeled probe for 60 min at 58°C. Sections were then hybridized in the same buffer overnight with either 2×106 cpm 5-HT$_7$ cRNA probe or 1×106 cpm 5-HT$_{2A}$ receptor cRNA probe at 58°C.

After hybridization, sections were treated with 10 mg/ml RNase A. Slices were mounted onto glass slides and exposed to autoradiographic film (Amersham Biosciences, Arlington Heights, IL) for 3 d (5-HT$_{2A}$ receptors) or 21 d (5-HT$_7$ receptors). Developed films were analyzed as scanned images using a Molecular Dynamics Personal Densitometer and ImageQuant software (Molecular Dynamics, Sunnyvale, CA). Changes in mRNA levels between sections were calculated by measuring the average optical density of the affixed region within the medial aspect of the prefrontal cortex. Background noise was determined by measuring optical density values in the genu of the corpus callosum and subtracted from all data. Graphed data represent arbitrary units on the basis of optical density values obtained from scanned images. Slices from all ages were processed in parallel.

Chemicals. (±)DOB, 5-carboxamidotryptamine (5-CT), 5-HT, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate salt (WAY 100635), and 6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yloxy)pyridin-3-yl carbamoyl] indoline (SB 242084) were purchased from Sigma (St. Louis, MO). Alexa Fluor 488 hydrazide was purchased from Molecular Probes (Eugene, OR). Tetrodotoxin (TTX) was purchased from Alomone Labs (Jerusalem, Israel). SB 269970 was a generous gift from SmithKline Beecham Pharmaceuticals (Harlow, UK). WAY 100478 was a generous gift from Wyeth-Ayerst Research (Princeton, NJ). *R*-(+)-α-(2,3-dimethoxyphenyl)-1-[4-fluorophenylethyl]-4-piperidinemethanol (MDL 100907) was a generous gift from Marion Merrel (Dow, Strasbourg, France) and from Dr. Kenner C. Rice (National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD).

Results

Electrophysiological characteristics of layer V pyramidal neurons in the developing prefrontal cortex

For these experiments, we focused on pyramidal neurons of layer V of the anterior cingulate and prelimbic subdivisions of the prefrontal cortex starting at P6 and extending to young adults. Pyramidal neurons were selectively targeted for whole-cell recording using DIC imaging and also identified by their electrophysiological characteristics (Connors and Gutnick, 1990). To test the reliability of this targeting procedure, we labeled a subset of these cells ($n = 19$) with the fluorescent dye Alexa 488 and reconstructed their dendritic arborization using laser scanning confocal microscopy (Fig. 1*A*). All labeled cells showed the morphological characteristics of cortical pyramidal neurons: an approximately pyramidal shaped soma from which protruded several basal dendrites and a single branching apical dendrite extending toward the brain surface.

The overall mean resting membrane potential of pyramidal neurons in the present study was −72 ± 0.53 mV. As illustrated in Figure 1*B*, there was a tendency for neurons to express more hyperpolarized potentials with increasing age. This small age-dependent change in resting membrane potential contrasted with the marked reduction in input resistance observed over the same period (Fig. 1*C*). In principle, such a reduction in input resistance with increasing age might be explained by a concurrent increase in expression of ion channels open at rest, such as the leak potassium channels. Consistent with this idea, we found in voltage-clamp experiments that the inward current induced by 2 mM barium, which blocks most leak potassium channels (Patel and Honore, 2001), became progressively larger with increasing age (P8, −24.1 ± 4.0 pA, $n = 5$; P11, −68.4 ± 7.9 pA, $n = 5$; P25, −100.9 ± 11.8 pA, $n = 9$; data not shown). Similar morphological and physiological changes across early postnatal development have been described previously in prefrontal cortex (Zhang, 2003a) and in cortex (Zhu, 2000; Tyzio et al., 2003) and was not studied further.

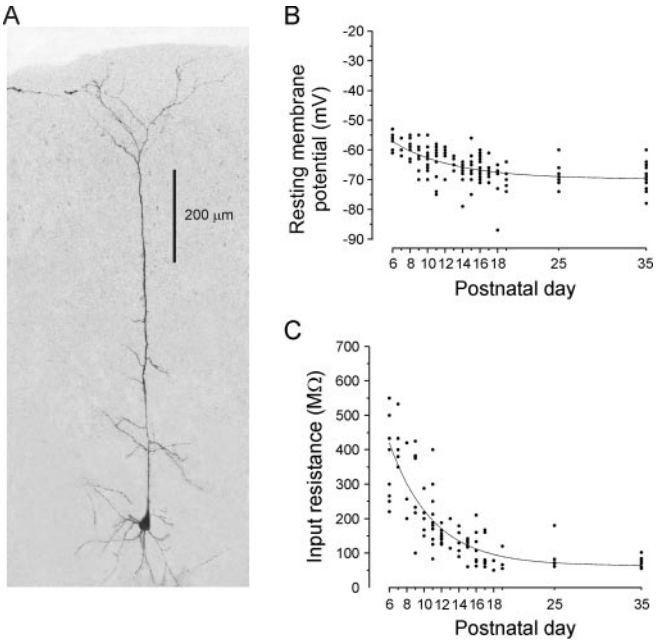

Figure 1. Electrophysiological properties of layer V pyramidal neurons of developing medial prefrontal cortex. *A*, Morphological reconstruction of one of the pyramidal neurons included in the present study. This neuron from a P12 animal was filled with Alexa 488 and reconstructed from a Z-stack using laser scanning confocal microscopy. *B*, Resting membrane potential of layer V pyramidal neurons across postnatal development. Note the tendency of these neurons to display more hyperpolarized membrane potentials with increasing age. *C*, Input resistance of layer V pyramidal neurons across postnatal development. The input resistance of these cells, determined using short hyperpolarizing current pulses in current clamp, markedly decreased during the first 2 weeks of postnatal life.

The effects of 5-HT on membrane potential of prefrontal cortical neurons exhibit a developmental switch

As illustrated in Figure 2, the ability of 5-HT to regulate membrane potential in layer V pyramidal neurons of the prefrontal cortex was found to exhibit a striking developmental regulation ($n > 200$ cells). In slices derived from young pups (<P15), applications of 5-HT consistently induced a robust depolarization–inward current (Fig. 2*A*). In the course of the third postnatal week, however, this depolarization subsided and was gradually replaced by a hyperpolarization–outward current (Fig. 2*B*). This hyperpolarization–outward current became the dominant response to 5-HT by the fourth postnatal week.

Figure 2*C* illustrates a systematic quantification of this shift. For these experiments, we used brief bolus applications of 5-HT in the presence of TTX (1 μM) to rapidly assess the net effect of 5-HT on membrane potential of layer V pyramidal neurons while eliminating the confound induced by concomitant increases in synaptic activity (see below). Using this method, 5-HT was found to elicit a consistent depolarization between the fifth and fifteenth postnatal day that averaged 3.9 ± 0.26 mV ($n = 43$ cells). This

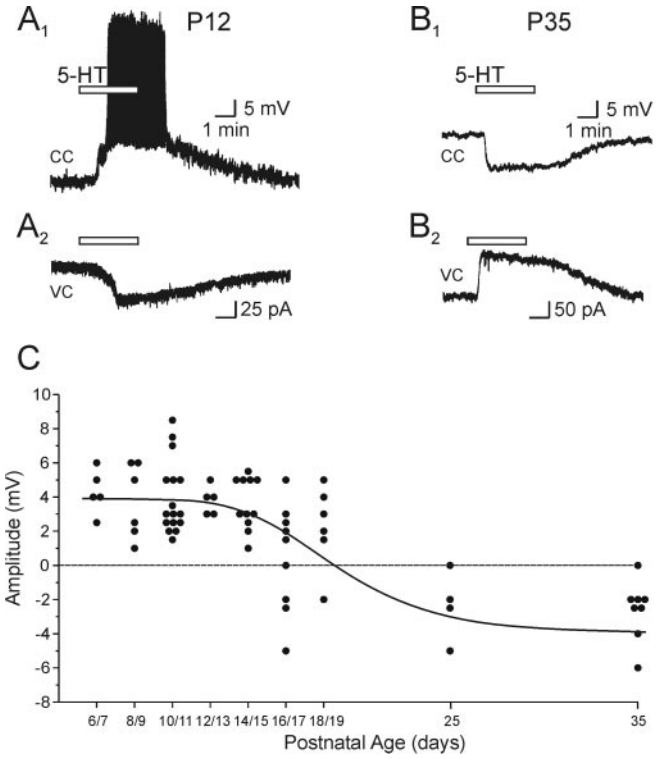

Figure 2. The effects of 5-HT on membrane excitability of layer V pyramidal neuron are regulated across postnatal development of the rat prefrontal cortex. A_1, In a P12 animal, bath administration of 5-HT (30 μM) induced a prominent depolarization of sufficient magnitude to elicit action potential discharge in this layer V pyramidal neuron, as revealed in this current-clamp (CC) recording. The resting membrane potential (Vm) of this neuron was −64 mV. A_2, The underlying inward current induced by 5-HT is shown in this current trace obtained in voltage-clamp (VC) mode from the same neuron. B_1, In a P35 animal, bath administration of 5-HT (30 μM) induced a membrane hyperpolarization (Vm, −65 mV) in a layer V pyramidal neuron. B_2, The underlying outward current is shown in this current trace obtained in voltage-clamp mode from the same neuron. *C*, Scatter graph showing the peak amplitude of the change in membrane potential of pyramidal neurons induced by drop application of 5-HT in the presence of TTX (1 μM). Data were collected from animals of all ages across the P6–P19 developmental window, binned at 2 d, and of P25 and P35 animals.

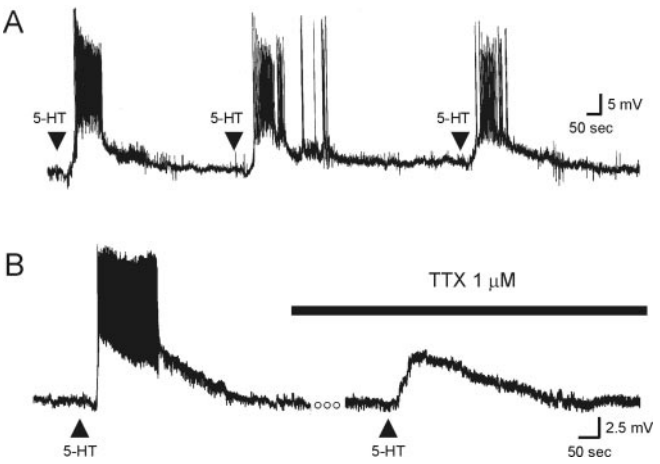

Figure 3. During the P6–P19 developmental period, 5-HT induces a robust, generally non-desensitizing membrane depolarization of layer V pyramidal neurons. *A*, Successive drop applications of 5-HT reliably depolarized and induced action potential discharge in this layer V pyramidal neuron from a P6 rat, as shown in this voltage trace (Vm, −66 mV). In this and subsequent figures, the arrow represents the time of agonist application. *B*, In this neuron from a P9 animal (Vm, −65 mV), application of 5-HT induced a membrane depolarization of sufficient magnitude to induce action potential discharge. TTX (1 μM) completely blocked the 5-HT-induced discharge of action potentials, revealing the underlying membrane depolarization. The open circles represent a period of 4 min.

depolarization was generally accompanied by either a small decrease ($n = 12$) or no change ($n = 8$) in input resistance (data not shown). Commencing at P16, an increasing fraction of cells displayed hyperpolarizing rather than depolarizing responses to 5-HT. By P25, most cells examined responded to 5-HT administration with a hyperpolarization that averaged 2.5 ± 0.5 mV ($n =$ 12 cells).

Concomitant with these effects of membrane potential, 5-HT also induced a strong increase in spontaneous synaptic activity at all ages tested (not shown). In the rest of this study, we report on experiments aimed at elucidating the mechanisms underlying the effects of 5-HT on membrane potential during development. A systematic analysis of the 5-HT-induced increase in spontaneous synaptic activity has been published previously (Béïque et al., 2004).

5-HT depolarizes and excites pyramidal cells during early postnatal development

The 5-HT-induced depolarization observed between P6 and P19 ranged from 1 to 8.5 mV and was often sufficient to bring the cell to threshold and initiate firing (Fig. 3*A*,*B*). In the current sample, 12 of 29 neurons tested in current clamp in the absence of TTX were depolarized enough to initiate spiking. With the relatively brief applications used for these experiments, no obvious signs of desensitization of the excitatory effects of 5-HT were observed in 10 of 13 neurons tested (Fig. 3*A*). This ability of 5-HT to induce action potential discharge was observed more frequently, but not exclusively, in neurons from younger animals. Administration of TTX to cells depolarized to spiking by 5-HT revealed robust 5-HT-induced depolarizations (Fig. 3*B*), suggesting that the direct, postsynaptic, depolarizing effect of 5-HT rather than the increase in spontaneous excitatory synaptic activity was primarily responsible for the spiking activity.

Activation of 5-HT$_{2A}$ receptors contributes to the 5-HT-induced depolarization

Previous work has shown that activation of different 5-HT receptor subtypes can trigger depolarizing or hyperpolarizing membrane potential responses in various CNS neurons (Andrade and Nicoll, 1987; Araneda and Andrade, 1991; Andrade and Chaput, 1991; Tanaka and North, 1993; Chapin and Andrade, 2001). Therefore, we hypothesized that the shift in the effects of 5-HT on membrane potential observed above could result from a developmentally regulated change in the functional expression of two or more 5-HT receptor subtypes. To test this idea, we conducted a pharmacological analysis of the effects of 5-HT.

5-HT can depolarize pyramidal cells in the adult rat cortex by activating 5-HT$_{2A}$ receptors (Davies et al., 1987; Pierce and Peroutka, 1990; Araneda and Andrade, 1991; Tanaka and North, 1993; Arvanov et al., 1999). We therefore tested whether this receptor subtype could mediate the 5-HT-induced depolarization seen in this region during the first 2–3 postnatal weeks. As illustrated in Figure 4*A*, brief applications of the broad spectrum 5-HT$_2$ agonist DOB (Titeler et al., 1985; Titeler et al., 1987) depolarized a significant fraction of pyramidal cells (~50%) and thus partly mimicked the effects of 5-HT (Fig. 4*A*) ($n = 45$ of 91 cells tested; P6–P19). However, in contrast to the effect of 5-HT, the DOB-induced depolarization was associated with a small but consistent increase in input resistance ($n = 5$). This ability of DOB to induce a membrane depolarization was blocked by the

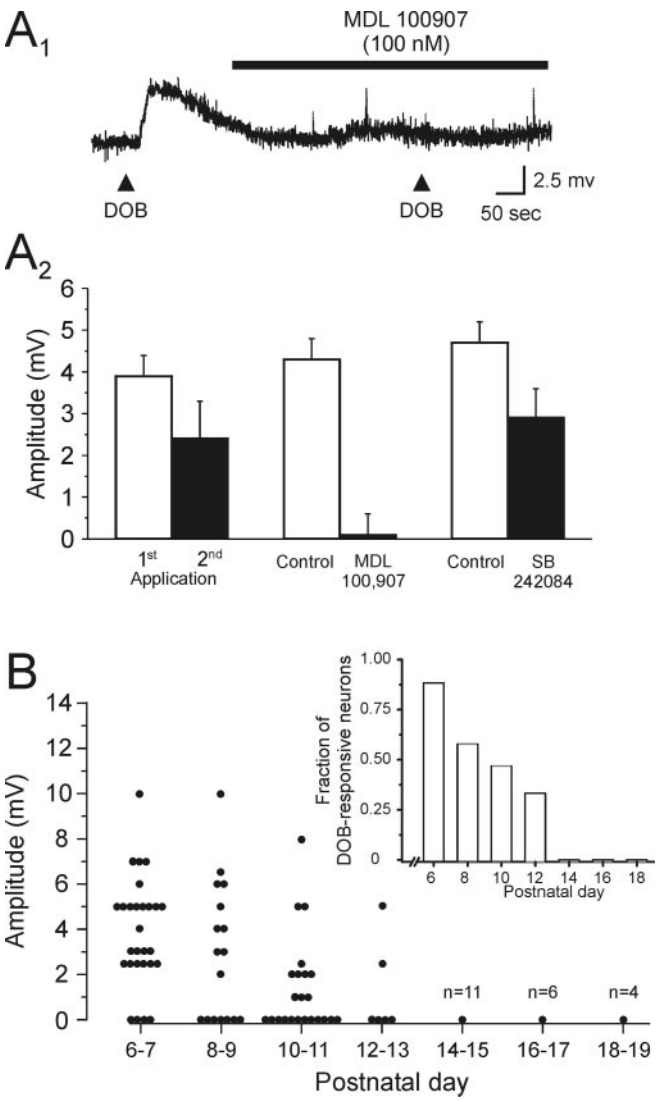

Figure 4. Activation of 5-HT_{2A} receptors depolarizes layer V pyramidal neurons of the rat prefrontal cortex. *A_1*, Application of the selective 5-HT_2 receptor agonist DOB induced a depolarization of this layer V pyramidal neuron (P11; Vm, −69 mV). This effect of DOB was blocked by the selective 5-HT_{2A} receptor antagonist MDL 100907 (100 nM). This recording was conducted in the presence of 1 μM TTX. *A_2*, Graph summarizing our pharmacological analysis of the DOB-induced depolarization. The response induced by DOB exhibited a small desensitization determined by two successive applications (8–12 min apart). The depolarizing response to DOB was completely blocked by bath administration of the selective 5-HT_{2A} receptor MDL 100907 (100 nM), whereas the reduction of the DOB-induced depolarization by the selective 5-HT_{2C} antagonist SB 242084 (100 nM) was similar to that expected from desensitization alone. *B*, Scatter graph showing the peak amplitude of the depolarization induced by application of DOB during the P6–P19 developmental period. Inset, Histogram showing the fraction of neurons that exhibited a depolarizing response to application of DOB across the same developmental window.

5-HT_2 antagonist ketanserin (1 μM; $n = 3$; data not shown), indicating the involvement of 5-HT_2 receptors in this response.

The 5-HT_2 receptor family is composed of three distinct subtypes (5-$HT_{2A\text{-}C}$). By using subtype-selective antagonists, we next sought to determine which of these accounted for the 5-HT_2 receptor depolarization observed here. Because 5-HT_{2B} receptors are not expressed at detectable levels in prefrontal cortex (Pompeiano et al., 1994), the main goal of these experiments was to distinguish between the involvement of 5-HT_{2A} and 5-HT_{2C} receptors. To accomplish this, we compared the abilities of the 5-HT_{2A} selective antagonist MDL 100907 and the 5-HT_{2C} selective antagonist SB 242084 to inhibit the DOB-induced depolarization. This comparison was complicated by the susceptibility of the DOB responses to partially desensitize (Fig. 4*A_2*). Therefore, we tested for an effect of MDL 100907 and SB 242084 beyond the decrement expected from desensitization alone. As illustrated in Figure 4, *A_1* and *A_2*, when the selective 5-HT_{2A} antagonist MDL 100907 (100 nM; $K_i = 0.85$ and 88 nM for 5-HT_{2A} and 5-HT_{2C} receptors, respectively) (Kehne et al., 1996; Roth et al., 2000) was administered between the first and second application of DOB, the ability of this agonist to elicit a membrane depolarization was completely suppressed ($n = 6$ cells; $p < 0.01$; paired Student's *t* test). In contrast, when the selective 5-HT_{2C} receptor antagonist SB 242084 (100 nM; $K_i = 1$ and 158 nM for 5-HT_{2C} and 5-HT_{2A} receptors, respectively) (Kennett et al., 1997; Roth et al., 2000) was administered, the mean depolarization elicited by a second application of DOB was comparable with that seen under control conditions (Fig. 4*A_2*). These results indicate that the depolarization induced by administration of DOB was predominantly, if not exclusively, mediated by activation of 5-HT_{2A} receptors.

Although the vast majority of pyramidal neurons of layer V responded to 5-HT with a depolarization during the P6–P19 developmental period, overall, only approximately half of the cells (45 of 91 cells tested) responded similarly to administration of DOB. During the course of these experiments, we observed that the ability of DOB to elicit a membrane depolarization was age dependent, such that only cells derived from younger animals were responsive to this agonist. In fact, as illustrated in Figure 4*B*, application of DOB induced a significant depolarization only in neurons derived from rats younger than P14. This decrease was reflected not only in the fraction of cells responding to DOB (Fig. 4*B*, inset) but also in the amplitude of the DOB response among responsive cells (Fig. 4*B*). These results indicated a strong developmental regulation of 5-HT_{2A} receptor function.

Activation of 5-HT_7 receptors also contributes to the 5-HT-induced depolarization

The results outlined above suggest that activation of 5-HT_{2A} receptors alone cannot account for the depolarizing actions of 5-HT observed during the first 3 weeks of life. Consistent with this idea, the depolarization induced by 5-HT was at least partly resistant to blockade by bath administration of the 5-HT_2 receptor antagonist ketanserin (1 μM; five of seven cells tested). These results indicated the presence of a second 5-HT receptor capable of depolarizing pyramidal cells in the developing prefrontal cortex.

Seeking to identify this second receptor, we tested 5-CT, a broad spectrum serotonergic agonist capable of activating a limited subset of 5-HT receptors, most notably those of the 5-HT_1 and 5-HT_{5-7} subtypes. Surprisingly, administration of 5-CT also elicited a membrane depolarization and thus mimicked the effect of 5-HT on pyramidal cells during the first three postnatal weeks (Fig. 5*A_1*,*B_1*) (51 of 59 cells tested). However, this depolarization differed from that elicited by DOB in some important respects. Most notably, it was associated with a small decrease in input resistance (8 of 12 cells tested) and displayed little, if any, desensitization ($n = 3$ cells). These results suggest that 5-HT acted on an additional, non-5-HT_{2A} receptor to depolarize pyramidal cells of the developing prefrontal cortex.

Previous studies in brain have shown that 5-CT, acting on 5-HT_7 receptors, can elicit a slow membrane depolarization analogous to that observed here (Cardenas et al., 1999; Chapin and Andrade, 2001). Therefore, we examined the ability of the highly selective 5-HT_7 receptor antagonist SB 269970 ($K_i = 1$ nM for

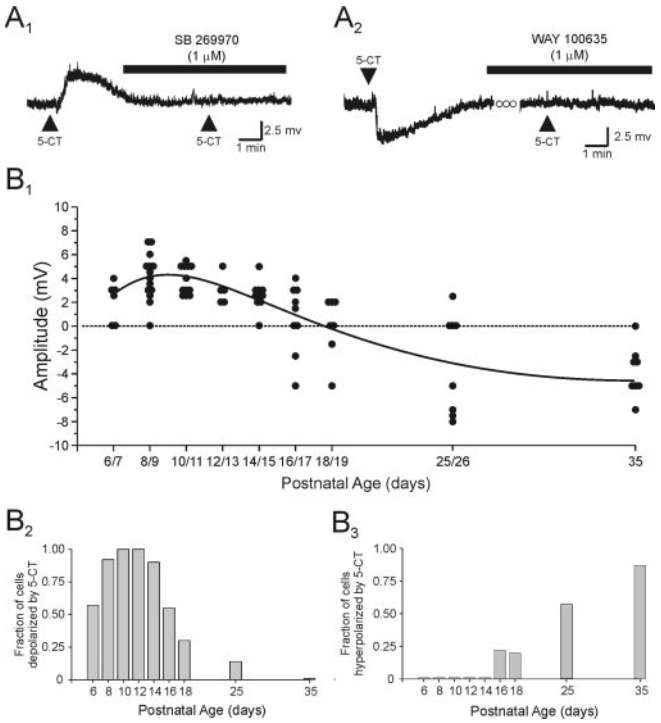

Figure 5. Activation of 5-HT$_7$ receptors induces a membrane depolarization, which is gradually replaced by a 5-HT$_{1A}$ receptor-mediated hyperpolarization with increasing age. *A$_1$*, In this neuron from a P10 animal, application of the 5-HT$_{1/7}$ receptor agonist 5-CT induced a depolarization that was completely blocked by bath application of the selective 5-HT$_7$ receptor antagonist SB 269970 (1 μM). This recording was conducted in the presence of 1 μM TTX. Vm, −70 mV. *A$_2$*, In this neuron from a P25 animal (Vm, −68 mV), application of 5-CT induced a hyperpolarization, which was blocked by bath application of the selective 5-HT$_{1A}$ receptor antagonist WAY 100 635 (1 μM). *B$_1$*, Scatter graph showing the peak change in membrane potential induced by application of 5-CT across postnatal development. *B$_2$*, Histogram showing the fraction of neurons tested that exhibited a membrane depolarization in response to application of 5-CT as a function of the age of the animal. *B$_2$*, Histogram showing the fraction of neurons that exhibited a membrane hyperpolarization in response to application of 5-CT as a function of the age of the animal.

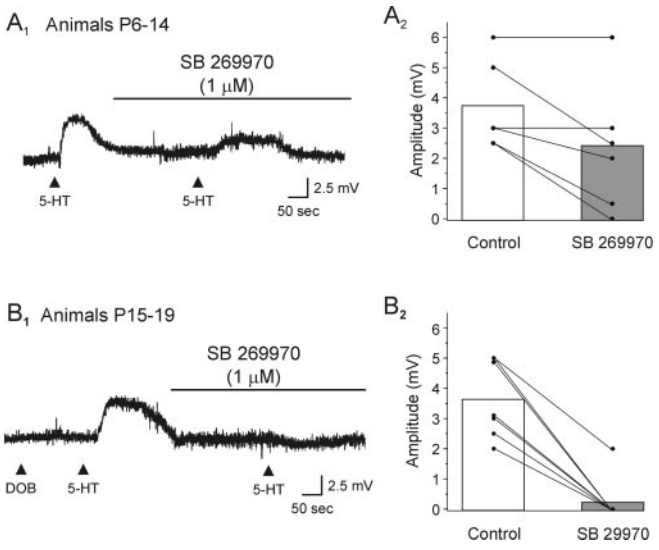

Figure 6. The 5-HT-induced depolarization is mediated by activation of both 5-HT$_{2A}$ and 5-HT$_7$ receptors. *A$_1$*, In this neuron from a P10 animal, the depolarization induced by the application of 5-HT was only partially blocked by SB 269970 (1 μM). Vm, −65 mV. *A$_2$*, Plot illustrating the effects of SB 269970 on the 5-HT-induced depolarization in slices derived from animals P6–P14. SB 269970 only partially antagonized the depolarization elicited by 5-HT in these slices. *B$_1$*, In this DOB-unresponsive neuron taken from a P17 animal, the depolarization induced by 5-HT was completely blocked by SB 269970 (1 μM). Vm, −71 mV. *B$_2$*, Plot illustrating the effects of SB 269970 on slices derived from animals P15–P19. SB 269970 (1 μM) essentially abolished the ability of 5-HT to depolarize pyramidal cells in these slices.

5-HT$_7$ receptors) (Hagan et al., 2000; Lovell et al., 2000) to inhibit the depolarizing action of 5-CT. As illustrated in Figure 5*A*, bath application of SB 269970 (1 μM) completely blocked the ability of 5-CT to depolarize pyramidal cells ($n = 14$), thus identifying the additional receptor involved as belonging to the 5-HT$_7$ subtype.

5-HT$_{2A}$ and 5-HT$_7$ receptors are coexpressed on pyramidal cells of the developing prefrontal cortex

Based on the results outlined above, we hypothesized that, early in postnatal development, the response mediated by 5-HT in an individual neuron would result from the coactivation of 5-HT$_7$ and 5-HT$_{2A}$ receptors, whereas later (P14–P19) it would result predominantly from the activation of 5-HT$_7$ receptors. Consistent with this idea, 5-HT-induced depolarizations were only partially sensitive to blockade by the 5-HT$_2$ receptor antagonist ketanserin and could be mimicked by both DOB and 5-CT in the same neuron (three of four neurons) only during the early postnatal period (<P14). In addition, we found that in animals younger than P14, the depolarization induced by application of 5-HT was only partially inhibited by bath administration of the selective 5-HT$_7$ receptor antagonist SB 269970 (1 μM) (Fig. 6*A*). In contrast, in animals P14–P19, the 5-HT-induced depolarization was completely blocked by SB 269970 in the vast majority of cells tested (six of seven cells tested) (Fig. 6*B*). These results support the idea that early in postnatal development (<P14), the depolarization induced by 5-HT generally involves the coactivation of both 5-HT$_{2A}$ and 5-HT$_7$ receptors, whereas later (between P15 and P19) the depolarizing response is mediated primarily by 5-HT$_7$ receptors.

The depolarization induced by 5-HT$_7$ receptors is gradually replaced by a 5-HT$_{1A}$ receptor-mediated hyperpolarization during postnatal development

As outlined above, although the overwhelming effect of 5-HT on pyramidal cell membrane potential in the P6–P15 developmental period was a membrane depolarization commencing at approximately P16, a progressively large fraction of cells began to display hyperpolarizing rather than depolarizing responses (Fig. 2*B*,*C*). Because adult pyramidal cells express robust 5-HT$_{1A}$ receptor-induced hyperpolarizations (Araneda and Andrade, 1991; Tanaka and North, 1993), we hypothesized that the hyperpolarizations appearing at P16–P19 could represent the beginning of a developmentally regulated increase in 5-HT$_{1A}$ receptor responsiveness. Consistent with this idea, the response to 5-CT, which is also a 5-HT$_1$ receptor agonist, shifted from a membrane depolarization to a hyperpolarization between P16 and P25, thus mimicking the shift seen with 5-HT (Fig. 5*B*). Most significantly, the hyperpolarizing responses elicited by 5-HT ($n = 5$) as well as 5-CT ($n = 4$) were completely blocked by bath administration of the selective 5-HT$_{1A}$ antagonists WAY 100635 (1 μM; $K_i = 0.5$ nM for 5-HT$_{1A}$ receptors) (Hamon et al., 1990) or WAY 100478 (1 μM; $K_i = 10–32$ nM for 5-HT$_{1A}$ receptors) (Roth et al., 2000). These results identified the receptor involved in the late developing hyperpolarization as being of the 5-HT$_{1A}$ subtype.

Interestingly, with only few exceptions between P16 and P18, administration of these 5-HT$_{1A}$ antagonists did not unmask depolarizing responses to 5-HT. Likewise, only in one case (P18) was the depolarizing response induced by 5-HT converted to a

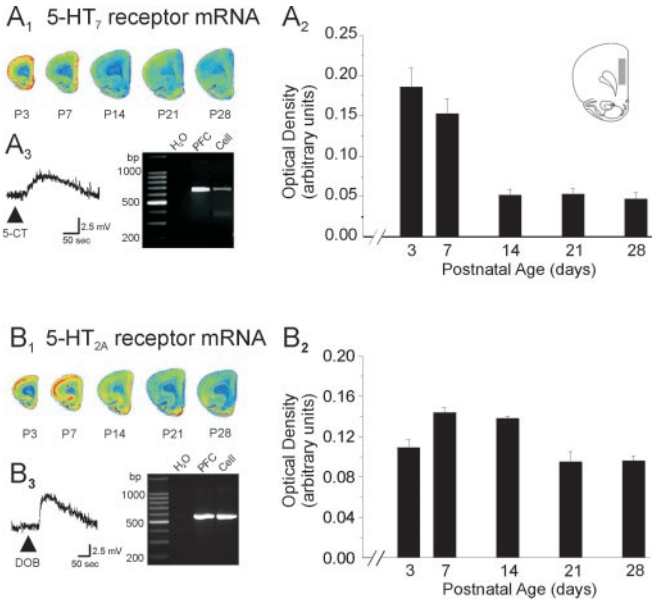

Figure 7. Expression of 5-HT_{2A} and 5-HT_7 receptors mRNA during the postnatal period. A_1, B_1, Pseudo-color autoradiographic image of cortical slices derived from rats of different ages are shown after hybridization with probes for the 5-HT_7 receptor mRNA (A_1) and 5-HT_{2A} receptor mRNA (B_1). The quantification of 5-HT_7 (A_2) and 5-HT_{2A} (B_2) receptor mRNA expression in the cingulate and prelimbic subdivisions of the medial prefrontal cortex is plotted across postnatal development. The area used to obtain the optical readings is illustrated in the inset of A_2. This area corresponds to the area from which the electrophysiological recordings were performed. Graphed data depict optical density measurements expressed in arbitrary units. Data represent the mean ± SEM of four animals per age group. A_3, Ethidium bromide-stained agarose gel showing the products of RT-PCR reactions amplifying 5-HT_7 receptor mRNA from whole prefrontal cortex and from the pyramidal cell of which the voltage response to 5-CT is shown. Vm, −64 mV; P15 animal. The H_2O lane depicts the results of a negative control using water as template. B_3, Ethidium bromide-stained agarose gel showing the products of RT-PCR reactions amplifying 5-HT_{2A} receptor mRNA from whole prefrontal cortex and from the pyramidal cell of which the voltage response to DOB is depicted. Vm, −71 mV; P10 animal.

small hyperpolarization by bath administration of SB 269970 (P18). These observations suggest a relatively rapid, within-cell transition from the expression of a 5-HT_7 receptor-mediated depolarization to a 5-HT_{1A} receptor-mediated hyperpolarization. Together, these results outline a developmentally regulated progression in 5-HT responsiveness involving the gradual replacement of the early 5-HT_{2A}–5-HT_7 receptor-mediated depolarization by a late developing 5-HT_{1A} receptor-mediated hyperpolarization in the rat prefrontal cortex.

Distinct mechanisms account for the decrease in 5-HT_{2A} and 5-HT_7 receptor-mediated depolarizations and the appearance of 5-HT_{1A} receptor-mediated hyperpolarizations during development

In principle, the gradual loss of 5-HT_{2A} and 5-HT_7 receptor-mediated depolarizations and the appearance of the 5-HT_{1A} receptor-mediated hyperpolarization could reflect changes in receptor expression, receptor-effector coupling, and the loss–gain of the downstream ion channels responsible for the changes in membrane potential. As a first step to distinguish between these possibilities, we used *in situ* hybridization and single-cell RT-PCR to directly test for changes in the expression of 5-HT_7 and 5-HT_{2A} receptor mRNA during the first postnatal month.

As illustrated in Figure 7*A*, *in situ* experiments showed that 5-HT_7 receptor mRNA levels in layer V of prefrontal cortex were greatest in the first few days after birth and remained elevated for the first postnatal week. However, 5-HT_7 receptor mRNA expression decreased rapidly after that period, attaining low to very low levels by P14–P28. This is consistent with previous observations indicating low levels of 5-HT_7 mRNA in adult prefrontal cortex (Ruat et al., 1993; Gustafson et al., 1996; Vizuete et al., 1997; Heidmann et al., 1998). Because pyramidal cells are the predominant neuronal type in layer V, it is likely that a significant fraction of the 5-HT_7 mRNA detected by the *in situ* hybridization reflects expression in these cells. Consistent with this idea, using single-cell RT-PCR, we could amplify 5-HT_7 receptor mRNA from layer V pyramidal neurons (19 of 23 cells from P7, P8, P9, and P15 animals). As illustrated in Figure 7A_3, 5-HT_7 receptor mRNA was detectable in a subset of these cells shown to be responsive to 5-CT before the mRNA harvesting procedure (9 of 11 cells). Together, these observations indicate that a large fraction of layer V pyramidal neurons expresses mRNA coding for 5-HT_7 receptors during early postnatal development.

In contrast to the expression pattern seen for 5-HT_7 receptors, 5-HT_{2A} receptor mRNA expression in the prefrontal cortex was highest between P7 and P14 and remained elevated throughout the first postnatal month (P3–P28) (Fig. 7*B*). This persistent expression appears to be at odds with the profound loss of the ability of 5-HT_{2A} receptor activation to elicit a membrane depolarization during the first 2–3 postnatal weeks. Such a discrepancy could be explained if 5-HT_{2A} receptors, as detected by *in situ* hybridization, were expressed in cells other than those we recorded. However, we could detect 5-HT_{2A} receptor mRNA using single-cell RT-PCR in pyramidal cells during the P6–P15 developing period (17 of 32 cells tested and 6 of 13 cells shown to be responsive to DOB) (Fig. 7B_3). These results suggest that the loss of 5-HT_{2A} receptor-induced depolarization seen during the early postnatal period cannot simply be accounted for by a loss of 5-HT_{2A} receptors but rather involves a different mechanism.

The waning of the 5-HT_{2A} receptor-mediated depolarization in the face of persistent 5-HT_{2A} receptor expression could result from a reduction in receptor-effector coupling or from the loss of the ion channels responsible for the depolarization. Our testing protocol may be expected to be very sensitive to changes in receptor coupling, because it relies on brief applications of a partial agonist (DOB). Therefore, we tested whether more prolonged applications of full 5-HT_{2A} agonists could rescue the 5-HT_{2A} receptor-mediated effect on membrane potential at a time when DOB was ineffective (P15–P19). For these experiments, we directly measured the 5-HT_{2A} receptor-induced inward current, because this procedure facilitated the averaging of the response over multiple cells. Bath administration of 5-HT (30 μM; in the presence of 1 μM SB 269970 to block 5-HT_7 receptors; $n = 7$) or α-methyl 5-HT (10 μM; $n = 13$), a preferential 5-HT_2 receptor agonist, elicited small but clearly detectable inward currents (Fig. 8*B*) (see below). The effect of 5-HT (in SB 269970; $n = 4$) and α-methyl 5-HT ($n = 6$) was blocked by MDL 100 907 (300 nM-1 μM; data not shown), indicating that they involved activation of 5-HT_{2A} receptors. As such, these observations support the idea that both the 5-HT_{2A} receptors and the channels responsible for the depolarization–inward current were still expressed in juvenile (P15–P19) pyramidal cells.

If this reduction in 5-HT_{2A} responses reflected a weakening in receptor-effector coupling, then it could be expected that manipulations that facilitate G-protein signaling would become more effective with increasing age. To test this prediction, we used the poorly hydrolysable GTP analog GTPγS, a compound that can be expected to amplify G-protein-mediated responses by limiting the rate of GTP hydrolysis by the G-α subunit. If the reduction in

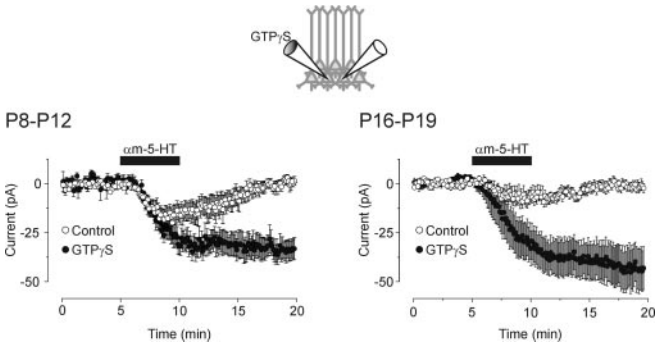

Figure 8. Intracellular GTPγS facilitates the ability of 5-HT_{2A} receptor to elicit an inward current in the developing prefrontal cortex. Left, Ensemble average of voltage-clamp recordings from P8–P12 slices showing that bath administration of αM-5-HT (10 μM) induced a small inward current in control cells (open circles). αM-5-HT (10 μM) induced a larger, nonrecovering, inward current in cells recorded with an intracellular recording solution supplemented with GTPγS (100 μM). Voltage-clamp recordings were simultaneously obtained from two close neighboring cells (as illustrated in the inset) with electrodes containing GTP alone or GTP plus GTPγS. Neurons were held at −70 mV, and the holding currents were sampled every 6 sec and then averaged across recordings. This plot is constructed from data obtained in six paired recordings. Right, Ensemble average of recordings obtained in P16–P19 slices showing that administration of αM-5-HT (10 μM) induced an inward current of much greater amplitude in cells perfused with intracellular GTPγS than in cells recorded with a control intracellular solution ($n = 13$ pairs). Note that the potentiation induced by GTPγS was itself of greater magnitude in slices derived from the older animals (P16–P19) than in those derived from younger animals (P8–P12).

5-HT_{2A} receptor responsiveness during development reflected a weakening of receptor-effector coupling, then it can be expected that GTPγS would facilitate the 5-HT_{2A} receptor-induced inward current more efficiently in slices derived from animals older than P14 than in slices from younger animals. In agreement with this conjecture, infusion of GTPγS only modestly potentiated the 5-HT_{2A} receptor-mediated inward in slices derived from P8–P12 animals (1.75-fold; measured at $t = 10$ min) (Fig. 8*A*). However, this same manipulation resulted in a much larger facilitation of the 5-HT_{2A} receptor-induced inward current in slices derived from P16–P19 animals (5.5-fold; $p < 0.05$) (Fig. 8*B*). Together, these results suggest that the decrease in 5-HT_{2A} receptor responsiveness observed during development does not result from a reduction in the expression of 5-HT_{2A} receptors or of the ion channels responsible for the depolarization but involve a weakening of the 5-HT_{2A} receptor-effector coupling.

In a final set of experiments, we examined the mechanisms underlying the late appearance of the 5-HT_{1A} receptor-mediated hyperpolarizations. Previous studies have shown that the 5-HT_{1A} receptor-mediated hyperpolarization seen in many regions of the brain is mediated by the activation of G-protein-coupled inwardly rectifying K+ channels (GIRKs; Kir 3.x) (Andrade and Nicoll, 1987; Colino and Halliwell, 1987; Ehrengruber et al., 1997; Luscher et al., 1997). Previous studies have shown that the expression of both 5-HT_{1A} receptors (Daval et al., 1987; Miquel et al., 1994) and GIRK potassium channels (Chen et al., 1997) in cortex increases during the postnatal period. Therefore, the appearance of 5-HT_{1A} receptor-induced depolarizations could have resulted in principle from the expression of either of these proteins, although close to maximal levels of GIRK expression are already seen by P10 (Chen et al., 1997). To distinguish between these possibilities, we took advantage of the fact that GIRK channels can be convergently regulated by 5-HT_{1A} and $GABA_B$ receptors (Andrade et al., 1986; Luscher et al., 1997), the latter being, in turn, expressed in cortex during the early postnatal period (Turgeon and Albin, 1994). This allowed us to test for the expression of GIRK channels independently of 5-HT_{1A} receptors. Activation of the $GABA_B$ receptors using baclofen (10–30 μM) consistently elicited a membrane hyperpolarization–outward current throughout postnatal development (P12–P25; data not shown; $n = 7$), indicating the expression of functional GIRK channels during this developmental epoch. These results suggest that the appearance of 5-HT_{1A} receptor-mediated hyperpolarization stems primarily from a developmental regulation of 5-HT_{1A} receptor expression per se rather than from regulation of downstream effectors.

Discussion

In the present study, we have examined the actions of 5-HT on membrane potential in layer V pyramidal neurons of the rat prefrontal cortex. We find a remarkable shift in the effects of 5-HT across development. During the first 2 postnatal weeks, 5-HT elicits a consistent membrane depolarization that can often reach threshold and initiate spiking activity. However, this effect gradually subsides during the third postnatal week, shifting toward a membrane hyperpolarization. It is this later effect that dominates the membrane response to 5-HT seen in adults. Results from a combined pharmacological, physiological, and molecular biological analysis indicate that this developmental progression is the result of coordinated changes in the expression and function of three distinct 5-HT receptor subtypes.

Early in development, the predominant effect of 5-HT on the membrane potential of pyramidal cells of layer V of prefrontal cortex was a slow membrane depolarization. We found that the main receptor contributing to the 5-HT-induced depolarization in developing prefrontal cortex was of the 5-HT_7 subtype. Thus, the 5-$HT_{1/6-7}$ agonist 5-CT mimicked the depolarizing effect of 5-HT through the first 2 postnatal weeks, and this effect was blocked by bath administration of the selective 5-HT_7 receptor antagonist SB 269970. This pharmacological identification is supported by results from *in situ* hybridization and single-cell RT-PCR experiments that demonstrated the presence of mRNA coding for the 5-HT_7 receptor in pyramidal cells derived from animals of this same age group.

However, activation of 5-HT_7 receptors cannot completely account for the depolarizing effects of 5-HT. Most notably, during the P6–P15 period, the effect of 5-HT was only partially inhibited by SB 269970, was mimicked by DOB, and was partially inhibited by the 5-HT_2 receptor antagonist ketanserin. These results indicate that a 5-HT_2 receptor also contributed to the response to 5-HT during this early time period. Consistent with the involvement of 5-HT_{2A} receptors, single-cell RT-PCR demonstrated the expression of 5-HT_{2A} mRNA in pyramidal neurons of layer V, and the DOB-induced depolarization was blocked by the selective 5-HT_{2A} receptor antagonist MDL 100907 but not the selective 5-HT_{2C} receptor antagonist SB 242084. These results identify 5-HT_{2A} receptors as secondary contributors to the depolarization induced by 5-HT during the postnatal period.

The effect of 5-HT on membrane potential exhibited a gradual shift from a depolarization to a hyperpolarization beginning in the third postnatal week. This late developing hyperpolarizing response becomes first detectable at P16–P17 and progresses steadily with age to become the dominant effect of 5-HT on membrane potential in adult animals (P35 and beyond). Pharmacological analysis of this response indicates that it was mimicked by 5-CT and blocked by the selective 5-HT_{1A} receptor antagonists WAY 100635 and WAY 100478, thus identifying the receptor involved as belonging to the 5-HT_{1A} subtype. These

results are consistent with previous results identifying 5-HT_{1A} receptor-mediated hyperpolarizations in adult prefrontal cortex (Araneda and Andrade, 1991; Tanaka and North, 1993).

Interestingly, different mechanisms seem to account for the changes in 5-HT_7, 5-HT_{2A}, and 5-HT_{1A} receptor function during development in prefrontal cortex. In the case of 5-HT_7 and 5-HT_{1A} receptors, these changes appear attributable to changes in receptor expression. Thus, early in development, 5-HT_7 receptor mRNA is abundantly expressed in prefrontal cortex, specifically in pyramidal cells, but this expression diminishes dramatically during the second and third postnatal weeks. This reduced mRNA expression is accompanied by a parallel decrease in 5-HT_7 receptor function detected by electrophysiological experiments, albeit with a delay of several days. Assuming a half-life for the 5-HT_7 receptor protein comparable with that seen for other 5-HT receptors in cortex (Pinto and Battaglia, 1994), the observed decrease in functional expression of 5-HT_7 receptors seems likely to be consequential to the decrease in 5-HT_7 receptor mRNA. A similar but opposite situation applies to 5-HT_{1A} receptors. Previous studies have shown that 5-HT_{1A} receptors are poorly expressed in the cerebral cortex immediately after birth, but that their expression increases rapidly during the postnatal period (Daval et al., 1987; Miquel et al., 1994). Consistent with these findings, 5-HT_{1A} receptor-mediated hyperpolarization does not become evident until the third postnatal week, despite the expression of GIRK channels during the preceding week (Chen et al., 1997; our results). As such, these results suggest that transcriptional regulation plays a determining role in shaping the pattern of 5-HT_7 and 5-HT_{1A} receptor function in developing prefrontal cortex.

In contrast to the changes in 5-HT_7 and 5-HT_{1A} receptor function, the reduction in the 5-HT_{2A} receptor-induced depolarization does not appear to result from changes in receptor expression, because 5-HT_{2A} mRNA and protein are abundantly expressed at all ages examined (Mengod et al., 1990; Roth et al., 1991; Morilak and Ciaranello, 1993; Lopez-Gimenez et al., 1997; Jakab and Goldman-Rakic, 1998; Mansour-Robaey et al., 1998; our study). Similarly, this reduction also does not appear to result from a loss of the ion channel responsible for the depolarization, because 5-HT_{2A} receptor activation can induce robust inward currents in the presence of intracellular GTPγS during the P15–P19 period. Rather, the reduction in 5-HT_{2A} receptor responsiveness appears to result, at least in part, from a weakening of the 5-HT_{2A} receptor-effector coupling with increasing age. However, other factors may also contribute to this loss of responsiveness. One additional and likely contributing factor is the decrease in membrane resistance observed during the first 2 weeks of life, which can be expected to result in a reduction in the ability of even a stable 5-HT_{2A} inward to depolarize these cells. Another possible factor could be a developmentally regulated change in subcellular distribution of 5-HT_{2A} receptors. If receptors became localized at sites progressively more electrotonically distal from the soma with increasing age, this could also potentially lead to an apparent loss of 5-HT_{2A} receptor electrophysiological signaling. However, anatomical studies have shown that 5-HT_{2A} receptors do not show a preferential distal distribution over dendrites in adults (Jakab and Goldman-Rakic, 1998), and therefore such a mechanism appears less likely to play a significant role. Future studies will be needed to more precisely evaluate the contribution of these and other potential mechanisms to the decrement in the ability of 5-HT_{2A} receptors to signal a depolarization during development.

Previous studies have reported that activation of 5-HT_{2A} receptors can induce a membrane depolarization of pyramidal neurons in the adult rat prefrontal cortex (Araneda and Andrade, 1991; Tanaka and North, 1993). Although these depolarizations were relatively modest and were seen in only a fraction of cells tested, they nevertheless appeared more readily detectable in these previous studies than in the current recordings. There are a number of methodological differences that could contribute to this apparent discrepancy. First, in the current study, we observed a small but significant 5-HT_{2A} receptor-mediated inward current when using agonists with full intrinsic activity. As such, this difference may be more quantitative than qualitative. Second, the use of different recording methods, "blind" sharp microelectrodes in the previous study, visual targeting of neurons for whole-cell recordings in the current, may have introduced a subtle sampling bias affecting the composition of the cell populations studied. Third, it is possible that the dialysis intrinsic to whole-cell recordings might alter the ability of 5-HT_{2A} receptors to signal a depolarization. Regardless of the exact mechanism accounting for this difference, none of these possibilities is inconsistent with the current results delineating a developmental shift in the ability of 5-HT_{2A} receptors to signal a depolarization nor with our mechanistic interpretation, namely that this developmental shift reflects, at least in part, a reduction in receptor-effector coupling.

Although the effects of 5-HT in adult prefrontal cortex have been extensively studied, only a few studies have examined the effects of 5-HT in regulating membrane excitability in this region during the postnatal period (Zhou and Hablitz, 1999; Lambe and Aghajanian, 2001; Zhang, 2003b). Notably, the large role played by 5-HT_7 receptors in developing prefrontal cortex, as well as the remarkable absence of 5-HT_{1A} receptor-mediated hyperpolarizations, has gone either unnoticed or unreported. This may reflect an emphasis on changes in spontaneous synaptic activity by 5-HT and the use, at least in part, of rats older than 3 weeks (Zhou and Hablitz, 1999; Lambe and Aghajanian, 2001). A recent study (Zhang, 2003b), published while this study was being prepared, described a 5-HT-induced depolarization comparable with that reported here but attributed it to the activation of 5-HT_{2A} receptors primarily on the basis of its sensitivity to ketanserin. It is possible that the contribution of 5-HT_{2A} receptors to this depolarization might have been overestimated in that study in light of the non-negligible affinities of ketanserin for 5-HT_7 receptors (Shen et al., 1993; Jasper et al., 1997; Adham et al., 1998).

In summary, the present results outline a physiological and mechanistic progression in the ability of 5-HT to regulate the membrane potential of developing layer V pyramidal neurons of the rat prefrontal cortex. Early in the postnatal period, 5-HT depolarizes pyramidal cells by activating receptors of the 5-HT_7 and secondarily 5-HT_{2A} subtypes. Beginning in the third postnatal week, however, this depolarizing action begins to subside and is gradually replaced by a hyperpolarizing effect mediated by receptors of the 5-HT_{1A} receptor subtype. Interestingly, different mechanisms appear to underlie these changes in serotonergic function. The decrease in 5-HT_7 responsiveness seen during the third postnatal week as well as the concomitant increase in 5-HT_{1A} receptor function are preceded by changes in mRNA expression and most likely reflect changes in receptor expression. In contrast, the gradual reduction in the ability of 5-HT_{2A} receptors to depolarize pyramidal cells during the first 2 postnatal weeks seems to reflect changes in receptor-effector coupling. Future studies will be needed to identify specific actions of each of these receptors on specific developmental processes and to determine whether their disturbance can lead to abnormal behavioral sequela in adulthood.

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