Heterogeneous Actions of Serotonin on Interneurons in Rat Visual Cortex

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Submitted 9 July 2002; accepted in final form 14 November 2002

Xiang, Zixiu and David A. Prince. Heterogeneous actions of serotonin on interneurons in rat visual cortex. J Neurophysiol 89: 1278–1287, 2003; 10.1152/jn.00533.2002. The effects of serotonin (5-HT) on excitability of two cortical interneuronal subtypes, fast-spiking (FS) and low threshold spike (LTS) cells, and on spontaneous inhibitory postsynaptic currents (sIPSCs) in layer V pyramidal cells were studied in rat visual cortical slices using whole-cell recording techniques. Twenty-two of 28 FS and 26 of 35 LTS interneurons responded to local application of 5-HT. In the group of responsive neurons, 5-HT elicited an inward current in 50% of FS cells and 15% of LTS cells, an outward current was evoked in 41% of FS cells and 81% of LTS cells, and an inward current followed by an outward current in 9% of FS cells and 4% LTS cells. The inward and outward currents were blocked by a 5-HT3 receptor antagonist, tropisetron, and a 5-HT1 receptor antagonist, NAN-190, respectively. The 5-HT-induced inward and outward currents were both associated with an increase in membrane conductance. The estimated reversal potential was more positive than −40 mV for the inward current and close to the calculated K+ equilibrium potential for the outward current. The 5-HT application caused an increase, a decrease, or an increase followed by a decrease in the frequency of sIPSCs in pyramidal cells. The 5-HT3 receptor agonist 1-(m-chlorophenyl)biguanide increased the frequency of larger and fast-rising sIPSCs, whereas the 5-HT1A receptor agonist (±)8-hydroxydipropylaminotetralin hydrobromide elicited opposite effects and decreased the frequency of large events. These data indicate that serotonergic activation imposes complex actions on cortical inhibitory networks, which may lead to changes in cortical information processing.

INTRODUCTION

Cortical GABAergic interneurons, through their synapses onto different somatic and dendritic locations, play critical roles in controlling the output functions of excitatory pyramidal cells (Somogyi et al. 1998). They are also major targets for ascending neurotransmitter systems such as serotonin (5-HT) (Freund et al. 1990; Freund and Gulyas 1997; Parra et al. 1998) and acetylcholine (Beaulieu and Somogyi 1991; Houser et al. 1983a; Saper 1984; Shute and Lewis 1967; Wainer et al. 1984), providing a mechanism by which small groups of neurons in subcortical nuclei can influence activities in large cortical networks. Cortical interneurons are heterogeneous in terms of gross morphology, positions in the cortex, electrophysiological properties, connections, and content of calcium-binding proteins and neuropeptides (DeFelipe 1993; Freund and Buzsaki 1996; Hendry et al. 1984a,b; Houser et al. 1983b; Jones 1993; Kawaguchi 1993; Kawaguchi and Kubota 1993, 1996; Kubota and Kawaguchi 1997). In layer V of the neocortex, there are several subtypes of electrophysiologically distinct interneurons, including fast-spiking (FS) cells with short-duration action potentials that are nonadapting during spike trains, and low threshold spike (LTS) cells, also termed “burst spiking nonpyramidal cells,” that generate rebound spikes or bursts of spikes when stimulated during hyperpolarization (Kawaguchi and Kubota 1993, 1997). These two subclasses are prominent in layer V of rat visual cortex (Xiang et al. 1998). FS and LTS cells not only exhibit different firing properties but also have distinctly different intracortical axonal projection patterns. The axons of FS interneurons in layer V tend to be distributed more horizontally, whereas those of LTS cells have more vertical arborizations (Jones and Hendry 1984; Kawaguchi and Kubota 1993; Xiang et al. 1998). These interneuronal subgroups make synaptic connections onto different somato-dendritic regions of the principle cells. FS cells synapse primarily onto somatic and proximal dendritic domains of pyramidal neurons, including those in layer V (Tamas et al., 1997; Thomson et al., 1996), whereas axonal terminals of LTS cells innervate more distal regions of pyramidal cell dendritic trees (Deuchars and Thomson 1995; Thomson et al., 1996). In addition to differences in intrinsic properties and output onto pyramidal neurons, the excitability of these subclasses of interneuron may also be differentially modulated by acetylcholine (Xiang et al. 1998) through activation of different types of postsynaptic receptors. These latter results raise the possibility that other ascending neurotransmitter systems might have similar differential effects. We therefore performed experiments focused on the actions of 5-HT on FS and LTS neocortical interneurons.

Serotonergic axons, mainly originating from neurons in the mesencephalic dorsal and median raphe nuclei (Dahlstrom and Fuxe 1964), make specific synaptic connections predominantly on interneurons in monkey prefrontal cortex (Smiley and Goldman-Rakic 1996) as well as on selective subgroups of nonpyramidal cells in other species (Acsady et al. 1993; Freund 1992; Freund et al. 1990; Hornung and Celio 1992). The functional influence of the serotonergic system is mediated by activation of a variety of 5-HT receptors, including G protein–coupled subtypes (5-HT1, 5-HT2, and 5-HT7) and a ligand-gated ion channel (5-HT3) (Derkach et al. 1989; Gerhardt and
van Heerikhuizen 1997; Hoyer et al. 1994). Activation of at least three distinct 5-HT receptors, including 5-HT₁₃ (Kawa 1994; McMahon and Kauer 1997; Roerig et al. 1997; Ropert and Guy 1991), 5-HT₂ (Gellman and Aghajanian 1994; Marek and Aghajanian 1994, 1996; Shen and Andrade 1998), and 5-HT₃ (Schmitz et al. 1995) affects excitability of interneurons. It is not clear whether serotonergic activation can differentially modulate excitability of various neocortical interneuronal subtypes. Such differential effects on cells with specific connections would provide an important mechanism for influencing activities in particular intracortical circuits.

We used whole-cell recording techniques to examine the effects of serotonin on membrane excitability of FS and LTS cells and resultant effects on inhibitory synaptic transmission onto layer V pyramidal neurons in rat visual cortex. We found that application of 5-HT caused excitation in half of FS cells and a small subset of LTS cells and inhibition in about another half of FS cells and the large majority of LTS cells. Excitation followed by inhibition was evoked by 5-HT application in small numbers of FS and LTS cells.

METHODS

All procedures were performed according to protocols approved by the Stanford Institutional Animal Care and Use Committee.

Slice preparation

Young Sprague-Dawley rats (P14–P17) were anesthetized with pentobarbital (55 mg/kg), decapitated, and brains removed and immersed in ice-cold oxygenated (95% O₂/5% CO₂) “cutting solution,” which was composed of (in mM): 230 sucrose, 2.5 KCl, 0.5 CaCl₂, 10 MgSO₄, 1.25 NaH₂PO₄, 26 NaHCO₃, and 10 d-glucose. Coronal visual cortical slices (350 μm) were cut from blocks of visual cortex (Oc1M/Oc1B) (Zilles and Wree 1985) in this solution using a vibratome and maintained in oxygenated artificial cerebrospinal fluid (ACSF) at 32°C for at least 1 h in an interface incubation chamber. The ACSF contained (in mM): 126 NaCl, 3 KCl, 2 CaCl₂, 2 MgSO₄, 1.25 NaH₂PO₄, 26 NaHCO₃, and 10 d-glucose. Slices were then transferred, one at a time, to the recording chamber, where they were superfused with ACSF (32–33°C) at a rate of approximately 2 ml/min.

Electrophysiological recordings

Whole-cell recordings were made from visually identified interneurons and pyramidal cells in layer V under infrared video microscopy with Nomarski optics. An EPC-7 patch amplifier (List) was used for current- and voltage-clamp recordings. Patch pipettes were prepared from borosilicate glass with a Flaming-Brown micropipette puller (Model P-80/PC, Sutter Instruments). In experiments in which effects of 5-HT on membrane conductance were examined, the pipette solution contained (in mM): 123 K-gluconate, 10 KCl, 1 MgCl₂, 1 CaCl₂, 10 HEPES, 11 EGTA, 3 ATP, and 0.2–0.4 GTP. The pH was adjusted to 7.3 with 1 M KOH and osmolarity was adjusted to 290–295 mOsm with water. Under these recording conditions, the calculated chloride equilibrium potential (E_Cl) was −15 mV, based on the Nernst equation with activity coefficients for extracellular Cl⁻ of 0.76 and intracellular Cl⁻ of 0.80, and taking into account the permeability of gluconate through Cl⁻ channels (Barker and Harrison 1988). Patch pipettes had resistances of 3–4 MΩ when filled with the above pipette solutions. Series resistance (Rs) typically ranged from 9 to 15 MΩ. Only data obtained from stable recordings during which there was <15% change in Rs were included in the analysis. In experiments in which effects of 5-HT on membrane conductance were examined, Rs was compensated by 55–70%.

After the electrophysiological recordings, slices containing biocytin-filled neurons were fixed and processed with standard avidin-biotin–peroxidase methods as described elsewhere in detail (Horikawa and Armstrong 1988; Tseng et al. 1991). The location and morphology of the filled cells were examined under the light microscope. A few neurons proved to be pyramidal in type and were not included in the analysis. The nonpyramidal nature and gross morphology of dendritic and axonal arborizations of successfully labeled interneurons was verified (Fig. 1).

Drug application

In experiments in which membrane excitability of interneurons and sIPSCs were examined, 5-HT (100 μM) and 1-(m-chlorophenyl)
biganide (mCPBG, 30 μM) were applied with pressure pulses (25–35 kPa; 25–200 ms) through a “puffer” pipette (2–4 μm in tip diam) that was placed approximately 50 μm from the recorded cell. The interval between applications was ≥2 min. 1-(2-Methoxyphenyl)-4-(4-[2-phthalimido]butyl)piperazine (NAN-190, 1–2 μM), 3-tropolyn-indole-3-carboxylate hydrochloride (tropisetron, 3–4 μM), and 8-hydroxy-di-n-propylamino tetralin (8-OH-DPAT, 1 μM) were applied through a multibarreled microperfusion pipette (tip size 200–300 μm) placed within 0.5 mm of the recorded cell. In experiments in which effects of 8-OH-DPAT were examined, the [K⁺]₀ was elevated to 8 mM to increase the frequency of impulse-dependent IPSCs. Other agents such as ionotropic glutamate receptor antagonists, 6,7-dinitro-quinoxoline-2,3-dione (DNQX, 20 μM) and 3-(2-carboxypiperazin-4-y1)-1-propanyl-1-phosphonic acid (AP-5, 50 μM); GABAₐ receptor antagonist, bicuculline (10 μM); and voltage-gated Na⁺ channel blocker, TTX (1 μM) were added to the perfusate and bath applied as required. Drugs were obtained from Sigma and RBI.

Data analysis

A Gateway computer equipped with PCLAMP (Axon Instruments) and Strathclyde Electrophysiology Software (courtesy of J. Dempster) was used to generate the pulses and digitize and record data on-line. Recordings were also digitized (44 kHz) with a Neurocorder DR-484 (Neuro Data Instruments) and stored on videotape for off-line analysis. Other software, including SCAN (J. Dempster), Origin (Microcal), and the locally written programs Metatape and Detector (Ulrich and Huguenard 1996), were used for data analysis.

Spontaneous IPSCs or miniature (m)IPSCs were automatically detected using the second derivative of the digitally filtered current traces (cutoff, 800Hz) as the trigger (Ulrich and Huguenard 1996). The detection threshold was set at 3 times rms. of the background noise. Statistical comparison of IPSC properties was performed using Student’s t-test, unless otherwise stated. Data are presented as means ± SE.

RESULTS

Effects of 5-HT on membrane excitability of FS and LTS interneurons

Recordings were made from 28 FS and 35 LTS layer V interneurons that exhibited distinct firing properties. FS cells fired a train of short-duration action potentials with little frequency adaptation when a suprathreshold depolarizing current step was applied under the current clamp (Fig. 1A). The spike half-width for selected successfully labeled FS cells was 0.61 ± 0.04 ms (n = 8), comparable to that reported by Kawaguchi and Kubota (1993) for FS parvalbumin-containing cells in layer V of rat frontal cortex. LTS cells generated a burst of spikes riding on the top of a characteristic low-threshold spike when membrane potential rebounded from a hyperpolarized potential (Fig. 1B1, arrow) or when a depolarizing current pulse was injected from a hyperpolarized holding potential (-86 mV in Fig. 1B2, arrow) (Foehring et al. 1991; Kawaguchi 1993, 1995; Xiang et al. 1998).

All of the successfully labeled FS cells had a basket cell–like morphology and LTS cells were nonpyramidal in their gross structure (Fig. 1C). As reported in our previous studies as well as those of others (Jones and Hendry 1984; Kawaguchi and Kubota 1993; Xiang et al. 1998), axons of FS interneurons in layer V tend to be confined within layer V, whereas those of LTS cells have more vertical arborizations and extend to the upper layers (Fig. 1C). Twenty-two of 28 FS cells (78.6%) and 26 of 35 LTS interneurons (74.3%) responded to locally applied 5-HT. There were no apparent differences in cell depth in the slice, proximity to the perfusion pipette, duration of the pressure pulse, or other variables between 5-HT–responsive and –nonresponsive cells. Under differential interference contrast (DIC) microscopy, the locally perfused drug-containing solution was observed to flow across the soma of the recorded cell each time 5-HT was ejected. Therefore it is likely that nonresponsive cells represented a cohort of interneurons that were unresponsive to 5-HT (cf. Férézou et al. 2002).

Under voltage clamp, local application of 5-HT (100 μM) elicited an inward current in 11 of 22 FS cells that were responsive to 5-HT (Fig. 2A1) and an outward current in 9 of 22 responsive FS neurons (Fig. 2B1). The mean inward current amplitude was 154.8 ± 38.3 pA for 9 FS cells that had series resistances <15 MΩ. The mean outward current amplitude was 51.5 ± 9.8 pA (n = 9). An inward current followed by an outward current was elicited in 2 of 22 cells (Fig. 2C1). The inward as well as outward currents were associated with an increase in membrane conductance indicated by larger amplitude downward current deflections in response to voltage ramps (Figs. 2 and 4, B3 and B4). To further characterize these actions, we tested the effects of selective receptor antagonists on the 5-HT–elicited currents. The peak inward current was reduced by 96.2 ± 6.2% after application of tropisetron (3 μM, n = 6), a selective 5-HT₁ receptor antagonist (Beubler et al. 1993; Pei et al. 1993; Yoshida et al. 1991) (cf. Fig. 2, A1, A2, C1, and C2), whereas the peak outward current was diminished by 88.1 ± 4.1% after application of NAN-190 (1 μM, n = 5).

FIG. 2. Responses of 3 FS cells (A–C) to focal application of 5-HT. A: 5-HT application evoked an inward current (A1) that was blocked by local perfusion of tropisetron (3 μM, A2). B: 5-HT application evoked an outward current in another FS cell (B1) that was blocked by NAN-190 (1 μM, B2). C: 5-HT application to a third FS neuron elicited an inward current followed by an outward current (C1). The inward current was blocked by local perfusion of tropisetron (3 μM, C2), and the inward and outward currents were blocked by combined local perfusion of tropisetron and NAN-190 (C3). The rapid downward deflections in each segment are current responses to voltage ramps from −55 to −60 to −115 to −120 mV. Arrowheads indicate the time of 5-HT application (100 μM) with a pressure pulse. Vₒ in cells of A–C: −55 to −60 mV. Perfusate contained 20 μM DNQX, 50 μM APV, and 10 μM bicuculline for cells of B and C.
indicating the involvement of 5-HT$_{1A}$ receptors. The inward current in response to 5-HT application (Fig. 3A1). The mean amplitude of the outward current was 38.5 ± 5.4 pA for 17 LTS cells that had series resistance < 15 MΩ. Only a small percentage of LTS cells (4/26) were excited (i.e., inward currents were elicited by 5-HT as in Fig. 3B1). The amplitude of the inward currents was 173.4 ± 52.0 pA (n = 4). One LTS cell showed an inward current followed by an outward current in response to 5-HT application (Fig. 3C1). As in the 5-HT–induced responses in FS cells, the outward and inward currents were both associated with increases in membrane conductance (Figs. 3 and 4, B1 and B2). The outward current was decreased by 87.1 ± 5.1% after NAN-190 application (1 μM, n = 4) (cf. Fig. 3, A1, A2, C1, and C3), indicating the involvement of 5-HT$_{1A}$ receptors. The inward current was reduced by 95.6 ± 1.6% following tropisetron application (3–4 μM, n = 3) (Fig. 3, B2 and C2), suggesting that it was mediated by 5-HT$_3$ receptors.

Table 1 summarizes the properties of 5-HT–induced responses in FS and LTS cells. When 5-HT was rapidly applied by local pressure pulses (20–35 kPa; 20–200 ms), the time to peak for 5-HT$_{1A}$ receptor–mediated outward current was about 20-fold longer than for 5-HT$_3$ receptor–mediated inward current (Table 1). These data are consistent with the expected slow kinetics for a G protein–coupled conductance change associated with 5-HT$_{1A}$ receptor activation, in contrast to a rapid ligand-gated conductance change elicited by 5-HT$_3$ receptor activation. Results indicated that 5-HT could modulate membrane excitability of both FS and LTS cells, however, almost all LTS cells were inhibited by 5-HT via activation of 5-HT$_{1A}$ receptors, whereas approximately equal numbers of FS cells were either excited or inhibited by 5-HT through activation of 5-HT$_3$ or 5-HT$_{1A}$ receptors, respectively. A small percentage of FS and LTS cells were sequentially excited and inhibited.

**Different ionic mechanisms underlie 5-HT-induced conductance changes**

To examine the possible ionic mechanisms underlying 5-HT-induced conductance changes, we plotted the current responses against ramp voltage before and after 5-HT application and obtained current difference (ΔI) versus voltage (V) plots by subtracting the control current from 5-HT current (ΔI = I$_{5-HT}$ – I$_{control}$) (Fig. 4). The reversal potential (E$_r$) for the 5-HT–induced outward current was estimated using the crossing points of the ΔI–V curve with the voltage abscissa at ΔI = 0 (Fig. 4, B1 and B3). The E$_r$ for the inward current was estimated by extrapolating the linear fitting curve to ΔI = 0 (Fig. 4, B2 and B4). The mean values of E$_r$ for the outward currents were −95.2 ± 1.4 mV for FS cells (n = 9) and −92.1 ± 1.6 mV for LTS cells (n = 17), both of which were close to the calculated K$^+$ equilibrium potential of −100 mV. The E$_r$ values for the inward currents in most cells were more positive than −40 mV, with a mean value of −28.0 ± 5.4 mV for FS cells (n = 7) and −32.6 ± 5.1 mV for LTS cells (n = 4). These values suggest that the inward currents were probably due to activation of a 5-HT$_3$ receptor/channel associated with a mixed cationic conductance. For two FS cells, the 5-HT–induced inward current did not appear to be associated with a linear membrane conductance change within the range of the test voltage ramp pulse (−55 to −115 mV).

**Effects of 5-HT on sIPSCs in layer V pyramidal cells**

5-HT regulates the excitability of FS and LTS cells in a complex manner as described above. Unitary IPSCs in layer V pyramidal cells evoked by FS cells are larger and faster rising than those evoked by LTS cells (Xiang et al. 2002), whereas those due to excitation of LTS interneurons would be expected to be smaller and have a slower time course, due to known primary projections of FS and LTS cell axons to perisomatic and distal dendritic areas of layer V pyramidal cells, respectively (Deuchars and Thomson 1995; Tamas et al., 1997; Thomson et al. 1996). We therefore expected that local application of 5-HT would have heterogeneous actions on basic parameters of sIPSCs in layer V pyramidal cells, depending in part on the extent of innervation of a particular postsynaptic cell by these two types of interneuron and in part on which of the above effects on FS and LTS cells predominated.

The effects of 5-HT (100 μM), applied via a “puffer” pipette near the recorded cell (see METHODS), were studied in 11 voltage-clamped pyramidal neurons to assess alterations in the parameters of sIPSCs. The recordings from the pyramidal cell in Fig. 5 show one type of response, namely increases in sIPSC frequency and amplitude following 5-HT application (Fig. 5, A and B). Shifts in distributions of sIPSC amplitude and 10–90% rise time after application of 5-HT were apparent (Fig. 5, C1 and C2) and indicated that 5-HT selectively increased the frequency of large-amplitude and fast-rising events. This was also evident when plots of sIPSC amplitude versus rise time in

![Figure 3](image-url)
control and following 5-HT application were compared (Fig. 5C3). The large increases in sIPSC frequency and amplitude associated with a decrease in rise time elicited by 5-HT were observed in 5 of 11 pyramidal neurons and are summarized in Fig. 5D. For those 5 cells, the sIPSC frequency increased from 9.3 ± 1.4 to 21.7 ± 4.1 Hz after 5-HT application and the amplitude increased from 31.7 ± 4.2 to 74.2 ± 14.8 pA, while the 10–90% rise time decreased from 1.35 ± 0.11 to 0.99 ± 0.06 ms. To estimate the amplitude and rise time of IPSCs that were selectively elicited by 5-HT, we subtracted the amplitude or rise time distribution in control from that in the presence of 5-HT. By this method, the amplitude of 5-HT–induced sIPSCs was estimated to be 29.2 ± 18.2 pA and the 10–90% rise time was 0.84 ± 0.03 ms (n = 5) (data not shown). These values were similar to those for unitary IPSCs obtained from our previous study using dual recording techniques in pairs of FS and pyramidal neurons in layer V of visual cortex (Xiang et al. 2002).

Opposite effects on sIPSC frequency and amplitude (i.e., increases in both parameters) were elicited by 5-HT in four other cells (Fig. 6). In two pyramidal neurons, 5-HT application elicited an increase followed by a decrease in the frequency of sIPSCs (Fig. 7, A–C). IPSC amplitude was increased during the frequency increase (Fig. 7D1), without a significant change in rise time (Fig. 7D2). All of the effects of 5-HT on IPSCs in pyramidal cells were reversible during washout (Figs. 5–7). These data are consistent with the results described above regarding modulation of excitability of FS and LTS cells by 5-HT.

In these experiments, we noted that puff application of 5-HT also induced an inward current in 6 of 11 pyramidal cells and an outward current in 5 of 11 cells. Serotonin was applied to 6 additional pyramidal cells in the presence of TTX to rule out indirect postsynaptic responses due to serotonergic activation of other neurons. Two of these 6 cells generated an inward current in response to 5-HT application, while 5-HT elicited an outward current in 2 others and no response in 2 neurons. Both inward and outward currents had slow time-to-peak and thus were likely mediated by 5-HT3 and 5-HT1A receptors, respectively, as previously reported (Davies et al. 1987).

Presumably, the 5-HT–induced increase in frequency of larger and faster-rising sIPSCs in pyramidal neurons largely reflected depolarization of a subset of FS cells through activation of 5-HT3 receptors, while the decrease in sIPSC frequency was likely due to hyperpolarization of other FS as well as LTS cells by activation of 5-HT1A receptors. To further test these possibilities, we examined the actions of the 5-HT3 receptor agonist mCPBG and 5-HT1A agonist 8-OH-DPAT on sIPSCs in pyramidal cells. As predicted from the above considerations, local brief application of mCPBG (30 μM) resulted in an increase in the frequency of larger and fast rising sIPSCs (n = 5, Fig. 8), whereas 8-OH-DPAT (1 μM) caused a decrease in frequency of large amplitude events (n = 7, Fig. 9). Local application of 5-HT (100 μM) did not alter the frequency or amplitude of mIPSCs recorded in the presence of 1 μM TTX (data not shown), indicating that the effects of 5-HT and agonists on sIPSC frequency were not due to direct actions on presynaptic terminals or the postsynaptic pyramidal neuron. The results suggested that the 5-HT–induced changes in frequency, rise time, and amplitude of sIPSCs in pyramidal cells mainly resulted from changes in membrane excitability of GABAergic interneurons and consequent effects on spike firing.

**DISCUSSION**

It is important to recognize the limitations of experiments involving exogenously applied 5-HT (or any transmitter) as an

**TABLE 1. Summary of 5-HT-induced responses**

<table>
<thead>
<tr>
<th>Current Type</th>
<th>Peak Amplitude, pA</th>
<th>n</th>
<th>Time to Peak, s</th>
<th>n</th>
<th>E_r, mV</th>
<th>n</th>
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<tbody>
<tr>
<td>FS cells</td>
<td></td>
<td></td>
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<tr>
<td>Inward</td>
<td>154.8 ± 38.3</td>
<td>9</td>
<td>0.38 ± 0.06</td>
<td>9</td>
<td>−28.0 ± 5.4</td>
<td>7</td>
</tr>
<tr>
<td>Outward</td>
<td>51.5 ± 9.8</td>
<td>9</td>
<td>9.94 ± 1.25</td>
<td>9</td>
<td>−95.2 ± 1.4</td>
<td>9</td>
</tr>
<tr>
<td>LTS cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Inward</td>
<td>173.4 ± 52.0</td>
<td>4</td>
<td>0.45 ± 0.09</td>
<td>4</td>
<td>−32.6 ± 5.1</td>
<td>4</td>
</tr>
<tr>
<td>Outward</td>
<td>38.3 ± 5.4</td>
<td>17</td>
<td>9.06 ± 0.82</td>
<td>17</td>
<td>−92.1 ± 1.6</td>
<td>17</td>
</tr>
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</table>

Values are ± SE. E_r, reversal potential; FS, fast-spiking; LTS, low threshold spike; n, number of responses.
FIG. 5. Local puff application of 5-HT caused an increase in frequency of sIPSCs in 5 of 11 pyramidal cells in layer V. A: time series showing sIPSC frequency in control, after application of 100 μM 5-HT, and washout from a representative cell. Each dot represents the average frequency of sIPSCs over 3 s here and in similar graphs of sIPSC frequency versus time in Figs. 6A and 7A. B: records of sIPSCs in control (a), after 5-HT application (b), and washout (c) taken from the time points, a, b, and c, as indicated in A. C: distributions of sIPSC amplitude (C1) and 10–90% rise time (C2) in control and after 5-HT application and plot of sIPSC amplitude versus 10–90% rise time during control and after 5-HT application (C3). Data were taken from the cell of A and B. Records of 12-s episodes during control and after 5-HT application were used for the analysis. D: bar graphs summarizing the effect of 5-HT on frequency (D1), amplitude (D2), and 10–90% rise time (D3) of sIPSCs for group data from 5 different cells. For each cell, equal length segments from control, 5-HT, and washout conditions were taken for analysis (4–12 s for different cells). $V_{m} = -80$ mV, $E_{Cl} = -15$ mV. Perfusate contained 20 μM DNQX and 50 μM APV. The recording conditions and data analysis methods employed here were the same as those used to obtain the data shown in Figs. 6–9, unless otherwise indicated.

FIG. 6. Local puff application of 5-HT caused a decrease in frequency of sIPSCs in 4 of 11 pyramidal cells. A: time courses of sIPSC frequency in control, after application of 100 μM 5-HT and washout from a representative cell. B: records of sIPSCs in control (a), after 5-HT application (b) and washout (c) taken from the time points, a, b, and c, as indicated in (A). C: bar graphs summarizing the effect of 5-HT on frequency (C1), amplitude (C2) and 10–90% rise time (C3) of sIPSCs for normalized group data ($n = 4$). Records of 10–30 s segments in control and after 5-HT application for each cell were used for data analysis.
approach to understanding effects of the endogenously released agent on cellular and network activities in a cortical structure. For example, 5-HT–containing axons originate from several brain stem nuclei (Dahlstrom and Fuxe 1964; Fuxe 1965) and terminate in topographically specific patterns (Kosofsky and Molliver 1987; Parnavelas and Papadopoulos 1989) that have regional and laminar specificities (Morrison et al. 1982; Morrison and Foote 1986; Wilson and Molliver 1991). These findings suggest possible differences in the effects of activation of different portions of the serotonergic system in various cortical subfields. Also, the majority of 5-HT axonal varicosities do not have postsynaptic specializations (DeFelipe et al. 1991; Seguela et al. 1989; Smiley and Goldman-Rakic 1996) and 5-HT neurotransmission may be predominantly paracrine (Bunin and Wightman 1999). It is therefore unclear which of the described actions of 5-HT on interneurons in these experiments are due to activation of synaptic versus extrasynaptic receptors. One further caution relates to the ages of the animals studied (P14–P17). Maturation of serotonergic innervation and density of 5-HT receptors increases progressively in rat cortex over the first few weeks of life (Daval et al. 1987; Dori et al. 1996; Nakazawa et al. 1992; Zilles et al. 1985). Such developmental changes might affect the size of responses, the number of 5-HT–sensitive cells, and perhaps the types of postsynaptic receptors activated in subgroups of interneurons.

Our results show that serotonergic activation exerts heterogeneous actions on the membrane excitability of two physiologically distinct interneuronal subtypes in the layer V of rat visual cortex. 5-HT induced a relatively slow outward current mediated by 5-HT1A receptors in almost all LTS cells and approximately half of the FS cells. It also elicited a fast inward current mediated by 5-HT3 receptors in another half of FS cells and a small percentage of LTS cells. Fast-spiking interneurons were identified on the basis of their gross anatomical structure (e.g., Fig. 1C) together with their responses to depolarizing current pulses, and it is possible that the seemingly heterogeneous effects of 5-HT on this cell type are due to the existence of more than one type of FS cell (Cauli et al. 1997; Gupta et al. 2000).

The 5-HT1A receptor–mediated response appeared to be associated with an increase in a K+ conductance, similar to that observed in dorsal raphe neurons (Bayliss et al., 1997; Lark-...
man and Kelly 1995) and subsets of pyramidal cells in cingulate cortex (Tanaka and North 1993) and hippocampus (Beck et al. 1996). Serotonin- and 8-OH-DPAT-induced hyperpolarizations, associated with increases in conductance in layer IV guinea pig pyramidal neurons, were also attributed to 5-HT1A receptor activation (Davies et al. 1987). Serotonin-induced hyperpolarization was also observed in a subpopulation of CA1 hippocampal interneurons, but it was not clear what type of 5-HT receptor was involved (Parra et al. 1998; Schmitz et al. 1995). However, evoked monosynaptic IPSPs in hippocampal pyramidal cells were inhibited by a 5-HT1A receptor agonist, 8-OH-DPAT, implying the presence of 5-HT1A receptors in hippocampal interneurons (Schmitz et al. 1995). Immunohistochemical studies in rat neocortex and hippocampus have indicated that 5-HT3 receptor-containing neurons are mainly GABAergic interneurons (Morales et al. 1996; Morales and Bloom 1997), which often contain cholecystokinin (CCK), calbindin (CB), and calretinin, but not somatostatin or parvalbumin (PV) (Morales and Bloom 1997). Baskets of serotonergic fibers in hippocampus are associated with CB- but not PV-containing interneurons (Freund 1992; Hormuz and Celio 1992). A recent report indicated that a small subset of regular spiking and irregular spiking CCK/vasoactive intestinal polypeptide–containing GABAergic interneurons in layers I, II, III, and V of rat sensorimotor cortex expresses 5-HT3 receptors and shows 5-HT3 receptor–mediated responses. Only rare interneurons were hyperpolarized and there was almost no 5-HT1A receptor expression or responsiveness in PV-containing FS cells (Ferézou et al. 2002). There is a subpopulation of neocortical CCK-containing interneurons that are FS cells (Cauli et al. 1997), but these apparently did not express 5-HT3A receptors. In contrast, our data indicate that approximately 75% of FS and LTS cells in layer V of visual cortex are 5-HT responsive and about one half of FS interneurons possess 5-HT3 receptors that are blocked by tropisetron. Differences in laminar or areal distribution of 5-HT receptors on interneurons may be factors that underlie these discrepant experimental results. A large proportion of FS cells contain PV (Cauli et al. 1997; Kawaguchi and Kubota 1993; Z Xiang, DA Prince, and I Parada, unpublished observations). Therefore it is likely that there is a population of 5-HT–responsive, PV-containing FS cells in layer V of rat visual cortex. In addition, we found biphasic responses mediated by 5-HT3 and 5-HT1A receptors in small numbers of LTS and FS cells, suggesting that individual interneurons can express more than one 5-HT receptor subtype. Further experiments are necessary to determine whether 5-HT–responsive and –nonresponsive FS and LTS cells have distinct profiles for calcium-binding proteins and peptides.

The 5-HT3 receptor–mediated excitation probably resulted from an increase in conductance for mixed cations, as described in hippocampal CA1 interneurons (McMahon and Kauer 1997), dentate gyrus basket cells (Kawa 1994), and neocortical layer I interneurons (Zhou and Hablitz 1999). We estimated that the reversal potential (Erev) for the 5-HT3 receptor–mediated inward current was more positive than −40 mV, but not very close to 0 mV, a value that would be anticipated for activation of 5-HT3 receptors/channels that are permeable to mixed monovalent cations (McMahon and Kauer 1997; Zhong et al. 1999). This could be attributed to the nonlinearity and inward rectification in I–V relation of 5-HT3 receptor–mediated current (Kawa 1994; McMahon and Kauer 1997; Zhong et al. 1999) and use of the linear extrapolation method to estimate Erev in this study (Fig. 4).

Layer V FS and LTS interneuronal subtypes synapse at different sites on layer V pyramidal cells, resulting, in part, in FS–pyramidal unitary (u)IPSCs being larger and faster-rising than LTS–pyramidal uIPSCs (Xiang et al. 2002). This finding, together with the heterogeneous actions of 5-HT on excitability of FS and LTS cells, accounts for the variable effects of 5-HT on sIPSCs in the pyramidal cells. We found that 5-HT application induced an increase in frequency of larger and fast-rising sIPSCs in some cases and a decrease in frequency of large events in others. These results are a consequence of 5-HT3 receptor–mediated fast excitation being more prominent in FS than in LTS cells, together with a 5-HT1A receptor–mediated slow hyperpolarization in some FS cells as well as almost all LTS cells. 5-HT did not alter mIPSCs (data not shown); however, it does reduce the amplitude of evoked IPSCs through activation of 5HT1A receptors (Koyama et al. 1999; Schmitz et al. 1995; and our unpublished observations). Thus the decrease in frequency of large-amplitude sIPSCs by 5-HT and 8-OH-DPAT can be attributed, in part, to a 5-HT1A receptor–mediated decrease in action potential–dependent GABA release from terminals. It is of interest that the effects of ACh on FS and LTS interneurons are more homogeneous and, to a significant degree, opposite those of 5-HT. ACh inhibits FS cells via muscarinic receptors and excites LTS interneurons by activating nicotinic receptors, resulting in a shift toward smaller, slower, and presumably more distal inhibitory events (Xiang et al. 1998). We
suggested that disinhibition of layer V pyramidal neurons due to the muscarinic inhibition of FS interneurons, together with the significant horizontal orientation of FS cells in layer V (Jones and Hendry 1984; Kawaguchi and Kubota 1993; Xiang et al. 1998), would result in an increase in intercoluminar interactions among pyramidal cells. By contrast, 5-HT inhibits almost all LTS cells and excites a significant proportion of FS neurons, effects that might result in an increase in somatic–proximal dendritic inhibitory inputs, a decrease in distal dendritic inhibition, and increases in intracolumnar versus intercolumnar pyramidal-to-pyramidal cell excitation. A similar effect of the ascending serotonergic system on the balance between somatic and dendritic inhibition has been proposed in the hippocampus (Gulyas et al. 1999).

In addition to postsynaptic effects on interneurons, both cholinergic and serotonergic systems have direct postsynaptic actions on pyramidal neurons and presynaptic terminals, so that their net effect on cortical excitability becomes hard to predict. For example, 5-HT can induce a direct slow excitation of pyramidal cells that is mediated by 5-HT2 and 5-HT1C receptors (Davies et al. 1987; Sheldon and Aghajanian 1991). By evoking fast excitation and slow inhibition in subgroups of interneurons mediated by 5-HT3 and 5-HT1A receptors, global activation of the ascending serotonergic system could lead to an initial inhibition of the pyramidal cells, followed by slow excitation due to disinhibition caused by hyperpolarization of interneurons and in part to direct excitation of the pyramidal cells. Thus serotonergic activation could gate cortical information processing in a time-dependent manner.

We thank Dr. J. R. Huguenard and I. Parada for invaluable advice and assistance during the course of these experiments.

This work was supported by the Fimley Research and Training Funds and National Institute of Neurological Disorders and Stroke Grants NS-39579 and NS-07280.

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