Frequency-Dependent Modulation of Retinogeniculate Transmission by Serotonin

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The relay of visual information converging in the lateral geniculate nucleus (LGN) en route to the visual cortex is modulated by projections from brainstem nuclei. The release of serotonin, one mediator of these effects, has been shown to act at a presynaptic site to inhibit neurotransmitter release at the retinogeniculate synapse, the connection between retinal ganglion cells and thalamocortical relay neurons in the LGN. To understand how serotonergic inhibition of synaptic transmission influences the transfer of information at this synapse, we examined the EPSCs and firing responses of relay neurons to 5-carboxytryptamine (5-CT), a 5-HT1 receptor agonist that preferentially activates the presynaptic over postsynaptic modulatory effects of serotonin. Bath application of 5-CT inhibits synaptic strength, relieves synaptic depression, and reduces the total synaptic charge transferred at the retinogeniculate synapse in mouse LGN brain slices. In contrast, 5-CT does not significantly alter the membrane potential response of relay neurons to trains of intracellular current injections. Here we show that presynaptic serotonergic modulation results in a frequency-dependent inhibition of relay neuron firing. At low-frequency stimulation, 5-CT markedly reduces charge transfer at the retinogeniculate synapse, thus inhibiting relay neuron firing. However, inhibition of firing by 5-CT is diminished during high-frequency stimulation, because relief from synaptic depression partially offsets the reduction in charge transfer. Thus, presynaptic serotonergic inhibition plays a powerful role in modulating the frequency range of visual information transmitted via the retinogeniculate synapse such that high-frequency inputs are more reliably transmitted than low-frequency inputs.

Key words: serotonin; thalamus; visual system; presynaptic modulation; high-pass filter; sleep

Introduction

Visual information, encoded in the firing patterns of retinal ganglion cells (RGCs), is modified in the dorsal lateral geniculate nucleus (LGN) by a number of factors that include brainstem neuromodulatory inputs, intrinsic inhibitory connections, corticthalamic feedback circuitry, and short-term synaptic plasticity (McCormick and Bal, 1994; Steriade et al., 1997; Chen et al., 2002). For example, during varying states of arousal or attention, brainstem inputs release a variety of neurotransmitters that modify the firing response of relay neurons by altering intrinsic membrane conductance (McCormick, 1992a). More recently, we have shown that robust presynaptic modulation can also occur at retinal axon terminals to alter both the strength and short-term plasticity of the retinogeniculate synapse (Chen and Regehr, 2003).

One neurotransmitter shown to modulate both presynaptic and postsynaptic aspects of the retinogeniculate synapse is serotonin. This neuromodulator is released in the LGN by projections from the dorsal raphe nucleus in the brainstem (De Lima and Singer, 1987; Papadopoulos and Parnavelas, 1990; Dinopoulos et al., 1995). Activation of postsynaptic serotonergic receptors results in a rightward shift of the voltage dependence of the hyperpolarization-activated cation current, \( I_h \). This depolarizes relay neurons by 1–3 mV and abolishes spontaneous rhythmic burst firing in individual relay neurons. Serotonin is thus thought to contribute to the activation of thalamocortical neurons during the transition from slow-wave sleep to awake states (McCormick and Pape, 1990a; McCormick, 1992b; Monckton and McCormick, 2002).

In contrast, several studies have shown that either iontophoretic application of serotonin to the LGN or stimulation of the dorsal raphe nucleus leads to inhibition of both spontaneous and evoked firing of relay neurons (Yoshida et al., 1984; Marks et al., 1987; Kayama et al., 1989). Consistent with these studies, we have shown that activation of presynaptic serotonergic receptors inhibits action potential (AP)-evoked calcium influx into retinal axon terminals, decreasing neurotransmitter release and short-term synaptic depression (Chen and Regehr, 2003). These inhibitory effects seem counterintuitive, given that the release of serotonin from dorsal raphé neurons is high during awake states and decreases during sleep states (McGinty and Harper, 1976; Lydic et al., 1983; Guzman-Marin et al., 2000). This raises the question of how a significant inhibition of synaptic transmission can be reconciled with the apparent necessity for precise transfer of visual information during awake states.
To understand the function of presynaptic serotonergic inhibition, we examined the effects of serotonin at the retinogeniculate synapse in LGN brain slices. We found that presynaptic serotonergic modulation of this synapse occurred primarily through 5-HT1B receptors (5-HT1BRs), resulting in inhibition of synaptic release and relief of synaptic depression. Moreover, the 5-HT1 receptor agonist 5-carboxytryptamine (5-CT) was relatively selective in activating presynaptic over postsynaptic serotonergic receptors at the retinogeniculate synapse. In experiments that correlated the EPSC response to the firing of the postsynaptic relay neuron, we found that, although reduction of synaptic strength by 5-CT had an overall inhibitory effect on relay neuron firing, inhibition in response to low-frequency stimulation (<20 Hz) was significantly greater than for high-frequency stimulation (100–200 Hz). Thus, presynaptic serotonergic modulation narrows the frequency range of information transmitted at the retinogeniculate synapse.

Materials and Methods

Slice preparation. Parasagittal brain slices containing both the optic tract and the dorsal lateral geniculate nucleus were obtained from p23–32 pups (Taconic, Germantown, NY) as described previously (Chen et al., 2002). Briefly, the brain was quickly removed and immersed in an oxygenated 4°C choline-based cutting solution containing the following (in mM): 87 NaCl, 25 NaHCO3, 37.5 choline chloride, 25 glucose, 2.5 KCl, 1.25 NaH2PO4, 7 MgCl2, and 0.5 CaCl2. The brain tissue was then mounted on the cutting stage of a vibrotome (Leica VT1000S; Leica, Nussloch, Germany) and submersed into oxygenated 4°C cutting solution. Slices (250 μm) were allowed to recover for 30 min at 30°C in an oxygenated choline-based cutting solution and then for another 30 min at 30°C in oxygenated saline containing the following (in mM): 125 NaCl, 26 NaHCO3, 1.25 NaH2PO4, 2.5 KCl, 1 MgCl2, 2 CaCl2, and 25 glucose.

Electrophysiology. Whole-cell voltage-clamp synaptic recordings from geniculate neurons were obtained using glass pipettes (1.2–1.5 MΩ) filled with an internal solution consisting of the following (in mM): 35 CsF, 100 CsCl, 10 EGTA, 10 HEPES, 0.1 methoxyverapamil, pH 7.4. This solution was designed to minimize the contributions from postsynaptic intrinsic membrane conductances and second messenger systems. The bath saline solution contained the GABAα receptor antagonist bicuculline (20 μM; Sigma, St. Louis, MO) and the GABAβ receptor antagonist (2S)-3-[(1S)-1-(3,4-dichlorophenyl)ethyl] amino-2-hydroxypropyl]-N-methyl-5-HT (H11021; Sigma, St. Louis, MO) and the GABAB receptor antagonist (3-(4-carboxypiperazine-4-yl)-propyl-1-phosphonic acid, anpirtoline (ANP), (±)-8-hydroxy-2-dipropylaminotetralin hydroxybromide (8-hydroxy-2-DPAT), α-methyl-5-HT (α-Met 5-HT), cinanserin, and ZD7288 (all from Tocris Cookson) were stored at −20°C and diluted 1:1000 into the bath reservoir immediately before application. Stock solutions of cyanoindolol and nonyloxytryptamine were dissolved in DMSO and diluted 1:1000 for the final concentration. Bath application of 1:1000 DMSO did not alter synaptic transmission. Constant bath flow during application of drugs was ensured by a perfusion pump (Gilmson Medical Electric, Middleton, WI) at flow rates of 2–4 ml/min. Dead space in the perfusion tubing was reduced to 1 ml, allowing rapid bath exchange of pharmacological agents.

Data acquisition and analysis. Recordings from both current-clamp and voltage-clamp experiments were acquired with an Axopatch 200B or Multiclamp 700A amplifier (Axon Instruments) filtered at 1 kHz and digitized at 10–20 kHz with an ITC-16 interface (Instrutech, Port Washington, NY). Data analysis was performed using Igor software (WaveMetrics, Lake Oswego, OR). The voltage threshold for action potential firing was measured as the membrane potential at which the dV/dt > 10 V/sec−1. Data are summarized as mean ± SEM, and statistics were calculated using the two-tailed Student’s t test (paired, where indicated) and ANOVA, where indicated.

Results

Serotonergic effects at the retinogeniculate synapse

We recorded from the retinogeniculate synapse from freshly prepared LGN brain slices of p23–32 Black Swiss mice in recording conditions that isolate the excitatory connection between the retina and thalamus from the inhibitory and cortical feedback circuitry (see Materials and Methods). The relatively few and robust inputs that each relay neuron receives during this late stage in development allowed us to reproducibly stimulate only one to three retinal inputs (Chen and Regeh, 2000). Figure 1A illustrates the modulatory effects of the 5-HT, receptor agonist, 5-CT, on the response to paired retinal fiber stimulation at the retinogeniculate synapse. On average, 50 nm 5-CT significantly decreased the amplitude of the peak AMPA receptor (AMPAR) and NMDA receptor (NMDAR) EPSC by 55.7 ± 10.2% (n = 5; p <...
AMPAR and NMDAR EPSC, respectively, and reduced PPR to 44 ± 7% (p < 0.03; n = 5 for AMPAR and NMDAR EPSC amplitude and PPR; 5-CT vs cyn).

5-HT1 receptors do not contribute significantly to postsynaptic serotonergic modulation

Previous studies have demonstrated that serotonergic modulation of the hyperpolarization-activated current, Ih, in thalamic relay neurons results in depolarization of the cell (Pape and McCormick, 1989; McCormick and Pape, 1990b). However, the specific 5-HT receptor subtype that mediates this effect in relay neurons has not been identified. To evaluate whether 5-HT1 receptors contribute to this postsynaptic response in addition to their role in presynaptic inhibition, we examined the effects of 5-CT on Ih in LGN neurons using an internal recording solution that is potassium based and contains a regenerative solution with ATP and GTP (see Experimental procedures). Unlike the CsF-based internal solution used for EPSC recordings (Figs. 1A, 2) that are designed to minimize the known postsynaptic effects of 5-HT, the K+-based solution used for these experiments also permits modulation of intrinsic relay neuron conductances. Figure 1B shows the Ih current, elicited by hyperpolarizing from −50 to −100 mV, in control conditions and during bath application of 5-CT (gray trace). This hyperpolarization-activated current was inhibited by the Ih channel inhibitor ZD7288. On average, 50 nM 5-CT enhanced the ZD7288-sensitive current to 119 ± 5% of control (n = 7 cells; p = 0.002, paired t test). However, the 5-CT-mediated effect on Ih current did not significantly alter the membrane potential (average resting membrane potential in control conditions, −79 ± 1 mV, vs in the presence of 5-CT, −80 ± 2 mV, n = 10, p = 0.4, paired t test), and a change of −2.6 ± 1.1 mV from an initial holding membrane potential of −55 mV, n = 8, p = 0.3). Therefore, activation of 5-HT1 receptors appears to have a larger presynaptic effect on synaptic transmission when compared with its postsynaptic effect on membrane potential. By using the selective 5-HT1 receptor agonist 5-CT, we could study the role of presynaptic serotonergic modulation on the relay of visual information in isolation from the postsynaptic effects of serotonin.

Pharmacological characterization of presynaptic serotonergic modulation

Several serotonin receptor subtypes have been identified in the thalamus, including 5-HT1A, 5-HT1B, 5-HT2A, 5-HT2C, and 5-HT7 (Boschert et al., 1994; Pompeiano et al., 1994; Gustafson et al., 1996; Kia et al., 1996; Gerard et al., 1997; Steriade et al., 1997; Sari et al., 1999; Upton et al., 1999; Lopez-Gimenez et al., 2001a,b). To determine which of these receptor subtypes mediate serotonergic modulation at a presynaptic site of the retinogeniculate connection, we took advantage of various 5-HT receptor agonists and antagonists. The robust effects of 5-HT1 receptor agonist and antagonist, 5-CT and cyanopindolol, respectively, at the retinogeniculate synapse suggested the involvement of this subclass of receptors. To distinguish between 5-HT1A and 5-HT1B receptors, we first examined the effects 5 µM 5-nonyloxytryptamine (5-NOT), an agonist with higher selectivity for the 5-HT1B over the 5-HT1A receptor (Glennon et al., 1996). Nonyloxytryptamine (5 µM) significantly inhibited the peak amplitude of evoked EPSCs to 48.8 ± 7.5% of control (n = 4; p < 0.05, paired t test) (Fig. 2A). Another selective 5-HT1B agonist, ANP (5 µM) (Schlicker et al., 1992), also inhibited synaptic strength to 58.9 ± 3.5% of control (n = 4; p < 0.05, paired t test) (Fig. 2D). In contrast, bath application of 8-hydroxy-
Synaptic transmission at the retinogeniculate synapse. We found that the effects of 5-CT on the retinogeniculate synapse were more easily reversible than 5-NOT; thus, we chose to use 5-CT for our subsequent experiments studying presynaptic serotonergic modulation.

Presynaptic serotonergic inhibition relieves paired-pulse depression

To further understand the role of relief of synaptic depression by presynaptic serotonergic modulation on the transmission of visual information, we examined in more detail the effect of 5-CT on the synaptic response to trains of retinal fiber stimulation. First, we examined the synaptic response to pairs of stimuli. Figure 3A shows the effects of 5-CT on the recovery from synaptic depression for pairs of EPSCs separated by ISIs varying from 10 to 200 msec. 5-CT greatly alleviated paired-pulse depression, particularly for short ISIs. For example, the paired-pulse ratio significantly increased from 20 ± 2% for an ISI of 20 msec in control conditions to 83 ± 4% (n = 5; p < 0.01, paired t test) in the presence of 5-CT (50 nM). A summary plot of the recovery from synaptic depression with and without 5-CT (open squares and filled circles, respectively) as a function of ISI is shown in Figure 3B. 5-CT was effective in relieving synaptic depression for pairs of stimuli separated by up to 1 sec in time (PPR for ISI 1 sec: control, 73 ± 2%; vs 5-CT, 84 ± 3%; n = 5; p = 0.01, paired t test). Comparison of the second EPSC amplitude of the synaptic response in the presence of 5-CT to the corresponding EPSC amplitude without the serotonergic agonist (EPSC_{5-CT}/EPSC_{CONTROL}) revealed an enhancement of synaptic strength up to ~150% of control at short ISIs (Fig. 3C). These findings suggest that activation of presynaptic serotonergic receptors could significantly alter the synaptic response over a wide range of presynaptic firing frequencies.

5-HT$_1$ receptor modulation during physiological stimulation

Given the strong relief from synaptic depression that followed presynaptic modulation with 5-CT, we next examined how this would affect synaptic transmission under more physiological conditions by recording the synaptic response to trains of retinal fiber stimuli at a bath temperature of 34–36°C. Figure 4 (left) shows the representative traces before (top) and during (bottom) bath application of 50 nM 5-CT for 10 Hz (A), 100 Hz (B), and 200 Hz (C) retinal fiber stimulation. On the right, the average control (black) and 5-CT (gray) traces are overlaid so that the relative amplitudes of each EPSC after the first EPSC in the train (EPSC$_2$/EPSC$_1$) can be compared. At all frequencies tested, 5-CT decreased the synaptic strength of the first EPSC. However, at higher frequencies (>50 Hz), the amplitudes of subsequent AMPAR EPSCs, which under control conditions were subject to strong depression (observe control traces in Fig. 4B,C), were enhanced, consistent with our studies using pairs of stimuli (Fig. 3). The effects of 5-CT on the EPSC amplitudes during a train of 15 stimuli are summarized in Figure 4D. The amplitude of each EPSC$_n$ in a train in the presence of 5-CT, normalized to the

DPAT (1 μM), a highly selective 5HT$_{1A}$ agonist (>10-fold greater affinity over 5HT$_{1B}$ receptors) that also activates 5-HT$_7$ receptors (pK$_{D}$ = 6.6) (Midlemiddin and Fozard, 1983; Engel et al., 1986; Wood et al., 2000), had little effect on the synaptic current at a concentration of 100 nM (n = 5; p > 0.05, paired t test) when compared with EPSCs in the presence of 5-CT (inhibited to 35.3 ± 2% of control, n = 5, p < 0.005) (Fig. 2B).

5-HT$_2$ receptors have also been shown to act as mediators of serotonergic modulation in the thalamus, in particular by causing excitation of local inhibitory interneurons (McCormick and Wang, 1991). To test whether 5-HT$_2$ receptors contribute to the presynaptic actions of serotonin on the excitatory retinogeniculate input, we examined the effects of α-methyl-5-HT, a broad 5-HT$_2$ receptor agonist, in the presence of the GABA$_A$ receptor antagonist bicuculline (Richardson et al., 1983; Baxter et al., 1995). As shown in Figure 2C, at a concentration of 100 nM, we observed no effect on the peak amplitude of evoked EPSCs. At a higher concentration (1 μM), however, the peak amplitude was significantly inhibited to 44.7 ± 8.7% of control (n = 4; p < 0.01, paired t test). Because α-methyl-5-HT has only ~1.3-fold specificity at 5-HT$_2$ over 5-HT$_1$ receptors (Engel et al., 1986; Baxter et al., 1995), it is conceivable that the drug elicited its effect by cross-reacting with other serotonergic receptors. To address this possibility, we applied, in succession, the specific 5-HT$_2$ antagonist cinanserin (25 μM) and the specific 5-HT$_7$ R antagonist cyanoindolol (5 μM) to the bath solution. Figure 2C shows that cyanoindolol, but not cinanserin, antagonized the effect elicited by α-methyl-5-HT, consistent with α-methyl-5-HT mediating its effect through 5-HT$_1$ receptors. A summary of the average effects of different 5-HT receptor agonists on the peak EPSC amplitude is shown in Figure 2D. Together, our pharmacological data support the role of 5-HT$_{1B}$ receptor-mediated inhibition of
SEROTONERGIC RECEPTOR ACTIVATION ALTERS SHORT-TERM PLASTICITY AT THE RETINOGENICULATE SYNAPSE

Figure 3. Serotonergic receptor activation alters short-term plasticity at the retinogeniculate synapse. **A**, 5-CT relieved synaptic depression especially for short ISIs. Superimposed pairs of AMPAR EPSCs recorded at varying ISIs were acquired under control conditions (top) and in the presence of 5-CT (bottom). The overlaid traces in Figure 4 also revealed another change in the synaptic response to trains by serotonergic presynaptic modulation. The time course of EPSC decay (τ) accelerated in the presence of 5-CT. The difference in the decay can be most clearly observed after the last EPSC in the train (EPSC15) when the decay is fit by a double exponential function. On average, 5-CT significantly accelerated the slow component of EPSC15 decay (τs) at 100 Hz (control, τs = 129 ± 28 msec, vs 5-CT, τs = 59 ± 11 msec; n = 6; p = 0.02, paired t test) but not at 10 Hz (control, τs = 27 ± 12 msec, vs 5-CT, τs = 14 ± 3; n = 7; p = 0.3, paired t test). In both control and 5-CT conditions, the average time course of EPSC15 decay at 100 Hz was prolonged when compared with the average time course of a single EPSC (control: first, τ1 = 22 ± 6 msec, vs 15th, τ1 = 129 ± 28 msec; n = 6, p = 0.01; 5-CT: first, τ1 = 11 ± 4 msec, vs 15th, τ1 = 59 ± 11 msec, n = 7, p = 0.02). Moreover, the average τ1 of a single EPSC in control conditions was not significantly different from that in the presence of 5-CT (p = 0.12; paired t test). Our data are consistent with a prolongation of EPSC decay time course during a train secondary to the accumulation of presynaptic residual calcium that results in enhanced delayed release and extended time course of transmitter in the synaptic cleft. The EPSC15 decay time course is decreased in the presence of a presynaptic modulator that acts by inhibiting presynaptic calcium, such as 5-CT (Rahamimoff and Yaari, 1973; Delaney et al., 1989; Van Der Kloot and Molgo, 1993; Ravin et al., 1997; Aturi and Regehr, 1998; Brenowitz et al., 1998).

Does the 5-CT-mediated inhibition of the first EPSC amplitude and acceleration of the time course of EPSC decay balance its enhancement of subsequent EPSC amplitudes (EPSC2–15) during high-frequency trains? We compared the average total synaptic charge transferred in control conditions to that in 5-CT. For the examples shown in Figure 4A–C, the total synaptic charge (Q) was 59, 29, and 21 pA*sec for 10, 100, and 200 Hz, respectively, in control conditions and 23, 15, and 16 pA*sec in the presence of 5-CT. Thus, at all frequencies tested, presynaptic serotonergic modulation resulted in a net decrease in the synaptic charge transferred. However, the relative decrease in synaptic charge was not constant over the frequency range (QCT/QCONT) was 0.39, 0.52, and 0.76, respectively, for 10, 100, and 200 Hz. Figure 4D compares the QCT/QCONT from seven individual relay neurons at 10 Hz to that at 100 Hz. The reduction of synaptic charge transferred was greater at low frequency compared with high frequencies for five of seven cells. On average, the QCT/QCONT was 0.5 ± 0.1 at 10 Hz and 0.7 ± 0.1 at 100 Hz (p < 0.03; paired t test). Thus, although the net synaptic charge was decreased in the presence of presynaptic serotonergic modulation, relief of synaptic depression contributed a frequency dependence to the inhibition of the total synaptic charge transferred at the retinogeniculate synapse.

To evaluate whether the effects of serotonergic modulation that we have characterized in regular trains of stimuli also apply to the synaptic response to irregular trains of stimuli that more closely resemble physiological presynaptic activity, retinal fibers were activated in a pattern that mimics the firing pattern of a
5-HT₁ receptor-mediated effects on postsynaptic firing

How do the decrease in synaptic charge and the relief of synaptic depression affect postsynaptic firing? If the safety factor at the retinogeniculate synapse is very high, such that individual EPSCs in a train inhibited by 5-CT can still drive the postsynaptic cell to fire, then one would expect firing to be enhanced for higher frequencies because of the strong relief of synaptic depression. Alternatively, if individual synaptic responses to retinal fiber stimulation are too weak to depolarize the postsynaptic neuron to threshold alone, then firing will depend more on the summation of EPSCs or the total synaptic charge transferred. To address this question, we examined the effects of 5-CT on the firing response of relay neurons to a physiological train of retinal fiber stimulation (see Experimental procedures). Because serotonin has been shown to contribute to the transition from a hyperpolarized bursting mode to a more depolarized tonic firing mode in relay neurons, we compared the responses of relay cells at two membrane potential ranges, −75 to −70 mV and −60 to −55 mV, chosen to simulate the membrane potential for bursting and tonic firing modes, respectively (Steriade et al., 1993, 1997; McCormick and Bal, 1994).

By switching from voltage-clamp to current-clamp modes while recording from the same cell, we could correlate the synaptic response and firing probability from a given relay neuron. A representative experiment is shown in Figure 6A in a counterclockwise direction. In voltage-clamp mode, the synaptic response elicited by a train of physiological stimuli using the K⁺-based internal solution was very similar to that using a CsF internal recording solution (compare Figs. 6Ai, left, to 5A). The recording amplifier was switched to the current-clamp mode with a hyperpolarized holding potential (Fig. 6Aii, left) and then the cell was depolarized to −55 mV (Figure 6Aiii, left). 5-CT was then bath applied at a holding potential of −55 mV (Fig. 6Aiii, right), and after the firing response had reached equilibrium (after 10 min), the cell was hyperpolarized (Fig. 6Aii, right). At the end of the experiment, the recording was then switched back to the voltage-clamp mode (Fig. 6Aii, right) in the presence of the serotoninergic receptor agonist to examine the degree of 5-CT inhibition on the synaptic currents. This experimental paradigm ensured that each retinal fiber stimulation resulted in a synaptic response and allowed us to compare the synaptic response to postsynaptic firing. Consistent with findings from previous studies, we found that, for a given retinal fiber train, the number of postsynaptic action potentials generated at a depolarized holding potential (−60 to −55 mV) was generally greater than the number of action potentials generated at more hyperpolarized potentials (−75 to −70 mV). Moreover, the spiking activity observed at depolarized potentials followed the pattern of input stimuli more closely than the burst-like spiking observed at more hyperpolarized potentials (Guido et al., 1995; Sherman, 1996; Blitz and Regehr, 2003).
Figure 6B illustrates that 5-CT inhibited relay neuron firing in a reversible manner. Representative traces of the firing response of a relay neuron to a physiological train of retinal fiber stimuli are shown before and during bath application of 5-CT as well as during washout of the 5-HT agonist in the presence of cyanopindolol at depolarized (Fig. 6B, left) and hyperpolarized (Fig. 6C, left) potentials, respectively. The time course of the experiment is shown on the right as a raster plot of relay neuron spikes. When 5-CT was applied to the bath solution, the number of evoked APs decreased at both holding potentials. This effect was readily reversible with bath application of cyanopindolol. Comparison of the effects of 5-CT on relay neuron firing revealed that the degree of spike inhibition was not dependent on relay neuron holding potential. 5-CT decreased the number of action potentials to 32.8 ± 17.4% (n = 5; p = 0.02, paired t test) and 52.4 ± 8.3% (n = 6; p = 0.002, paired t test) of control spikes at depolarized and hyperpolarized holding potentials, respectively (inhibition at −55 vs −70 mV not significantly different; p = 0.31). Moreover, spike voltage threshold was not significantly altered by 5-CT (−52.9 ± 0.4 vs −52.2 ± 0.6 mV for control and 5-CT, respectively; n = 21 neurons; p = 0.1, paired t test).

To determine whether possible postsynaptic effects of 5-CT contributed to its inhibition of relay neuron firing, we examined the effects of the serotonergic receptor agonist on the postsynaptic membrane potential in the absence of the presynaptic input. Figure 7 illustrates the membrane potential response of a relay neuron to a series of current injections designed to mimic EPSPs before (black trace) and during (gray trace) bath application of 50 nM 5-CT. The time course of membrane potential decay after five depolarizing current injections at 100 Hz, characterized by a double exponential fit, was not significantly altered in the presence of 5-CT (control: τfast = 4 ± 1 msec, τslow = 33 ± 2 msec; 5-CT: τfast = 6 ± 1 msec, τslow = 44 ± 7 msec; n = 4; p ≥ 0.2 for both time constants, paired t test). In addition, comparison of the area underlying the membrane potential changes in response to current injections revealed no significant changes in the presence of the 5-HT1 receptor agonist (control, 0.35 ± 0.07 mV·sec; 5-CT, 0.41 ± 0.09 mV·sec; n = 4; p = 0.1, paired t test). These data are consistent with a postsynaptic site of action of 5-CT that results in a decrease in the total synaptic charge transferred at the retinogeniculate synapse and concomitant inhibition of relay neuron firing.

Serotonergic inhibition of relay neuron firing is frequency dependent

During regular trains of stimulation, the decrease in the total synaptic charge transferred in the presence of 5-CT, when compared with control conditions, was greater for lower frequencies than for higher frequencies (Fig. 4E). To determine whether presynaptic serotonergic modulation of postsynaptic firing is also frequency dependent, we examined the response of the synapse before and during bath application of 5-CT over a range of stimulation frequencies. Again, the membrane potential of relay neurons was depolarized to a range between −60 and −55 mV to mimic the tonic mode of firing. Figure 8A shows representative firing responses of relay neurons recorded before (left) and during bath application of 5-CT (right). The synaptic response of each cell was recorded in voltage clamp at the beginning and end of the experiment to ensure that each presynaptic stimulation elicited a synaptic response (Fig. 6A). As described previously, the firing response to trains of retinal fiber stimulation varied from cell to cell (Blitz and Regehr, 2003). Thus, we examined the relative change in firing in response to 5-CT. 5-CT reduced the firing of the postsynaptic cell when stimulated with a train of 15 stimuli at 10 Hz, diminishing the number of action potential spikes to 35.8 ± 11% (n = 12 cells) of control. However, the inhibitory effect of 5-CT was significantly less at higher frequencies, reducing the number of spikes to 66.8 ± 5% of control for 200 Hz trains (n = 15 cells; p = 0.04). The fraction of action potentials elicited in the presence of 5-CT, relative to control conditions, is plotted as a function of retinal fiber stimulation frequency in Figure 8B. Thus, consistent with the frequency-dependent decrease in synaptic charge, inhibition of relay neuron firing by presynaptic serotonergic modulation was also frequency dependent.
modes.

Ai, Bath temperature, 34 –36°C

A single injection is followed by a 100 Hz train of five injections. The membrane potential current ramps injections depolarized the membrane potential of a representative relay neuron.

The role of baseline level of synaptic depression on presynaptic serotonergic modulation

The stimulation paradigm that we used in the experiments shown in Figures 4 and 8 allows for complete recovery from synaptic depression between trials. This type of activity pattern would be consistent with the firing characteristics of several classes of RGCs in mice that have been shown to exhibit long periods of silence during darkness (ON cells) or light (OFF cells) (Pang et al., 2003). After a period without retinal ganglion cell spiking, the strength of the first retinogeniculate EPSP in response to a change in light stimulation would be much greater than the subsequent EPSPs and would thus contribute to a large portion of the total synaptic charge transferred from RGCs to relay neurons. We were also interested in understanding how presynaptic activity conditions that result in a baseline state of synaptic depression at the retinogeniculate synapse would influence 5-CT modulation of EPSC amplitudes and relay neuron firing. Thus, we first examined the effects of 5-CT on EPSC amplitude in response to a stimulation paradigm where a conditioning train of five stimuli at 50 Hz preceded different frequencies of retinal fiber trains. Fifty Hertz was selected because the amplitudes of the subsequent EPSCs after the first EPSC are similar between control conditions and in the presence of 5-CT (Fig. 4D). Figure 9A–C illustrates the synaptic response to a change in stimulus frequency from a baseline of 50 Hz to 15 stimuli at 10, 50, and 100 Hz for control conditions (top trace) and in the presence of 5-CT (bottom trace). On the right of each panel, synaptic currents in control conditions (black traces) and in the presence of 5-CT (gray traces) are overlaid to compare the amplitudes and time course of the EPSCs in response to the 15 stimuli after the conditioning train. Consistent with our previous data using regular trains, 5-CT enhanced the EPSCs at 15 stimuli after the conditioning train at 100 Hz but reduced the amplitudes at 10 Hz. A summary of the relative EPSC amplitude in the presence of 5-CT compared with the amplitude of the corresponding EPSC in control is shown in Figure 9D. However, when comparing the total synaptic charge transferred, measured from the onset of the train after the conditioning train, we found that synaptic charge is still reduced in the presence of 5-CT agonist (Fig. 9E). $Q_{\text{CONT}}/Q_{\text{CT}}$ was significantly greater at 100 versus 10 Hz for 10 individual cells (average $Q_{\text{CONT}}/Q_{\text{CT}}$ for 10 Hz, 0.44 ± 0.03; for 100 Hz, 0.70 ± 0.03; p < 0.001, paired t test). Thus, even when the amplitude of the first EPSC was not significantly larger than subsequent EPSCs in a train, the serotonergic agonist inhibited the total amount of charge. Comparison of the overlaid synaptic response in Figure 9A–C (right) revealed the basis of the decrease in synaptic charge. Consistent with our data using regular trains (Fig. 4), 5-CT accelerated the decay of each EPSC in a train when compared with control conditions. However, the decrease in synaptic charge was frequency dependent, secondary to the enhancement of later EPSC amplitudes during high-frequency trains.

Figure 10 illustrates how the 50 Hz conditioning train affects relay neuron firing in the presence of 5-CT. The degree of inhibition of spiking by 5-CT was significantly greater at 10 versus 100 Hz stimulation (Fig. 10B). In fact, relay neuron firing in response to 100 Hz retinal fiber stimulation was not significantly altered in 5-CT when compared with control conditions ($n = 11$; 0.001, paired t test).
The frequency dependence of inhibition by 5-CT was steeper when there was a baseline level of depression at the retinogeniculate synapse. To address whether a small change in postsynaptic I_h receptor conductance could contribute to the observed frequency-dependent inhibition of relay neuron firing by 5-CT, we compared the firing of relay neurons in the presence of 5-CT (50 nM) to that after a 10 min bath application of 5-CT and the I_h receptor antagonist ZD7288 (10 μM, a concentration that effectively inhibits the I_h current in our preparation as illustrated in Fig. 1 B). We found that the addition of ZD7288 did not alter the frequency-dependent effects of 5-CT. The ratio of the number of spikes in 5-CT alone versus the number of spikes in 5-CT and 10 μM ZD7288 is 0.9 ± 0.5, 0.9 ± 0.1, and 0.9 ± 0.1 for 10, 50, and 100 Hz, respectively (n = 4 and p > 0.4 for all frequencies; paired t test). Thus, the frequency dependence of the inhibition of relay neuron firing by 5-CT is mediated through presynaptic and not postsynaptic modulatory effects of serotonin.

Discussion

Our pharmacological studies demonstrate that presynaptic 5HT_1 receptors, especially that of the 5HT_1B subtype, can exert a powerful inhibitory influence on glutamatergic transmission at the retinogeniculate synapse. These findings are consistent with the facts that RGCs express 5-HT_1B receptors in axon terminals of the LGN (Boschert et al., 1994) and that presynaptic inhibition through 5-HT_1B receptors occurs at other targets of RGCs, including the suprachiasmatic nucleus (Pickard et al., 1999) and the optic tectum (Mooney et al., 1994, 1996). Here, we take advantage of the relative selectivity of the 5-HT_1 receptor agonist, 5-CT, for presynaptic over postsynaptic effects of serotonin to examine the role of presynaptic serotonergic modulation at the retinogeniculate synapse.

Frequency-dependent relief from depression versus inhibition of synaptic strength

This study demonstrates that, through inhibition of synaptic strength and a shortening of the decay time course of EPSCs, presynaptic modulation by serotonin results in a reduction of charge transferred across the retinogeniculate synapse. The acceleration of the EPSC decay by 5-CT is likely secondary to a reduction in the accumulation of presynaptic calcium, thus decreasing delayed release and transmitter accumulation in the synaptic cleft during prolonged trains (Atluri and Regehr, 1998). It is less likely that the change in the EPSC decay is secondary to the postsynaptic changes in intrinsic membrane properties because 5-CT did not significantly alter the membrane potential of the postsynaptic neuron (both from the resting potential as well as when the membrane potential was initially held at −55 mV), the spike voltage threshold, or accelerate the membrane potential decay of relay neurons in response to intracellular current injections. Moreover, application of the I_h receptor antagonist, ZD7288, did not change the frequency-dependent effects of 5-CT on relay neuron firing.

The serotonin-mediated decrease in total synaptic charge transferred across the retinogeniculate synapse results in a decrease in postsynaptic firing over a wide range of stimulation frequencies. However, counteracting this inhibitory serotonergic effect is a frequency-dependent relief from synaptic depression that, at higher stimulation frequencies, enhances the charge transfer, relative to lower frequencies, at the retinogeniculate synapse. The mechanisms underlying relief from depression most likely involve a calcium-mediated decrease in release probability that reduces the depletion of available vesicles, although we cannot rule out that other presynaptic processes, such as relief of G-protein-coupled receptor activation, may also play a role (Park
At the end-bulb synapse, a single EPSC reduced to 10% of its modulation between the two sensory synapses is the safety factor. More than at higher frequencies, thus limiting the frequency retinogeniculate synapse reduces postsynaptic firing at lower synapse. In contrast, presynaptic serotonergic modulation of the range of presynaptic activity that is transmitted at the end-bulb enhancing postsynaptic firing at high stimulation frequencies.

Initial strength could still reliably drive the firing of the postsynaptic nucleus magnocellularis cell (Brenowitz et al., 1998a). In contrast, at the retinogeniculate synapse, we found that synaptic inputs >0.8 and 0.4 nA in amplitude were needed to elicit an action potential from holding potentials of −70 and −55 mV, respectively, in thalamic relay neurons (data not shown). Because the average AMPAR EPSC evoked at 34–36°C in our experiments was −2.2 ± 0.3 nA (ranging from −0.9 to −5.7 nA; n = 18), the safety factor at the retinogeniculate synapse is significantly lower than that at the auditory synapse. Thus, single action potentials invading the presynaptic terminal of the retinogeniculate synapse are less likely to drive postsynaptic firing in the presence of 5-CT. Instead, temporal summation of EPSCs in a train is more important to the efficacy of transmission of visual information across the synapse. This is consistent with the in vivo finding that successive retinal spikes are more effective in driving relay neuron firing (Mastronarde, 1987; Usrey et al., 1998). Therefore, in addition to the presynaptic modulation of short-term plasticity, postsynaptic temporal summation in response to high-frequency retinal fiber stimulation must also contribute to the differential inhibition of low- versus high-frequency activity by serotonin.

The difference in the net effect of presynaptic modulation between the auditory and visual synapses highlights the fact that relief of depression that results in an enhancement of AMPAR amplitudes during a train does not necessarily predict an enhancement of postsynaptic firing. The effects of presynaptic modulation can vary between different synapses, depending on the relative contributions of changes in synaptic strength, short-term synaptic plasticity, postsynaptic firing threshold and changes in delayed release and transmitter clearance in the synaptic cleft.

**Physiological significance of presynaptic modulation**

Serotonergic modulation has multiple effects at the retinogeniculate synapse. Counteracting the presynaptic 5HT_{1B} receptor-mediated inhibition of neurotransmitter release is the activation of postsynaptic I_{	ext{N}} current, resulting in elimination of spindle waves and rebound burst firing, thus increasing the probability of tonic mode firing (McCormick and Pape, 1990a; Lee and McCormick, 1996; Monckton and McCormick, 2002). However, we found that in both burst and tonic modes, relay neuron firing is reduced by presynaptic serotonergic modulation. Thus, in our recording conditions, the presynaptic inhibitory effect of serotonin can act in the presence of the postsynaptic excitatory effect. These findings are consistent with previous in vivo experiments that show that iontophoretic application of serotonin to the LGN or stimulation of the dorsal raphe nucleus decreases the firing rate of relay cells (Curtis and Davis, 1962; Rogawski and Aghajanian, 1980; Yoshida et al., 1984; Kayama, 1985; Marks et al., 1987; Funke and Eysel, 1993).

Inhibition of neurotransmission by serotonin may at first seem paradoxical, given that serotonergic tone is highest during the awake state and gradually declines through slow-wave sleep.
reaching a minimum during rapid eye movement states (Jacobs and Fornal, 1999). This raises the question of how presynaptic serotonergic modulation contributes to the transfer of information from the retina to the cortex. One possible explanation may lie in the frequency dependence of presynaptic inhibition. RGCs in mice have been shown to fire at rates ranging from 200 to 500 Hz in response to transitions between light and dark (Stone and Pinto, 1993; Nirenberg and Meister, 1997). Here, we show that activation of presynaptic 5-HT1 receptors acts to preferentially favor the transmission of higher versus lower frequency RGC firing via the retinogeniculate synapse. By effectively limiting the relay of visual information to those RGCs that respond to a change in light stimuli with a robust discharge of action potentials, presynaptic serotonergic modulation may be one mechanism that improves the “signal-to-noise” of the transmitted information. Changes between the tonic and burst modes, as well as postsynaptic modulatory actions of neurotransmitters, have been shown previously to affect relay neuron “filtering” properties at other thalamic synapses (Mukherjee and Kaplan, 1995; Castro-Alamancos and Calcagnotto, 2001; Castro-Alamancos, 2002). Here, we show that presynaptic inhibition can also strongly affect the temporal response properties of a thalamic synapse.

Our data does not address the possibility that serotonergic modulation takes place at other locations in the LGN. In fact, previous studies have shown an inhibitory serotonergic effect in the LGN through excitation of GABAergic neurons of the perigeniculate nucleus (McCormick and Wang, 1991; Funke and Eysel, 1993), GABAergic interlaminar interneurons (Sanchez-Vives et al., 1996), and through mild excitation of local GABAergic interneurons (Pape and McCormick, 1995). The inhibitory circuitry has been shown to contribute to the center-surround antagonism of receptive fields of LGN neurons (Eysel et al., 1986; Norton et al., 1989; Fjeld et al., 2002).

We studied the modulatory role of serotonin at the retinogeniculate synapse in isolation from GABAergic inhibitory influences, ascending brainstem connections, and corticothalamic feedback projections that have all been shown to be important in thalamic processing of visual information (McCormick and Bal, 1994, 1997; Steriade et al., 1997). This reductionist approach allowed us to examine the contributions of serotonergic presynaptic modulation at the excitatory retinogeniculate connection. In the LGN of intact, awake animals, however, it is likely that the presynaptic and postsynaptic effects of serotonin at the retinogeniculate synapse work in concert with other neuromodulators, as well as with inhibitory and cortical feedback circuits to refine the thalamic relay neuron response to visual information.

References


Figure 10. Differential inhibition of relay neuron firing by 5-CT is also present at synapses with a recent history of activity. A, The firing responses of relay neurons to a train of 15 stimuli at 10 Hz (top), 50 Hz (middle), and 100 Hz (bottom) after five conditioning stimuli at 50 Hz. Representative traces from the same neuron in control conditions (left) and during bath application of 50 nM 5-CT (right). The black bars under each trace indicate the time of the conditioning stimuli. B, Summary of the ratio of spike number in response to the last 15 stimuli in the presence of 5-CT to that in control conditions is plotted as a function of stimulation frequency (n = 10–11 cells for each frequency). The asterisks indicate that inhibition at 10 Hz is significantly different from that of 100 Hz (p = 0.008), Bath temperature, 34–36°C.


