Neuromodulatory Role of Serotonin in the Ferret Thalamus

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Monckton, James E. and David A. McCormick. Neuromodulatory role of serotonin in the ferret thalamus. J Neurophysiol 87: 2124–2136, 2002; 10.1152/jn.00650.2001. Serotonergic fibers broadly innervate the thalamus and may influence the sleep wake cycle, attention, and other processes through modulation of neurons in this structure. However, the actions of serotonin in the dorsal thalamus have been investigated in detail only in the dorsal lateral geniculate nucleus. In the present study, we examined the action of serotonin in several different regions of the ferret dorsal thalamus, including the associative nuclei, using the in vitro slice preparation and intracellular recording techniques. In nearly all nuclei examined, the predominant action of serotonin was one of hyperpolarization and inhibition of the tonic firing mode. The magnitude of the hyperpolarizing response decreased with age and varied greatly across and somewhat within nuclei maintaining the following relationship (in descending order of magnitude): lateral posterior, lateral dorsal, pulvinar, mediodorsal, center median, anteroventral, central lateral, ventral basal, and medial geniculate. This hyperpolarization is elicited through two mechanisms: one direct and the other via local interneurons. The direct action occurs through an increase in potassium conductance mediated through the 5-HT1A receptor. This conclusion is supported by the findings that it persists in the presence of tetrodotoxin and block of GABAergic synaptic transmission, the reversal potential shifts in a Nernstian fashion with changes in extracellular potassium concentration, and the response is antagonized by the 5-HT1A selective agonist WAY100635 and mimicked by the application of the 5-HT1A–selective agonist 8-OH DPAT. The second mechanism by which 5-HT evoked a hyperpolarization was through the activation of local interneurons. In slices in which GABA receptors were not blocked, 5-HT application increased the frequency and amplitude of spontaneous inhibitory postsynaptic potentials (IPSPs), which persisted in the presence of tetrodotoxin and block of GABAergic synaptic transmission. However, in vivo single unit studies of the lateral geniculate, when in the hyperpolarized state, is strongly influenced by the properties of two distinct ionic channels: Ih and If (for review see McCormick and Bal 1997). The first, Ih, is a low-threshold calcium channel that is inactivated at membrane potentials above approximately −65 mV. At hyperpolarized membrane potentials, the interaction of these two ionic currents can cause thalamocortical cells to discharge rhythmic bursts of action potentials either through intrinsic mechanisms or in response to the arrival of barrages of inhibitory postsynaptic potentials (IPSPs), such as during the generation of sleep spindles (reviewed in McCormick and Bal 1997; Steriade et al. 1993). In addition, at these hyperpolarized levels, excitatory post synaptic potentials can result in the activation of low-threshold Ca2+ spike-mediated bursts (Jahnsen and Llinas 1984a,b; McCormick and Feeney 1990). The transition from sleep to waking is associated with a general depolarization of thalamocortical neurons, which partially or completely inactivates low-threshold Ca2+ current and therefore reduces the probability of generation of bursts of action potentials (reviewed in McCormick and Bal 1997). Multiple electrophysiological and pharmacological studies suggest that the ascending systems from the brain stem are largely responsible for this change in firing mode of thalamocortical neurons (reviewed by McCormick 1992). Characterizing how these neuromodulators affect the properties of thalamic neurons is therefore critical to the understanding of the ascending modulation of thalamocortical function.

A significant effort has been made to understand the role of 5-HT in the primary visual sensory thalamic nucleus, the dorsal lateral geniculate. In vivo single unit studies of the lateral geniculate nucleus have demonstrated a decrease in spontaneous firing rate, response to visual stimuli, and responsiveness to optic tract stimulation following local application of 5-HT or electrical stimulation of the dorsal raphe (Curtis and Davis 1962; Kayama et al. 1989; Marks et al. 1987; Rogawski and Aghajanian 1980; Yoshida et al. 1984). These studies suggest that the ascending systems from the brain stem are largely responsible for this change in firing mode of thalamocortical neurons (reviewed by McCormick 1992). Characterizing how these neuromodulators affect the properties of thalamic neurons is therefore critical to the understanding of the ascending modulation of thalamocortical function.

INTRODUCTION

The thalamus is often thought of as a variable gate that regulates the flow of information to the cerebral cortex and that this regulation is mediated in part by the input of a variety of ascending activating systems from the brain stem and hypothalamus. These ascending activating systems release a number of neurotransmitters including serotonin (5-hydroxytryptamine, 5-HT), norepinephrine, acetylcholine, and histamine (for review, see McCormick 1992; Steriade and McCarley 1990). These neuromodulators influence the state of the thalamus by altering specific ionic channels in thalamic neurons through activation of G protein–coupled receptors (reviewed by McCormick 1992). The electrophysiological activity of thalamocortical neurons, when in the hyperpolarized state, is strongly influenced by the properties of two distinct ionic channels: Ih and If (for review see McCormick and Bal 1997). The first, Ih, is a low-threshold calcium channel that is inactivated at membrane potentials above approximately −65 mV. At hyperpolarized membrane potentials, the interaction of these two ionic currents can cause thalamocortical cells to discharge rhythmic bursts of action potentials either through intrinsic mechanisms or in response to the arrival of barrages of inhibitory postsynaptic potentials (IPSPs), such as during the generation of sleep spindles (reviewed in McCormick and Bal 1997; Steriade et al. 1993). In addition, at these hyperpolarized levels, excitatory post synaptic potentials can result in the activation of low-threshold Ca2+ spike-mediated bursts (Jahnsen and Llinas 1984a,b; McCormick and Feeney 1990). The transition from sleep to waking is associated with a general depolarization of thalamocortical neurons, which partially or completely inactivates low-threshold Ca2+ current and therefore reduces the probability of generation of bursts of action potentials (reviewed in McCormick and Bal 1997). Multiple electrophysiological and pharmacological studies suggest that the ascending systems from the brain stem are largely responsible for this change in firing mode of thalamocortical neurons (reviewed by McCormick 1992). Characterizing how these neuromodulators affect the properties of thalamic neurons is therefore critical to the understanding of the ascending modulation of thalamocortical function.

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1.25 NaH$_2$PO$_4$, 2 CaCl$_2$, 26 NaHCO$_3$, and 10 dextrose and was cellular labeling of recorded neurons and beveled on a Sutter Instrument sodium (30 mg/kg) and killed by decapitation. A modifi

METHODS

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RESULTS

Morphology

To more accurately determine the location of nuclei in the ferret thalamus, the brains from two, 2-month-old ferrets were fixed, sectioned, mounted on slides, and stained with cresyl violet. Using a cat atlas of the thalamus and basal telencephalon as a reference (Berman and Jones 1982), various thalamic nuclei of particular interest to this study were identified (e.g., Fig. 1A). In addition, to further facilitate the localization of neurons in their prospective nuclei, each recorded cell was filled with 2% biocytin, and in most cases the section containing the neuron was counterstained with cresyl violet (e.g., Fig. 1B). Examination of cresyl violet–stained cortical sections readily revealed the various thalamic nuclei of interest (Fig. 1A). Of the 312 neurons recorded for this study, 297 were characterized as thalamocortical cells based either on their electrophysiological properties (presence of a characteristic strong rebound low-threshold Ca$^{2+}$ spike and moderate spike duration) (see Pape and McCormick 1995) and/or on their morphological features (Fig. 1B). With the exception of the thalamocortical neurons filled in the Centre Median nuclei, the morphology of these cells was typical of thalamocortical neurons, including the presence of extensive smooth dendrites that lacked the filiform appendages typical of local interneurons (Guillery 1966). The majority of thalamocortical cells resembled those described as “class 1 cells” first described by Guillery: multipolar, with numerous dendrites projecting out radially in a relatively straight fashion (Guillery 1966). Centre Median neurons exhibited fewer dendrites and dendritic branches with each dendrite being thinner in diameter, possessing small fine dendritic appendages. In many of these filled thalamocortical cells, an axon could be followed to the edge of the slice, and local axon collaterals were not observed.

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Serotonin application evokes a hyperpolarizing response in nearly all thalamic nuclei examined

Intracellular recordings were obtained in 10 different nuclei throughout the thalamus. The dominant action of 5-HT in many thalamic nuclei other than the LGNd was a strong and prolonged hyperpolarization (Fig. 2). During this study, we noticed an age-dependent decrease in the 5-HT–induced hyperpolarization in pulvinar neurons, although even in adults this response was still prevalent. The amplitude of the 5-HT–induced hyperpolarizing response in pulvinar neurons was $-4.5 \pm 1.1$ (SE) mV at 7–12 wk of age ($n = 97$) and decreased to $-3.0 \pm 1.4$ mV at 20–35 wk of age ($n = 10$). The response in the 7- to 12-wk range appears to be relatively consistent, and therefore we restricted our comparison of the 5-HT response induced in different nuclei to this age range. The thalamic nuclei that were examined at 7–12 wk of age include the pulvinar ($n = 97$), lateral dorsal (LD, $n = 9$), and lateral posterior nuclei (LP, $n = 9$), the anteroventral nucleus (AV, $n = 7$), the intralaminar/medial nuclei (central lateral nucleus, CLN, $n = 13$; centre median, CM, $n = 10$; mediodorsal, MD, $n = 6$), and the primary sensory nuclei (lateral geniculate nucleus, LGNd, $n = 7$; medial geniculate nucleus, MGN, $n = 5$; and ventrobasal, VB, $n = 4$; Fig. 2; Table 1). With the notable exception of some neurons of the lateral geniculate nucleus, there were no depolarizing responses to serotonin recorded in these nuclei. The primary sensory nuclei exhibited markedly smaller hyperpolarizations than did the more associative nuclei. The relative order of magnitude of hyperpolarizing response to 5-HT was: LD, LP, pulvinar, MD, CM, AV, CLN, VB, MGN, LGNd. Compensating for the hyperpolarization with the intracellular injection of current revealed that it is associated with an increase in apparent input conductance (Fig. 2E).

Serotonin hyperpolarizes thalamocortical neurons through a direct postsynaptic action

We examined the mechanisms of the 5-HT–induced hyperpolarizations in thalamocortical cells in the pulvinar, although
some recordings were also obtained in other nuclei, as mentioned. Responses in the majority of cells to which 5-HT was applied revealed two distinguishing features that suggested at least two hyperpolarizing mechanisms. The 5-HT response profile included a fast component composed of IPSPs and a slower, larger magnitude component. To study the slower component of the hyperpolarization in isolation, we blocked the postsynaptic actions of local interneurons with the bath application either of the GABAA antagonists bicuculline (50–150 μM) or picrotoxin (100 μM) and either or the GABA_B antagonists CGP35348 (200 μM) or CGP56999A (0.5 μM). Additionally the Na+/K+ channel blocker, TTX (1 μM in bath) was often used to block the action potential–dependent release of neurotransmitters. The block of postsynaptic GABA receptors and action potential generation did not block the slow hyperpolarizing response to 5-HT, indicating that it is a direct postsynaptic effect (Fig. 3; n = 101). Switching to single electrode voltage clamp revealed that the hyperpolarization was associated with a 5-HT–induced outward current (Fig. 3C).

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>N</th>
<th>Response Amplitude</th>
</tr>
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<tbody>
<tr>
<td>Lateral dorsal</td>
<td>9</td>
<td>-6.9 ± 2.1</td>
</tr>
<tr>
<td>Lateral posterior</td>
<td>9</td>
<td>-5.1 ± 1.7</td>
</tr>
<tr>
<td>Pulvinar</td>
<td>97</td>
<td>-4.5 ± 1.7</td>
</tr>
<tr>
<td>Mediodorsal</td>
<td>6</td>
<td>-4.1 ± 1.6</td>
</tr>
<tr>
<td>Centre median</td>
<td>10</td>
<td>-3.7 ± 2.0</td>
</tr>
<tr>
<td>Anteroventral</td>
<td>7</td>
<td>-3.4 ± 1.3</td>
</tr>
<tr>
<td>Central lateral</td>
<td>13</td>
<td>-2.4 ± 1.4</td>
</tr>
<tr>
<td>Ventrobasal</td>
<td>4</td>
<td>-0.8 ± 1.1</td>
</tr>
<tr>
<td>Medial geniculate</td>
<td>5</td>
<td>-0.7 ± 0.9</td>
</tr>
<tr>
<td>Lateral geniculate</td>
<td>7</td>
<td>-0.2 ± 1.1</td>
</tr>
</tbody>
</table>

Values in Response Amplitude are means ± SE. These values reflect the naive response to serotonin in current clamp.
5-HT–induced hyperpolarization is consistent with the opening of a K⁺ channel

To elucidate the underlying ionic mechanisms of the hyperpolarization, the current-voltage relationship of the 5-HT response in thalamocortical neurons was examined. Slow voltage ramps performed in voltage clamp from −60 to −110 mV revealed a 5-HT–elicited outward current with a reversal potential around \( E_K \) (see Fig. 4). The potassium dependence of this current is supported by the shift in the reversal potential from −97.4 ± 2.7 mV to −71.6 ± 2.4 mV (\( n = 8 \)) with change in the concentration of potassium from 2.5 to 8 mM in the bathing media. This represents a 50.9-mV/10-fold change in [K⁺]o, similar to that predicted by the Nernst equation (61.4-mV/10-fold change at 35°C). To be consistent, all recordings examining the shift in reversal potential with change in potassium concentration were performed in the lateral pulvinar.

Pharmacological evidence supports the role of the 5-HT₁A receptor in the 5-HT–induced hyperpolarizing response

The hyperpolarizing action of 5-HT is dramatically attenuated or abolished by the local application of the 5-HT₁A–specific antagonist WAY100635 (\( n = 27; 10–100 \mu M \) in the micropipette). In the cell of Fig. 5, the application of 5-HT resulted in both a prolonged hyperpolarization and an apparent increase in IPSPs (Fig. 5A). Local application of the GABAₐ antagonist bicuculline methiodide (150 \( \mu M \) in micropipette) and of the GABAₐ antagonist CGP35348 (200 \( \mu M \)) resulted in a block of the 5-HT–evoked IPSPs and revealed a slow, direct hyperpolarizing action of 5-HT (Fig. 5B). The direct hyperpolarization activated by 5-HT was abolished by the local application of the 5-HT₁A antagonist WAY100635 (10–100 \( \mu M \) in micropipette; Fig. 5C; \( n = 17 \) pulvinar, 5 nonpulvinar), thus providing strong evidence for 5-HT₁A receptor being the mediator of the response.

Block of the hyperpolarizing 5-HT response with WAY100635 revealed another smaller response in a minority of cells (2/17 cells; 2 in pulvinar and an additional cell in VB; Fig. 6). In these cases, 5-HT application elicited a reduction in \( R_{in} \) with little or no change in \( V_m \). This response is similar to that examined previously in the LGNd and known to result from modulation of the hyperpolarization-activated cation current \( I_h \) (McCormick and Pape 1990). The presence of this unmasked decrease in \( R_{in} \) in the present study suggests that \( I_h \) is modulated through a receptor other than 5-HT₁A.

Application of the 5-HT₁A–selective agonist, 8-OHDPAT (10–100 \( \mu M \) in micropipette), in the presence of TTX or GABA receptors antagonists elicited a slow hyperpolarization in current clamp and an outward current in voltage clamp that partly or completely occluded the 5-HT–induced hyperpolarization (\( n = 9 \); not shown).

Serotonin indirectly inhibits thalamocortical neurons through the excitation of local interneurons

Close examination of the membrane potential in many thalamocortical cells revealed spontaneous IPSPs (Fig. 7, A and B). Local application of 5-HT resulted in an increase in the frequency of these IPSPs along with a prolonged hyperpolarization of the membrane potential (Fig. 7A). Local application of the GABAₐ receptor antagonist bicuculline completely blocked 5-HT–induced hyperpolarizing phasic events, con-
firming that they are GABAergic IPSPs (Fig. 7B), but left the direct, hyperpolarizing action of 5-HT intact.

A subset of pulvinar cells (n = 9) was identified as putative interneurons based on either their electrophysiological characteristics (lack of a strong and pronounced rebound low-threshold Ca$^{2+}$ spike and burst discharge and the presence of relatively short-duration action potentials) (Pape and McCormick 1995) and/or their morphological features (n = 8/9; see following text). Application of 5-HT to these cells resulted in a depolarization (n = 9/9) that was sufficiently large to evoke action potentials (8/9 neurons; Fig. 8A). This depolarization was associated with a small decrease in apparent input conductance (Fig. 8B). In voltage-clamp mode, the 5-HT response appeared as an inward current that persisted for several minutes (Fig. 8C). Intracellular injection of biocytin revealed that neurons that respond to 5-HT in this fashion possessed morphological features that have previously been associated with local GABAergic neurons in the thalamus, with both dendritic and axon-like processes that ramify locally. Closer examination of the dendritic processes revealed numerous filiform appendages in which a swelling was attached to the parent dendrite by a very thin process, as is common in local interneurons in the thalamus (Fig. 9; n = 8) (Guillery 1966).

**DISCUSSION**

With the exception of the dorsal lateral and medial geniculate nuclei, we have found the primary action of 5-HT in the ferret thalamus to be inhibitory. The magnitude of this inhibition varies between nuclei and even to some extent within nuclei. However, there is a gradient in the magnitude of the hyperpolarization across the associative nuclei whose relationship may be represented as follows: LD, LP, Pulvinar, MD, CM, AV, CLN, VB, MGN. Frank hyperpolarizing responses to 5-HT were not observed in LGN thalamocortical cells (n = 9) and only rarely observed in the MGN (n = 2/5), and in these two cases, the hyperpolarization was very small (<1.5 mV). Instead, in these nuclei, the application of 5-HT often elicited a small depolarization and increase in apparent input conductance, which has been shown previously to result from an enhancement of the hyperpolarization-activated cation current $I_h$ (McCormick and Pape 1990).

The inhibition observed in the other nuclei examined occurs through two mechanisms: direct postsynaptic action mediated by the 5-HT$\textsubscript{1A}$ receptor and an indirect increase in IPSPs, apparently through the excitation of local GABAergic interneurons. The direct action is mediated through an increase in a K$^+$ conductance. Support for this assertion comes from the outward direction of the current as measured in voltage clamp, an increase in input conductance, and a reversal potential shift that followed an increase in extracellular potassium concentration in a near Nernstian fashion. The conclusion that this modulation of the potassium current is governed by 5-HT$\textsubscript{1A}$ receptor activation is supported by the finding that the response is antagonized by the specific 5-HT$\textsubscript{1A}$ antagonist WAY-100635 (Fletcher et al. 1993, 1995; Foster et al. 1995). Additionally,
the application of the 5-HT1A-specific agonist, 8-OHDPAT (Gozlan et al. 1983), activated the hyperpolarizing response and partially antagonized the response to 5-HT. A partial agonist action of 8-OHDPAT has been previously reported in the hippocampus (Andrade 1992; Andrade and Nicoll 1987a,b; Stanhope and Dourish 1996). The 5-HT–induced hyperpolarizing response observed in the pulvinar is independent of GABA release, since it persists in the presence of TTX, and GABA_A and GABA_B antagonists. The postsynaptic responses reported here are consistent with those previously associated with the 5-HT1A receptor in the hippocampus (Aghajanian and Lakoski 1984; Andrade 1992; Andrade and Nicoll 1987a,b; Colino and Halliwell 1987; Sprouse and Aghajanian 1988), the raphe nucleus (Sprouse and Aghajanian 1987), and the cerebral cortex (McCormick and Williamson 1989; Sheldon and Aghajanian 1990). These and other studies have shown that 5-HT can open a particular subclass of K^+ channel (GIRKs) through the activation of the G_i/G_o family of G proteins (Luscher et al. 1997; see Aghajanian and Andrade 1997).

Immunohistochemical and autoradiographic studies reveal a relatively low density of 5-HT1A receptors in the thalamus of rat, cat, monkey, and human (Chalmers and Watson 1991; Dillon et al. 1991; Hall et al. 1997; Hume et al. 1994; Ito et al. 1999; Khawaja et al. 1995; Kia et al. 1996; Pompeiano et al. 1992), while the density of 5-HT1A receptors in ferret thalamus has not been reported. Possible explanations for the apparent discrepancy between this relatively low level of 5-HT1A receptors in the thalamus and our present results are age- or species-dependent differences. Indeed, we have found that the application of 5-HT to thalamocortical neurons of monkey and cat pulvinar maintained in vitro does not lead to a hyperpolarization, but rather to a robust enhancement of the hyperpolarization-activated cation current I_h (Monckton and McCormick 1999). In addition, we have also observed that the amplitude of the hyperpolarizing response to serotonin in ferret thalamus is age dependent, decreasing with age, although it is still present in the adult (unpublished observations). This decrease in amplitude of the hyperpolarizing response is consistent with age dependent decreases in the density of (3H) 8-OHDPAT binding in the rat thalamus (Daval et al. 1987). Together, these studies suggest that there may be considerable heterogeneity in the postsynaptic actions of serotonin between various thalamic...
FIG. 6. A: serotonin can activate 2 distinct responses in ventrobasal thalamocortical neurons. A: application of serotonin to this VB neuron results in a hyperpolarization and an anomalous increase in membrane conductance. B: the local application of WAY100635 (10 μM) blocks the 5-HT–induced hyperpolarization and leaves a response that is similar to that associated with 5-HT–induced enhancement of $I_h$ (McCormick and Pape 1990; Pape and McCormick 1989).

FIG. 7. Serotonin can strongly increase the occurrence of IPSPs in thalamocortical cells. A: application of serotonin to a pulvinar thalamocortical neuron results in a hyperpolarization and an increase in the frequency and amplitude of IPSPs. The regions labeled are expanded in the panels below so as to illustrate the 5-HT–induced barrage of IPSPs. The local application of bicuculline (500 μM in micropipette) and CGP35348 (2 mM) resulted in a block of the IPSPs (B), but did not block the 5-HT–induced hyperpolarization.
nuclei in different species, and therefore this heterogeneity must be taken into account.

**Intralaminar and medial nuclei differ from primary sensory nuclei in response to serotonin**

The finding that serotonin hyperpolarizes principal neurons in most of the thalamic regions examined in the ferret is somewhat surprising in light of in vitro work in the MGN and LGNd. In both of these nuclei in the guinea pig, ferret, and cat, local application of 5-HT results in a small depolarization accompanied by a decrease in input resistance (Lee and McCormick 1996; McCormick and Pape 1990). Both the decrease in $R_{in}$ and depolarization are due to a shift in the voltage dependency of $I_n$ such that it is active at more depolarized levels. It has been assumed that this response is mediated by stimulation of adenylyl cyclase, since application of membrane-permeable forms of cAMP, activation of adenylyl cyclase, or inhibition of phosphodiesterase all result in a similar shift of the voltage dependence of $I_n$ (McCormick and Pape 1990). This response to 5-HT appears to be mediated by 5-HT7 receptors (Chapin and Andrade 2001a,b), which are known to stimulate adenylyl cyclase (reviewed in Barnes and Sharp 1999; Boess and Martin 1994; Lucas and Hen 1995) and are present in the thalamus (Gerard et al. 1997; Gustafson et al. 1996; To et al. 1995).

In contrast to the primary sensory relay nuclei, the application of 5-HT to ferret thalamocortical cells in the medial, intralaminar, and other associative nuclei resulted in a pronounced hyperpolarization. Interestingly, even when the 5-HT1A, and GABA$_A$ receptors were blocked, only a few cells in the more associative nuclei showed evidence of a change in $I_n$, suggesting that there may be heterogeneity in this postsynaptic response to 5-HT in the ferret thalamus.

Even though we did not observe hyperpolarizing responses to 5-HT in the ferret LGNd, several previous in vivo studies have shown that 5-HT inhibits the activity of thalamocortical neurons in this nucleus. In vivo studies that iontophoretically applied 5-HT to neurons in the LGNd or caused it to be released through stimulation of the dorsal raphe demonstrated decreases in firing rates of extracellularly recorded thalamocortical neurons (Kayama et al. 1989; Marks et al. 1987; Rogawski and Aghajanian 1980; Yoshida et al. 1984). The decrement was observed in spontaneous activity, visually evoked activity, or responses to electrical stimulation of the optic tract. The serotonergic antagonist, methysergide, in most cases blocked this inhibition. These findings were taken to suggest that the role of 5-HT in the thalamus was one of inhibition. The mechanism by which this inhibition is achieved was not clearly delineated and could have arisen from increased release of GABA by interneurons, direct postsynaptic action on the principal cells, or by presynaptic inhibitory action on excitatory synapses. Indeed, blockade of GABA$_A$ receptors by iontophoretically applied bicuculline to the LGNd can nearly block the serotonin-induced suppression of visually evoked firing (Funke and Eysel 1995). In vivo single-unit recordings of the GABAergic neurons in the perigeniculate nucleus reveal that 5-HT excites these cells (Funke and Eysel 1993). Similarly, application of 5-HT to GABAergic neurons of the thalamic reticular nucleus (nRt) or perigeniculate nucleus (PGN) in vitro reveal a prolonged excitatory response mediated by membrane depolarization resulting from the closure of K$^+$ channels (McCormick and Wang 1991). Importantly, application of 5-HT to interlaminar interneurons in the ferret LGNd also resulted in a pronounced excitation of these cells. These GABAergic neurons appear to function, in all aspects, as displaced PGN/NRT neurons (Sanchez-Vives et al. 1996). Previous studies of the action of 5-HT on local interneurons in the cat LGNd have reported some mild excitatory responses (Pape and McCormick 1995), and our present results demonstrate that at least some local GABAergic neurons in the mammalian thalamus can be strongly excited by serotonin. Establishing the subclasses of GABAergic interneurons in the LGNd may shed light on these differences. The fact that catecholamines can excite GABAergic interneurons in the cat LGNd (To et al. 1995) but not in the ferret LGNd (Gerard et al. 1997; Gustafson et al. 1999; Boess and Martin 1994; Lucas and Hen 1995) supports the idea that there may be functional differences between these two species. Future studies using transgenic approaches may help to address this question.
ron within the mammalian thalamus that are excited by 5-HT remains a task for the future. Other studies have demonstrated an excitation of local GABAergic neurons in the cerebral cortex (Sheldon and Aghajanian 1990), hippocampus (Piguet and Galvan 1994), septum (Alreja 1996), and deep cerebellar nuclei (Cumming-Hood et al. 1993). However, not all types of GABAergic interneurons in the forebrain are excited by 5-HT, and some are inhibited (see Schmitz et al. 1995). In general, the excitation of GABAergic neurons is mediated by 5-HT2 receptors, which when activated, result in the closure of a K+ channel (McCormick and Wang 1991; reviewed in Aghajanian and Andrade 1997). This mechanism remains to be investigated in detail in thalamic local GABAergic interneurons. Both 5-HT2A and 5-HT2C receptors are present in the thalamus (Burnet et al. 1995; Pompeiano et al. 1994). The 5-HT2A receptor is particularly prevalent in the thalamic reticular nucleus, whose GABAergic neurons are strongly excited by 5-HT. Pharmacological evidence suggests that the 5-HT2A receptor mediates the excitation of GABAergic interneurons in the cerebral cortex by 5-HT (Marek and Aghajanian 1994).

Together, these results suggest that the 5-HT2A receptor may be mediating the excitation of local interneurons by 5-HT in the thalamus: a hypothesis that remains to be examined.

The possibility that 5-HT may also inhibit thalamocortical neuronal activity through a presynaptic mechanism is supported by the finding that this neurotransmitter has a potent presynaptic inhibitory effect on the activity of retinal, but not corticogeniculate, synapses (U. Kim and D. A. McCormick, unpublished observations). Similar presynaptic inhibitory effects of 5-HT on retinal transmission occur in the suprachiasmatic nucleus (Pickard et al. 1999), as well as the release of glutamate or GABA in the amygdala (Cheng et al. 1998; Koyama et al. 1999), raphe (Li and Bayliss 1998), and hippocampus (Schmitz et al. 1995).

Possible influence of serotonin in thalamic function

These findings suggest that the actions of 5-HT in the thalamus, and its influence on thalamocortical activity, are likely to be complex. The thalamus is innervated by serotono-
ergic neurons in the dorsal and median raphe (Azmita and Segal 1978; Vertes 1991). Neurons in these nuclei discharge with a relatively stable slow frequency that increases in relation to the sleep to wake transition and perhaps in relation to movement (Jacobs and Fornal 1999). This increased release of 5-HT throughout the thalamus may result in a small depolarization of thalamocortical neurons in the principal relay nuclei (e.g., LGNd, MGN, VB) through a modulation of the voltage dependence of $I_h$, while exciting both local as well as PGN/nRt GABAergic interneurons. We have shown previously that these actions can result in an abolition of sleep-related rhythms in thalamic slices (Lee and McCormick 1996). However, our present results also indicate that the release of 5-HT may lead to a hyperpolarization of thalamocortical cells in many other, widespread, thalamic nuclei as well as a decrease in the release of excitatory neurotransmitters from prethalamic fibers, such as those from the retina. Presumably, the overall action of 5-HT in the thalamus will depend on multiple factors including the site of release, concentration, and postsynaptic receptor-effector mechanisms.

The activity and responsiveness of thalamocortical neurons and the GABAergic cells of the nRt/PGN changes dramatically with the sleep-wake cycle, and these changes are controlled in large part by the actions of neuromodulatory transmitters from the brain stem and hypothalamus, including 5-HT (reviewed in McCormick 1992; Steriade and McCarley 1990). Sleep-wake changes are reflected in single thalamocortical cells and nRt neurons, since both of these types of neurons exhibit two distinct firing modes. At hyperpolarized membrane potentials, such as during slow-wave sleep, they can generate rhythmic bursts of action potentials through the activation of the low-threshold Ca$^{2+}$ current $I_T$. In contrast, at more depolarized membrane potentials, such as often occurs during the awake, attentive state, or during rapid eye movement (REM) sleep, thalamic neurons can generate trains of action potentials in the tonic firing mode (Steriade and McCarley 1990). The accurate transmission of visual information through the LGNd is dramatically decreased during slow-wave sleep states, in part owing to the generation of intrinsic cellular and thalamocortical rhythms and the hyperpolarized state of the neurons (McCormick 1992). In addition, the hyperpolarized state during slow-wave sleep not only facilitates the generation of normal thalamocortical rhythms, but also some abnormal rhythms, such as the 3 cycle per second spike-wave discharge of absence seizures (see McCormick and Bal 1997; McCormick and Contreras 2001).

Although many neurotransmitters (e.g., acetylcholine, noradrenaline, histamine, and glutamate) clearly have a role in the “activation” of thalamocortical networks through depolarization of thalamocortical and thalamic reticular cells, the role for serotonin in ascending activation is less clear. Although thalamocortical neurons are thought to change from a predominance of burst firing during slow-wave sleep to a predominance of single spike firing during waking, the release of serotonin may actively antagonize this transition in some nuclei, through an increase in K$^+$ conductance. Perhaps this hyperpolarizing influence of serotonin counterbalances the depolarizing influences of other neuromodulators, such that a “push-pull” mechanism for adjusting the membrane potential of thalamocortical neurons is established. In addition, the spatial distribution of serotonin’s hyperpolarizing influence in the thalamus will also be important in determining the overall influence of this modulatory transmitter in setting the waking state of forebrain systems.

The action of 5-HT in GABAergic thalamic neurons of the thalamic reticular and perigeniculate nuclei is more consistent with the known changes in excitability of these cells in the sleep-wake cycle. Like thalamocortical neurons, the transition to the waking state is associated with a decrease in burst firing in nRt neurons (Steriade et al. 1986). The depolarization of nRt/PGN neurons that underlies this switch may be mediated in part by the actions of 5-HT (McCormick and Wang 1991; Pinault and Deschenes 1992). Changes in the excitability of local GABAergic neurons in the thalamus during the transition to the awake, attentive state are not yet known. In the present study, we demonstrate that at least some local GABAergic neurons may be excited by 5-HT, and therefore undergo an increase in excitability on increased release of the modulatory neurotransmitter, although this modulation must be considered in reference to the actions of other neurotransmitters (Pape and McCormick 1995).

In summary, although the actions of many neuromodulatory transmitters in the thalamus can be construed to promote the awake, attentive state, the actions of 5-HT are less clear. Increases in excitability of GABAergic neurons may facilitate center-surround mechanisms in receptive field processing, or regulate the influences of other neurotransmitters that actively inhibit GABAergic neurons in the thalamus, such as acetylcholine. Only a detailed knowledge of the plethora of neuromodulatory agents affecting the thalamus and their joint actions can lead to a true understanding of the modulatory influence of each, including 5-HT.

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REFERENCES


NEUROMODULATORY ROLE OF SEROTONIN


