

# 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-HT<sub>1A</sub> 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-HT<sub>1A</sub> antagonist WAY100635 and mimicked by the application of the 5-HT<sub>1A</sub>-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) occurring in thalamocortical neurons. Application of 5-HT to physiologically or morphologically identified interneurons evoked a prolonged suprathreshold depolarization. Our results suggest that serotonergic inputs act differentially across the thalamus in a complex manner involving direct and indirect mechanisms. It appears that 5-HT has a greater direct postsynaptic inhibitory influence in the posterior, medial, and intralaminar nuclei than in the primary sensory nuclei.

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## 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:  $I_h$  and  $I_T$  (for review see McCormick and Bal 1997). The first,  $I_h$ , is a hyperpolarization-activated cation current, and the second,  $I_T$ , 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 postsynaptic potentials can result in the activation of low-threshold  $Ca^{2+}$  spike-mediated bursts (Jahnsen and Llinas 1984a,b; McCormick and Feeseer 1990). The transition from sleep to waking is associated with a general depolarization of thalamocortical neurons, which partially or completely inactivates the low-threshold  $Ca^{2+}$  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 a general inhibitory action of 5-HT in the LGNd. However, *in vitro* intracellular studies did not reveal a direct inhibitory action of 5-HT on thalamocortical neurons (Lee and McCor-

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mick 1996; McCormick and Pape 1990). These *in vitro* studies revealed that locally applied 5-HT results in a small depolarization and a shift in the voltage dependency of the hyperpolarization-activated cation current,  $I_h$ , such that it is active at more depolarized levels. This shift in the voltage dependence of  $I_h$  has the functional consequences that the spontaneous generation of sleep spindles is suppressed (Lee and McCormick 1996). In contrast to the actions on thalamocortical neurons, the application of 5-HT to the GABAergic neurons of the perigeniculate nucleus or the thalamic reticular nucleus results in a strong excitation of these cells (McCormick and Wang 1991; Sanchez-Vives et al. 1996), suggesting that this may be one mechanism by which 5-HT inhibits the activity of thalamocortical cells *in vivo* (e.g., Funke and Eysel 1995). Given the multitude of serotonergic receptors in the thalamus (Chapin and Andrade 2001a,b; Kia et al. 1996; Lopez-Gimenez et al. 1998, 2001; Mengod et al. 1996; Pompeiano et al. 1994), it is expected that the actions of 5-HT in this structure are considerably more complex than previously anticipated. In particular, it is unknown how representative the action of 5-HT in the dorsal lateral geniculate nucleus (LGNd) is with respect to other thalamic nuclei. Therefore to further examine the role of 5-HT in the modulation of thalamic function, we chose to examine the actions of this neuromodulator in various thalamic nuclei, with particular focus on the pulvinar, which is thought to play an important role in visual salience and attention (Robinson and Cowie 1997). We report here that 5-HT can activate both a direct hyperpolarization of thalamocortical neurons through an increase in  $K^+$  conductance as well as an indirect inhibition through the activation of local GABAergic interneurons.

Additional information about these and related findings may be obtained at <http://www.mccormicklab.org>.

## METHODS

For the preparation of slices, ferrets of either gender between the ages of 6 and 24 wk were deeply anesthetized with pentobarbital sodium (30 mg/kg) and killed by decapitation. A modification of the technique by Aghajanian and Rasmussen (1989) was used and described herein to increase slice viability. The brain was rapidly removed and placed into cold (4–8°C) artificial cerebrospinal fluid (ACSF) with sodium replaced by sucrose maintaining a constant osmolarity of 307 mOsm. After blocking, the tissue was affixed with cyanoacrylate in the appropriate plane and cut into 400- $\mu$ m sections using a DSK microslicer (model DTK-1000; Ted Pella). The slices were transferred to an interface style tissue chamber (Fine Science Tools), where they were allowed to incubate for a period of 2 h. During the first 20 min of this incubation a 50/50 mixture of the sucrose-containing and normal bathing solutions was used to provide a more gradual transition from the cutting solution. The normal bathing medium contained (in mM) 124 NaCl, 2.5 KCl, 2.0 MgSO<sub>4</sub>, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 2 CaCl<sub>2</sub>, 26 NaHCO<sub>3</sub>, and 10 dextrose and was aerated with 95% O<sub>2</sub>-5% CO<sub>2</sub> to a final pH of 7.4. The bath temperature was maintained at 34–35°C.

Intracellular recording electrodes were made using a Flaming Brown micropipette puller (Model P-80; Sutter Instruments) from medium-walled glass (IBF100; World Precision Instrument). Micropipettes were filled with 2 M potassium acetate with 5 mM KCl and 2% biocytin ( $\epsilon$ -biotinoyl-L-lysine; Molecular Probes) for intracellular labeling of recorded neurons and beveled on a Sutter Instrument beveler to the desired resistance of 60–90 M $\Omega$ . Biocytin-filled neurons were visualized through standard avidin-biotin-horseradish per-

oxidase reaction (ABC Vectastain kit; Vectastain) processed with diaminobenzidine (Sigma) as described by Horikawa and Armstrong (1988). The number of interneurons recorded was less than expected from the percentage of GABAergic cells in the ferret thalamus. Presumably this reduced percentage of interneuronal recordings resulted from the small size of these cells and a significant electrode bias.

Intracellular recordings were made using an Axoclamp-2A amplifier (Axon Instruments) in current- and voltage-clamp modes. While in current clamp the voltage output was filtered with a 10-kHz low-pass filter, whereas in voltage clamp the current output was filtered with a 0.3-kHz filter. The switching frequency of the voltage clamp was 2.8–3.5 kHz, and the output of the headstage was monitored continuously on an oscilloscope to assure an adequate settling time. The current and voltage signals were digitized using a Neurodata digitizer and recorded to VHS tape. Voltage-clamp ramps were executed at a rate of 8.6 mV/s with the PClamp 5 computer program (Axon Instrument) and analyzed with Clampan and Origin 5.0 (Microcal Software) software on an IBM style PC.

Neurotransmitter agonists and antagonists were applied either through bath infusion or locally with a custom-designed picospritzer using General Valve solenoid controlled pressure pulses actuated manually or by a Master-8 Stimulator (AMPI; Israel).

5-HT HCl, 5-HT sulfate, WAY100635, 8-OHDPAT, and bicuculline methiodide were all obtained from Research Biochemicals International (Natick, MA), whereas tetrodotoxin, CsCl, and BaCl were obtained through Sigma. The GABA<sub>B</sub> antagonists, CGP35348 and CGP56999A, were kindly donated by Novartis (Basel, Switzerland).

## 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 coronal 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<sup>2+</sup> 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.

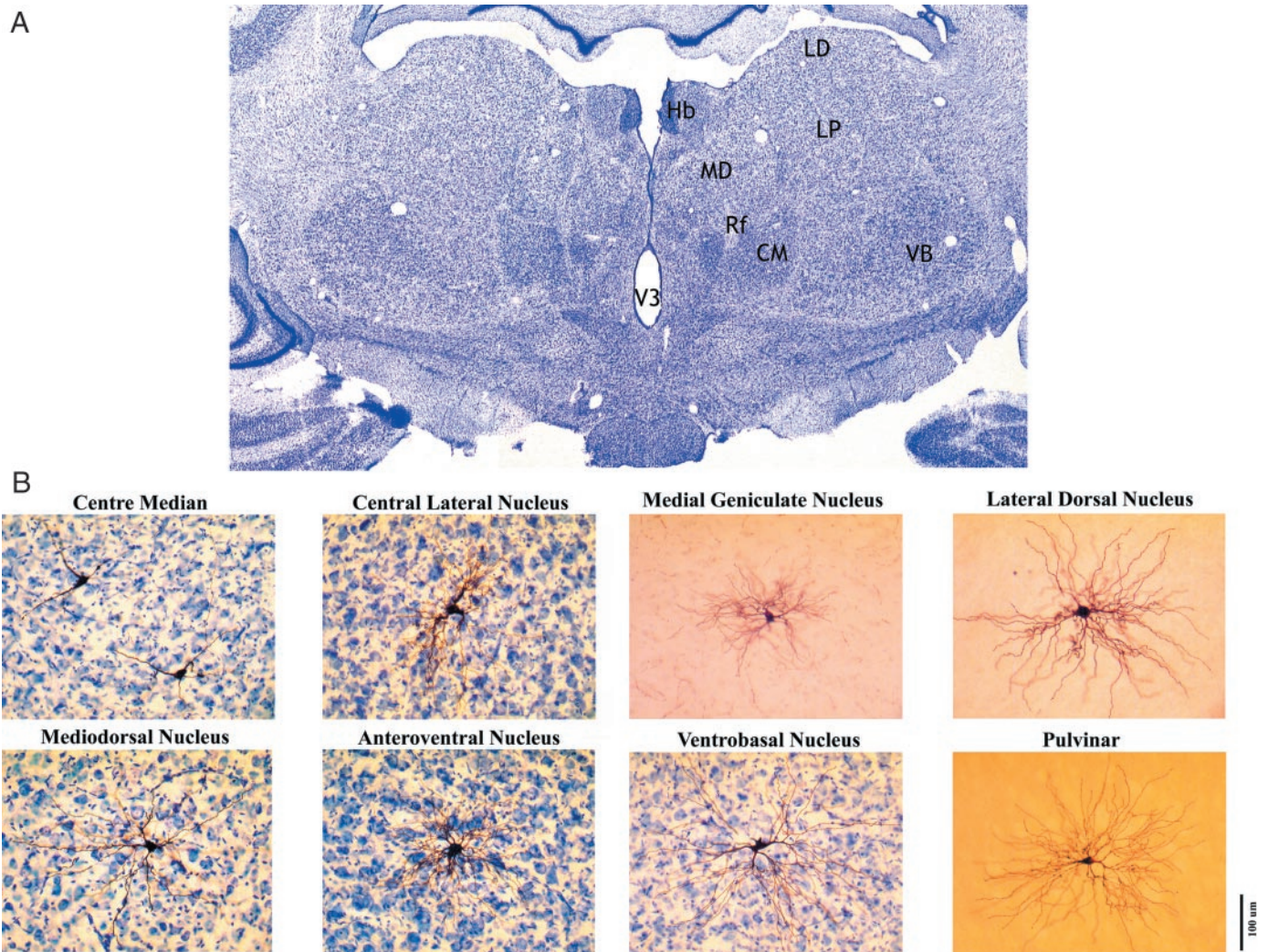


FIG. 1. Location and morphology of recorded neurons. *A*: coronal section of the ferret thalamus stained with cresyl violet to illustrate several of the nuclei from which neurons were recorded. Labeled nuclei are centre median (CM), habenula (Hb), lateral posterior (LP), tractus retroflexus (Rf), ventrobasal (VB), third ventricle (V3), medial dorsal (MD), and lateral dorsal (LD) nuclei. *B*: examples of biocytin-filled thalamocortical neurons in several of the nuclei in which the action of serotonin was investigated. In most cases, the sections were counterstained with cresyl violet to facilitate the identification of the nucleus from which the neuron was recorded.

#### *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

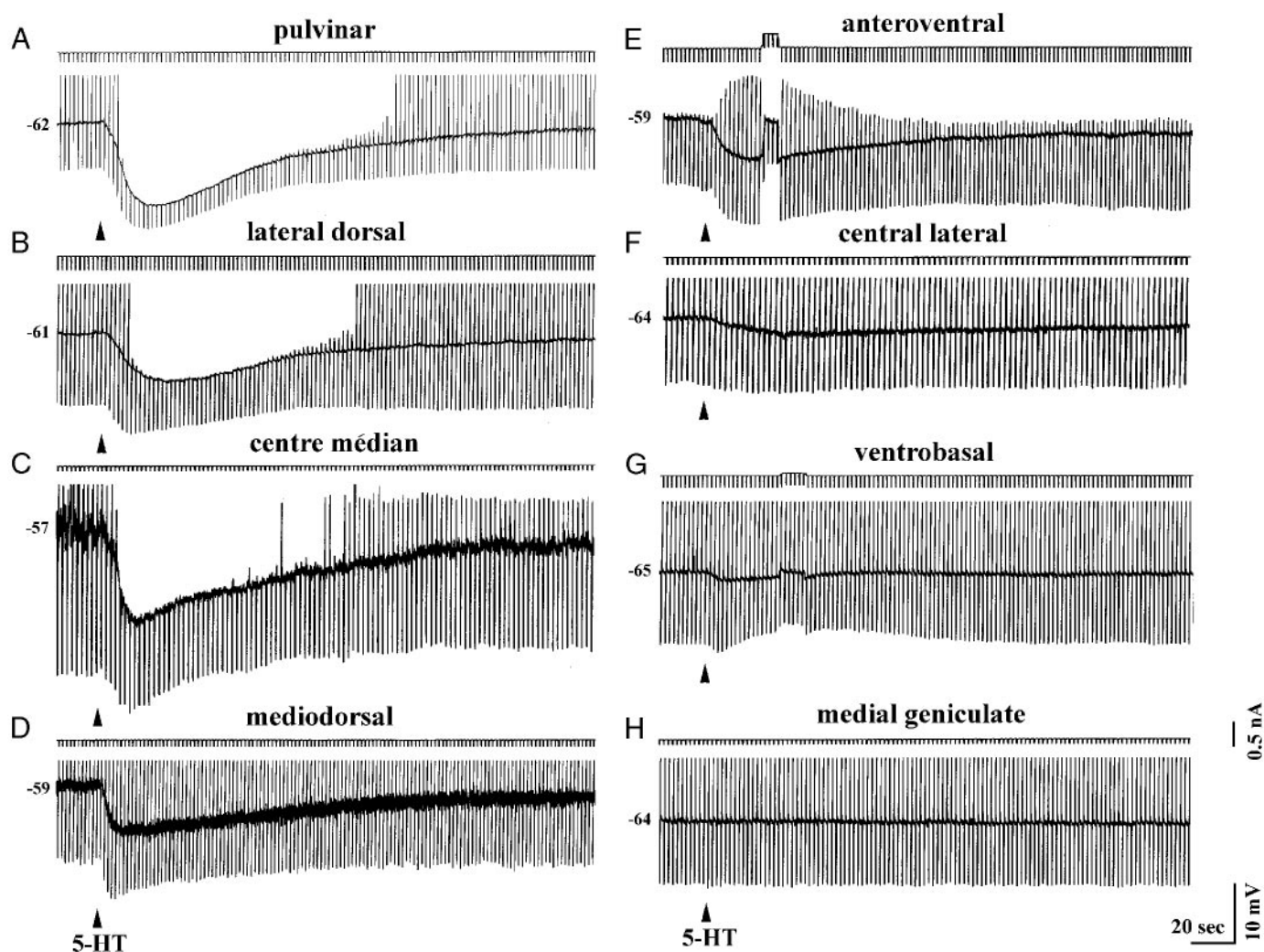


FIG. 2. Serotonin hyperpolarizes thalamocortical neurons in several different nuclei. Each trace illustrates a response, recorded in current clamp mode, to the local application of serotonin (250  $\mu$ M in micropipette). Responses illustrated are from pulvinar (A, in 20  $\mu$ M bicuculline); lateral dorsal (B, in 20  $\mu$ M bicuculline); centre median (C); mediodorsal (D); anteroventral (E); central lateral nucleus (F); ventral basal (G); and medial geniculate nuclei (H). In all the recordings the *top trace* is the current step command, and the *bottom trace* corresponds to the measured voltage. Compensating for the change in membrane potential with the intracellular injection of current reveals that the hyperpolarizing response is associated with an increase in membrane conductance (E). Hyperpolarizing responses to 5-HT were not found in medial or lateral geniculate thalamocortical cells. Although in this figure the response to 5-HT in the pulvinar is larger than the lateral dorsal nucleus, on average this was not the case (see RESULTS).

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

TABLE 1. Response amplitude of thalamic nuclei to 5-HT

Nucleus	N	Response Amplitude
Lateral dorsal	9	$-6.9 \pm 2.1$
Lateral posterior	9	$-5.1 \pm 1.7$
Pulvinar	97	$-4.5 \pm 1.7$
Mediodorsal	6	$-4.1 \pm 1.6$
Centre median	10	$-3.7 \pm 2.0$
Anteroventral	7	$-3.4 \pm 1.3$
Central lateral	13	$-2.4 \pm 1.4$
Ventrobasal	4	$-0.8 \pm 1.1$
Medial geniculate	5	$-0.7 \pm 0.9$
Lateral geniculate	7	$-0.2 \pm 1.1$

Values in Response Amplitude are means  $\pm$  SE. These values reflect the naive response to serotonin in current clamp.

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 GABA<sub>A</sub> antagonists bicuculline (50–150  $\mu$ M) or picrotoxin (100  $\mu$ M) and either or the GABA<sub>B</sub> antagonists CGP35348 (200  $\mu$ M) or CGP56999A (0.5  $\mu$ M). Additionally the Na<sup>+</sup> channel blocker, TTX (1  $\mu$ M 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).

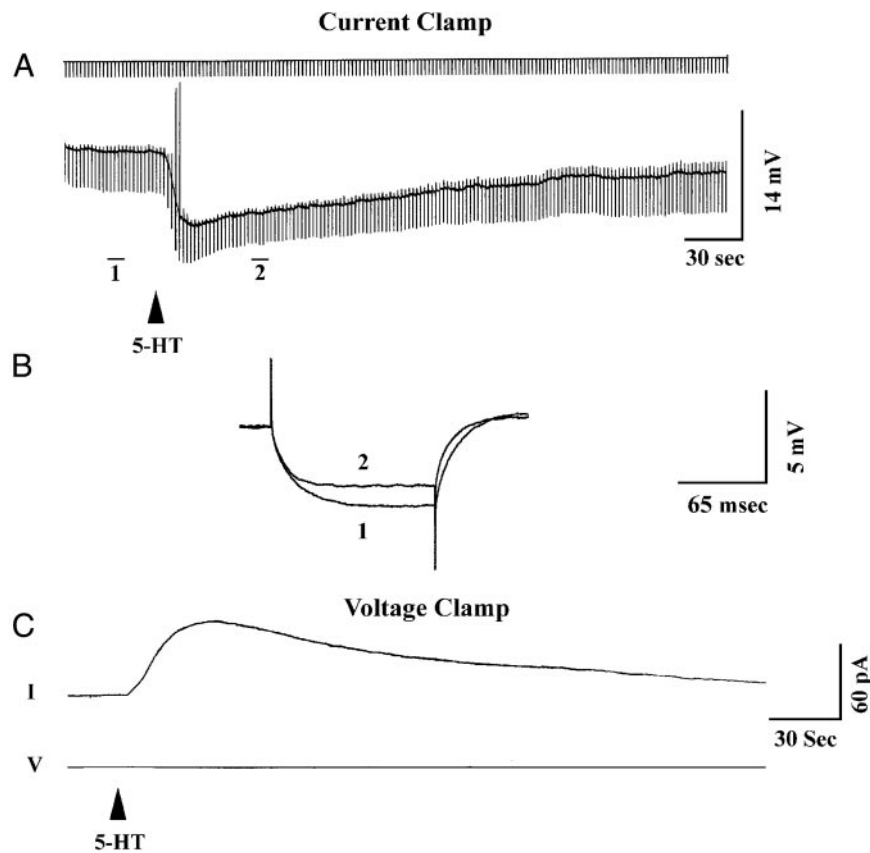


FIG. 3. The action of serotonin persists following block of GABAergic responses. Application of serotonin (200  $\mu$ M) following the bath application of tetrodotoxin (1  $\mu$ M), bicuculline (100  $\mu$ M), and CGP35348 (200  $\mu$ M) resulted in a prolonged hyperpolarization (A) that was associated with an apparent increase in membrane conductance (B). In voltage-clamp mode, serotonin elicited a prolonged outward current (C; cell was held at  $-60$  mV). Data shown here were obtained from a thalamocortical cell in the pulvinar nucleus.

*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  $-97.4 \pm 2.7$  mV to  $-71.6 \pm 2.4$  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  $35^\circ\text{C}$ ). 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-HT<sub>1A</sub> 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-HT<sub>1A</sub>-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 GABA<sub>A</sub> antagonist bicuculline methiodide (150  $\mu$ M in micropipette) and of the GABA<sub>B</sub> 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-HT<sub>1A</sub> antagonist WAY100635 (10–100  $\mu$ M in micropipette; Fig. 5C;  $n = 17$  pulvinar, 5 nonpulvinar), thus providing strong evidence for 5-HT<sub>1A</sub> 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-HT<sub>1A</sub>.

Application of the 5-HT<sub>1A</sub>-selective agonist, 8-OHDPAT (10–100  $\mu$ M in micropipette), in the presence of TTX or GABA receptor 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 GABA<sub>A</sub> receptor antagonist bicuculline completely blocked 5-HT-induced hyperpolarizing phasic events, con-

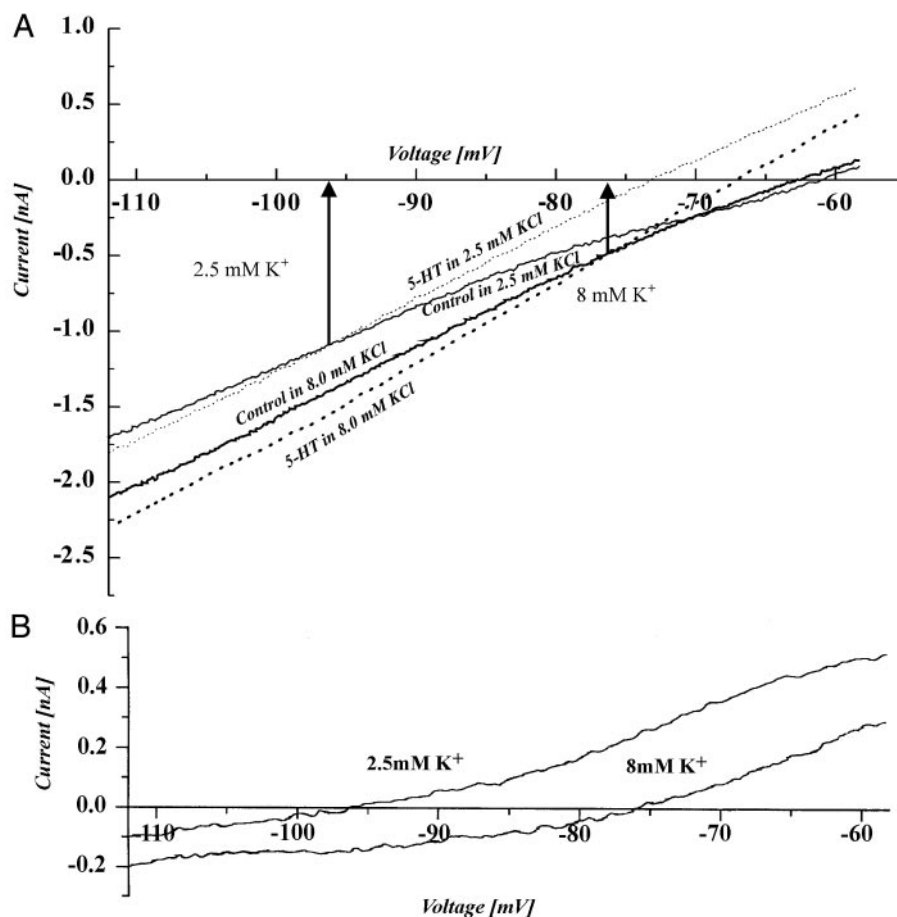


FIG. 4. Current-voltage ( $I$ - $V$ ) relationship of a pulvinal thalamocortical neuron in control and after serotonin application in normal (2.5 mM) and high potassium (8 mM) conditions. Serotonin induces an outward current that reverses at approximately  $-97$  mV in 2.5 mM  $K^+$  (A) and at  $-77$  mV in 8 mM  $K^+$  (A). Subtracting the  $I$ - $V$  relationship before and after serotonin reveals the voltage dependence of the serotonin-induced current (B). All traces illustrated represent the average of 5 voltage ramps each executed at a rate of 8.6 mV/s. Cesium chloride (10 mM in micropipette) was applied locally to block any contribution to the response by voltage activated changes in  $I_h$ .

firming that they are GABAergic IPSPs (Fig. 7B), but left the direct, hyperpolarizing action of 5-HT intact.

A subset of pulvinal 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, Pulvinal, 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<sub>1A</sub> 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<sub>1A</sub> receptor activation is supported by the finding that the response is antagonized by the specific 5-HT<sub>1A</sub> antagonist WAY-100635 (Fletcher et al. 1993, 1995; Foster et al. 1995). Additionally,

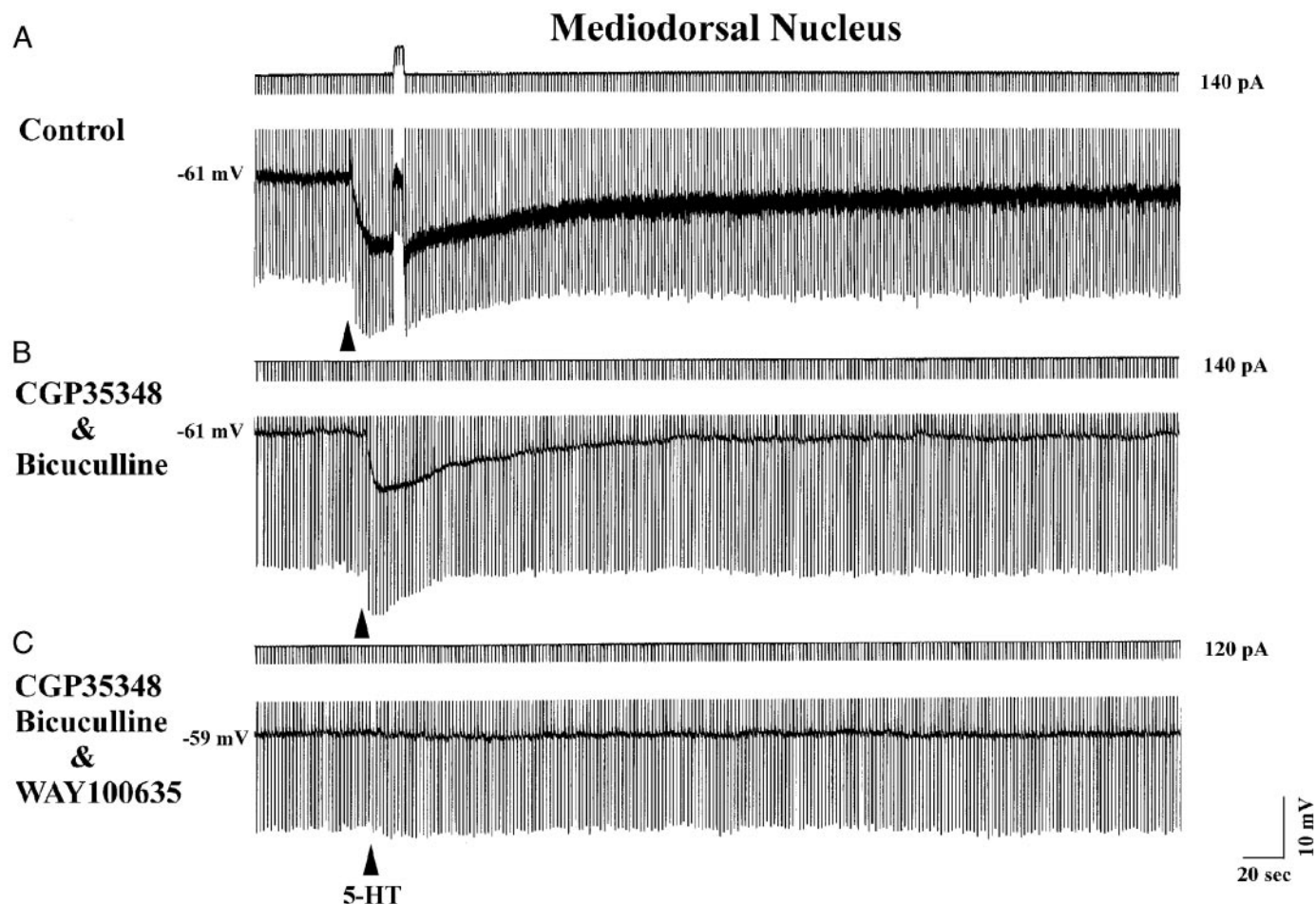


FIG. 5. *A*: serotonin elicits a hyperpolarization in a mediodorsal neuron and an increase in the amplitude and frequency of inhibitory postsynaptic potentials (IPSPs). *A*: the 5-HT-evoked IPSPs appear as an increase in the thickness of the membrane potential during the hyperpolarization. Compensating for the serotonin-induced hyperpolarization with the intracellular injection of current reveals that the hyperpolarization is associated with an increase in membrane conductance (*A*). *B*: bath application of bicuculline (150  $\mu$ M) and CGP35348 (200  $\mu$ M) abolishes the 5-HT-evoked IPSPs and reveals the direct, postsynaptic hyperpolarizing response to serotonin. *C*: the local application of WAY100635 (10  $\mu$ M in micropipette), a specific 5-HT<sub>1A</sub> antagonist, abolishes the hyperpolarizing response to serotonin. Note that after WAY100635 application there is no residual change in  $R_{in}$  with serotonin.

the application of the 5-HT<sub>1A</sub>-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<sub>A</sub> and GABA<sub>B</sub> antagonists. The postsynaptic responses reported here are consistent with those previously associated with the 5-HT<sub>1A</sub> 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<sup>+</sup> channel (GIRKs) through the activation of the G<sub>i</sub>/G<sub>o</sub> family of G proteins (Luscher et al. 1997; see Aghajanian and Andrade 1997).

Immunohistochemical and autoradiographic studies reveal a relatively low density of 5-HT<sub>1A</sub> 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-HT<sub>1A</sub> receptors in ferret thalamus has not been reported. Possible explanations for the apparent discrepancy between this relatively low level of 5-HT<sub>1A</sub> 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 pulvinar 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 (<sup>3</sup>H) 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

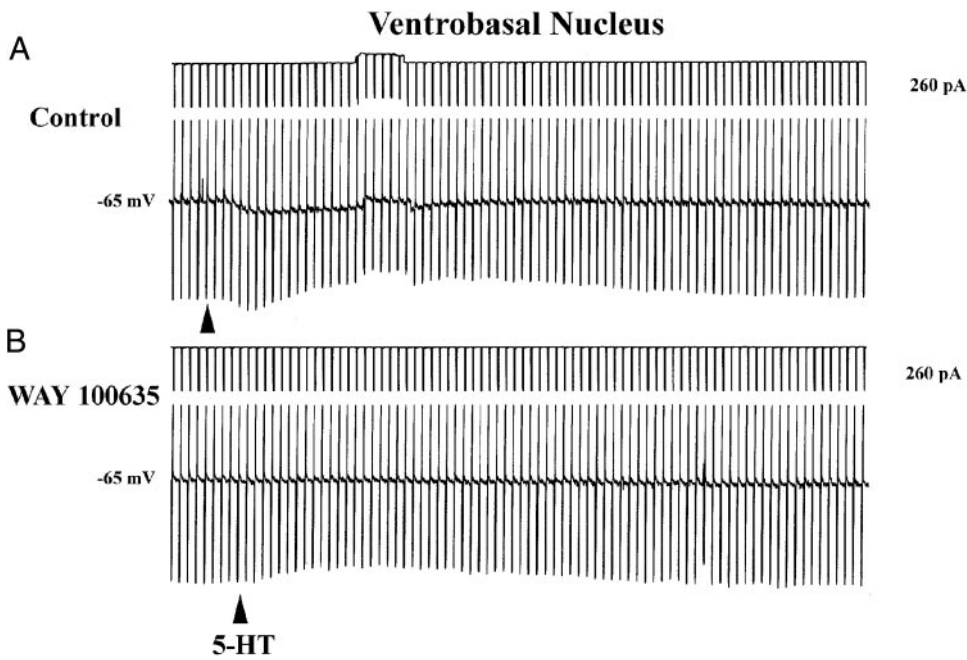


FIG. 6. A: serotonin can activate 2 distinct responses in ventrobasal thalamocortical neurons. A: application of serotonin to this VB neuron results in a hyperpolarization and an anomalous increase in membrane conductance. B: the local application of WAY100635 (10  $\mu$ M) blocks the 5-HT-induced hyperpolarization and leaves a response that is similar that associated with 5-HT-induced enhancement of  $I_h$  (McCormick and Pape 1990; Pape and McCormick 1989).

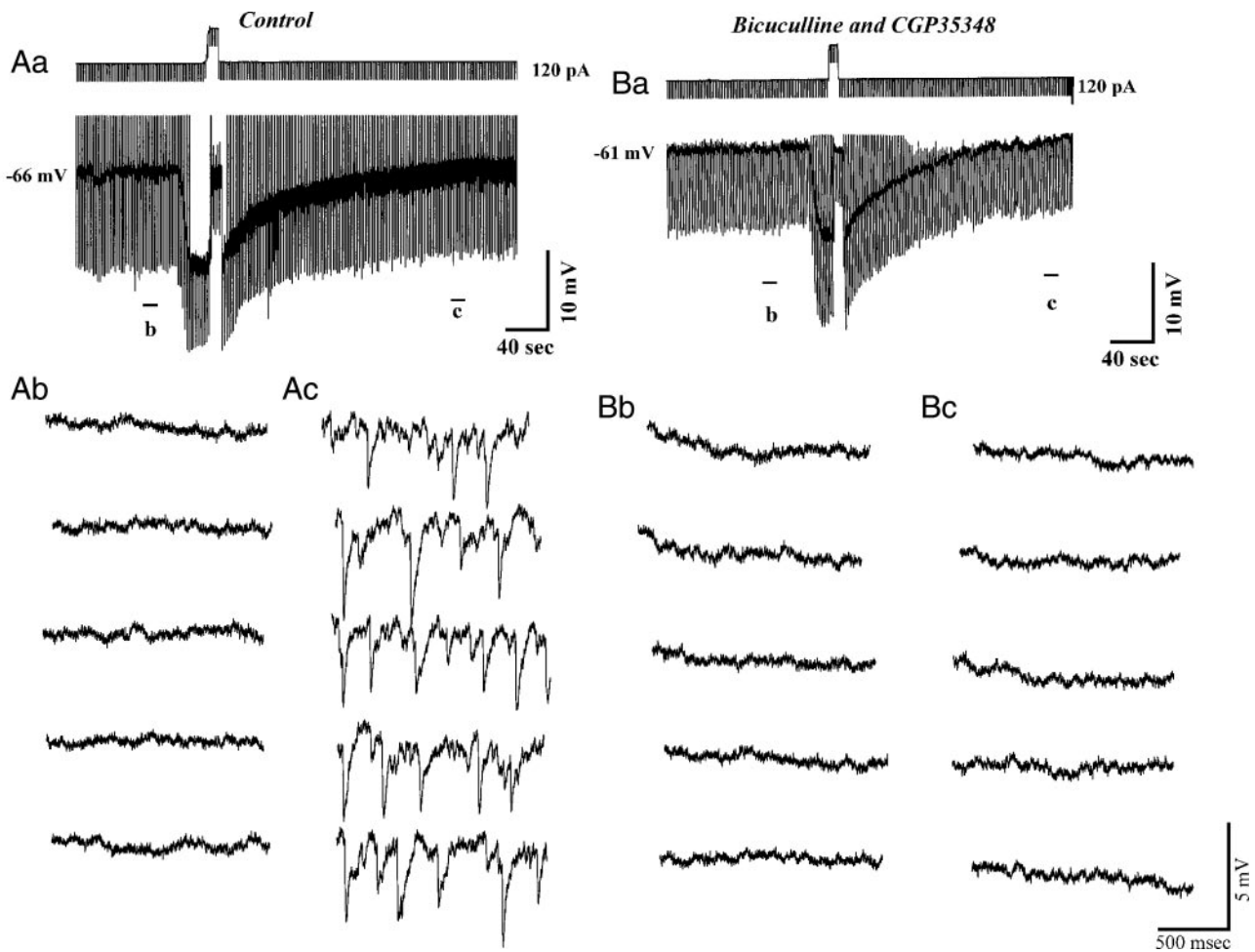


FIG. 7. Serotonin can strongly increase the occurrence of IPSPs in thalamocortical cells. A: application of serotonin to a pulvina thalamocortical neuron results in a hyperpolarization and an increase in the frequency and amplitude of IPSPs. The regions labeled are expanded in the panels below so as to illustrate the 5-HT-induced barrage of IPSPs. The local application of bicuculline (500  $\mu$ M in micropipette) and CGP35348 (2 mM) resulted in a block of the IPSPs (B), but did not block the 5-HT-induced hyperpolarization.

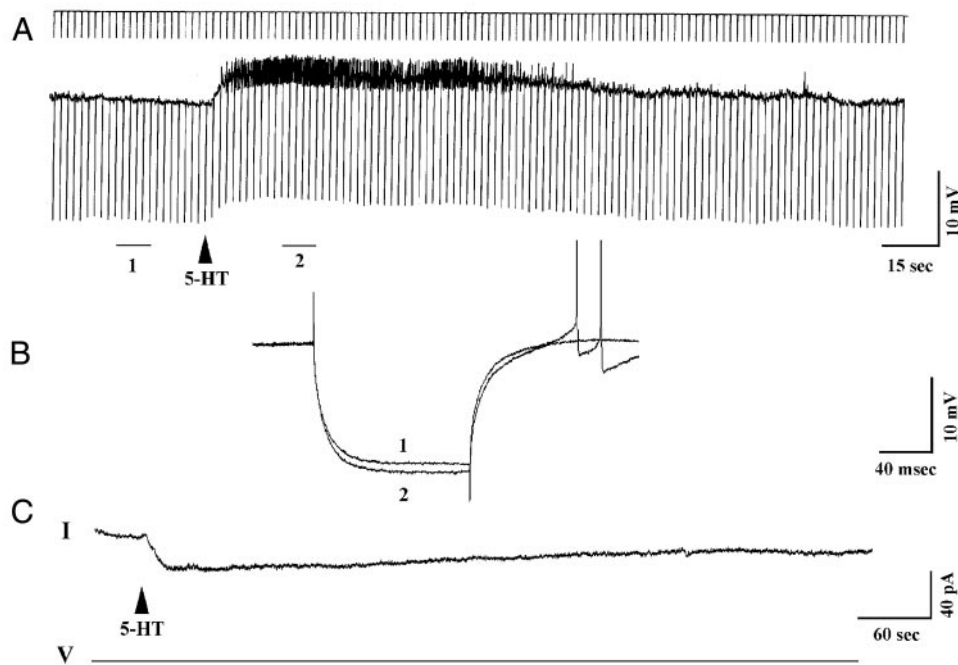


FIG. 8. Serotonin can excite local interneurons in the thalamus. *A*: local application of serotonin to a putative interneuron elicits a depolarization sufficient, in this example, to bring the cell to spike threshold. This depolarization is accompanied by an apparent increase in input resistance (*B*). *C*: voltage-clamp recordings reveal an apparent inward current presumably due to a reduction in a potassium current. Morphological properties of the interneuron that was depolarized by serotonin in *A* are shown in Fig. 9, *A* and *B*.

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  $I_h$  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  $I_h$  (McCormick and Pape 1990). This response to 5-HT appears to be mediated by 5-HT<sub>7</sub> 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-HT<sub>1A</sub> and GABA<sub>A+B</sub> receptors were blocked, only a few cells in the more associative nuclei showed evidence of a change in  $I_h$ , 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 intervening interneurons, direct postsynaptic action on the principal cells, or by presynaptic inhibitory action on excitatory synapses. Indeed, blockade of GABA<sub>A</sub> 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<sup>+</sup> 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 interneu-

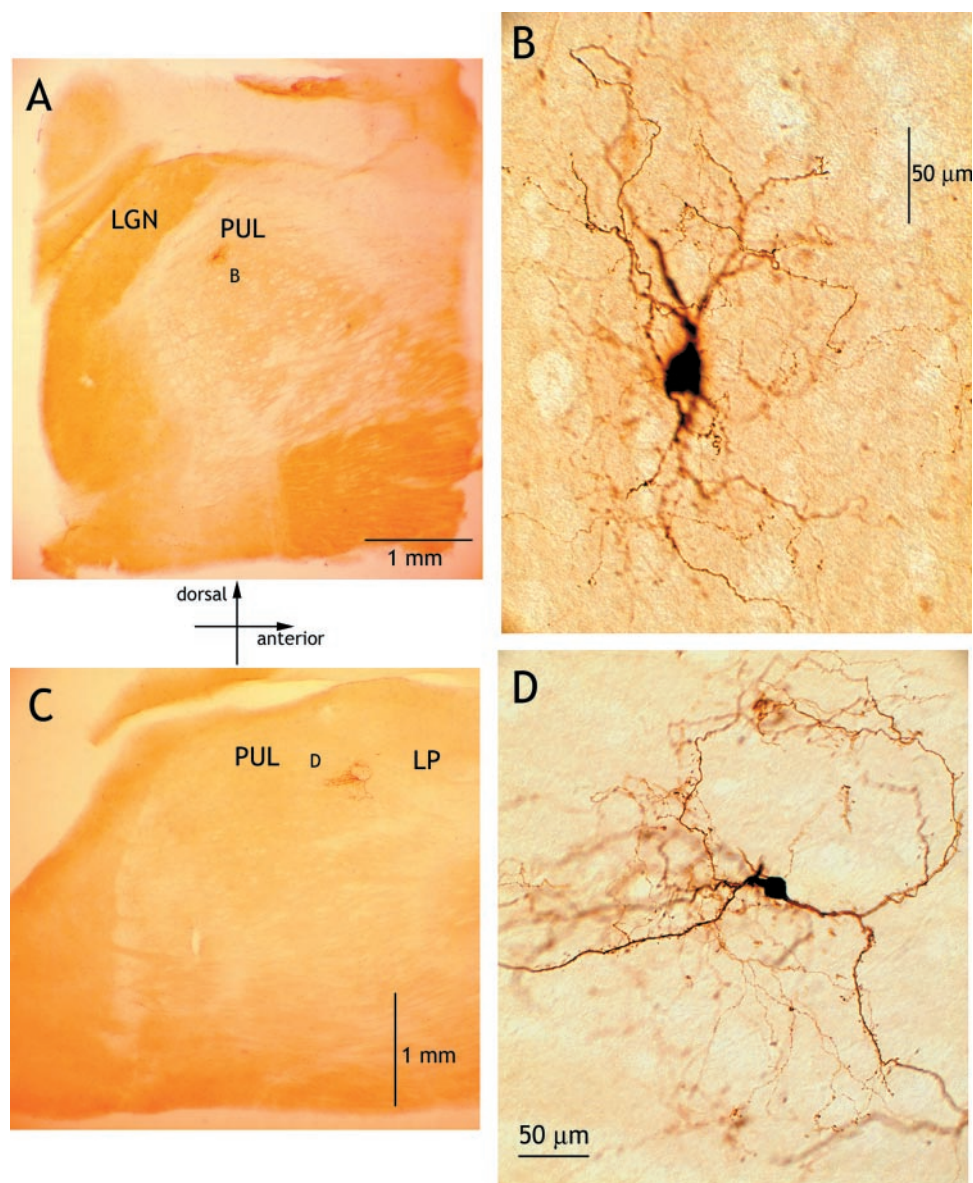


FIG. 9. Photomicrograph of 2 biocytin-filled interneurons that responded to serotonin with a depolarization. Low magnification images (A and C) of parasagittal sections illustrate the location of these 2 cells. The Lateral geniculate nucleus (LGN), pulvinar (PUL), and lateral posterior nucleus (LP) are labeled as landmarks, and orientation of the slice is illustrated. The leftmost edge of the slice is the posterior limit of the thalamus. The rightmost edge of the picture in A and C is either the anterior cut edge of the slice (A) or just posterior to that (C). Higher magnification images illustrate the dendritic and axonal arbors (B and D). In the higher magnification images one can see extensively ramifying thin processes and filiform appendages, indicating that these cells were interneurons. The response of the cell in B to serotonin is illustrated in Fig. 8.

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 (Pigué 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-HT<sub>2</sub> receptors, which when activated, result in the closure of a K<sup>+</sup> 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-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors are present in the thalamus (Burnet et al. 1995; Pompeiano et al. 1994). The 5-HT<sub>2A</sub> receptor is particularly prevalent in the thalamic reticular nucleus, whose GABAergic neurons are strongly excited by 5-HT. Pharmacological evidence suggests that the 5-HT<sub>2A</sub> 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-HT<sub>2A</sub> 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 serotoner-

gic 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, norepinephrine, 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 spa-

tial 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

- AGHAJANIAN GK AND ANDRADE R. Electrophysiology of 5-HT receptors. In: *Serotonergic Neurons and 5-HT Receptors in the CNS. Handbook of Experimental Pharmacology*, edited by Baumgarten HG and Gothert M. New York: Springer, 1997, vol. 129, p. 499–535.
- AGHAJANIAN GK AND LAKOSKI JM. Hyperpolarization of serotonergic neurons by serotonin and LSD: studies in brain slices showing increased  $K^+$  conductance. *Brain Res* 305: 181–185, 1984.
- AGHAJANIAN GK AND RASMUSSEN K. Intracellular studies in the facial nucleus illustrating a simple new method for obtaining viable motoneurons in adult rat brain slices. *Synapse* 3: 331–338, 1989.
- ALREJA MA. Excitatory actions of serotonin on GABAergic neurons of the medial septum and diagonal band of Broca. *Synapse* 22: 15–27, 1996.
- ANDRADE R. Whole-cell and perforated-patch recording of serotonin responses in the rat hippocampus. *J Chem Neuroanat* 5: 339–341, 1992.
- ANDRADE R AND NICOLL RA. Pharmacologically distinct actions of serotonin on single pyramidal neurons of the rat hippocampus recorded in vitro. *J Physiol (Lond)* 394: 99–124, 1987a.
- ANDRADE R AND NICOLL RA. Novel anxiolytics discriminate between postsynaptic serotonin receptors mediating different physiological responses on single neurons of the rat hippocampus. *Naunyn-Schmiedeberg's Arch Pharmacol* 336: 5–10, 1987b.
- AZMITA EC AND SEGAL M. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J Comp Neurol* 179: 641–668, 1978.
- BAL T, VON KROSIGK M, AND MCCORMICK DA. Synaptic and membrane mechanisms underlying synchronized oscillations in the ferret lateral geniculate nucleus in vitro. *J Physiol (Lond)* 483: 665–685, 1995.
- BARNES NM AND SHARP T. A review of central 5-HT receptors and their function. *Neuropharmacology* 38: 1083–1152, 1999.

- BERMAN EG AND JONES AL. *The Thalamus and Basal Telencephalon of the Cat: A Cytoarchitectonic Atlas With Stereotaxic Coordinates*. Madison, WI: Univ. of Wisconsin Press, 1982.
- BOESS FG AND MARTIN IL. Molecular biology of 5-HT receptors. *Neuropharmacology* 33: 275–317, 1994.
- BURNET PWJ, EASTWOOD SL, LACEY K, AND HARRISON PJ. The distribution of 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptor mRNA in human brain. *Brain Res* 676: 157–168, 1995.
- CHALMERS DT AND WATSON SJ. Comparative anatomical distribution of 5-HT<sub>1A</sub> receptor mRNA and 5-HT<sub>1A</sub> binding in rat brain—a combined in situ hybridisation/in vitro receptor autoradiographic study. *Brain Res* 561: 51–60, 1991.
- CHAPIN EM AND ANDRADE R. A 5HT(7) receptor mediated depolarization in the anterodorsal thalamus. I. Pharmacological characterization. *J Pharmacol Exp Ther* 297: 395–402, 2001a.
- CHAPIN EM AND ANDRADE R. A 5HT(7) receptor mediated depolarization in the anterodorsal thalamus. II. Involvement of the hyperpolarization-activated current  $I_h$ . *J Pharmacol Exp Ther* 297: 402–409, 2001b.
- CHENG LL, WANG SJ, AND GEAN PW. Serotonin depresses excitatory synaptic transmission and depolarization-evoked  $Ca^{2+}$  influx in rat basolateral amygdala via 5-HT<sub>1A</sub> receptors. *Eur J Neurosci* 10: 2163–2172, 1998.
- COLINO A AND HALLIWELL JA. Differential modulations of three separate K-conductances in hippocampal CA1 neurons by serotonin. *Nature* 328: 73–77, 1987.
- CUMMING-HOOD PA, STRAHLENDORF HK, AND STRAHLENDORF JC. Effects of serotonin and the 5-HT<sub>2/1C</sub> receptor agonist DOI on neurons of the cerebellar dentate/interpositus nuclei: possible involvement of a GABAergic interneuron. *Eur J Pharmacol* 236: 457–465, 1993.
- CURTIS DR AND DAVIS R. Pharmacologic studies upon neurons of the lateral geniculate nucleus of the cat. *Br J Pharmacol* 18: 217–246, 1962.
- DAVAL G, VERGE D, BECERRIL A, GOZLAN H, SPAMINATO U, AND HAMON M. Transient expression of 5-HT<sub>1A</sub> receptor binding sites in some areas of the rat CNS during postnatal development. *Int J Dev Neurosci* 5: 171–180, 1987.
- DILLON KA, GROSS-ISSEROFF R, ISRAELI M, AND BIEGON A. Autoradiographic analysis of serotonin 5-HT<sub>1A</sub> receptor binding in the human brain postmortem: effects of age and alcohol. *Brain Res* 554: 56–64, 1991.
- FLETCHER A, BILL DJ, BILL SJ, CLIFFE IA, DOVER GM, FORSTER EA, HASKINS JT, JONES D, MANSELL HL, AND REILLY Y. WAY-100635: a novel, selective antagonist at presynaptic and postsynaptic 5-HT<sub>1A</sub> receptors. *Eur J Pharmacol* 237: 283–291, 1993.
- FLETCHER A, FORSTER EA, BILL DJ, BROWN G, CLIFFE IA, HARTLEY JE, JONES DE, MCLENACHAN A, STANHOPE KJ, CRITCHLEY DJP, CHILDS KJ, MIDDLFELL VC, LANFUMEY L, CORRADETTI R, LAPORTE AM, GOZLAN H, HAMON M, AND DOURISH CT. Electrophysiological, biochemical, neurohormonal and behavioural studies with WAY-100635, a potent, selective and silent 5-HT<sub>1A</sub> receptor antagonist. *Behav Brain Res* 73: 337–353, 1995.
- FOSTER EA, CLIFFE IA, BILL DJ, DOVER GM, JONES D, REILLY Y, AND FLETCHER AA. A pharmacological profile of the selective silent 5-HT<sub>1A</sub> receptor antagonist, WAY100635. *Eur J Pharmacol* 281: 81–88, 1995.
- FUNKE K AND EYSEL UT. Modulatory effects of acetylcholine, serotonin and noradrenaline on the activity of cat perigeniculate neurons. *Exp Brain Res* 95: 409–420, 1993.
- FUNKE K AND EYSEL UT. Possible enhancement of GABAergic inputs to cat dorsal lateral geniculate relay cells by serotonin. *Neuroreport* 6: 474–476, 1995.
- GERARD C, MARTRES M-P, LEFEVRE K, MIQUEL M-C, VERGE D, LANFUMEY L, DOUCET E, HAMON M, AND MESTIKAWY SE. Immuno-localization of serotonin 5-HT<sub>6</sub> receptor-like material in the rat central nervous system. *Brain Res* 746: 207–219, 1997.
- GOZLAN H, EL MESTIKAWY S, PICHAT L, HLOWINSKI J, AND HAMON M. Identification of presynaptic serotonin autoreceptors using a new ligand: (<sup>3</sup>H)PAT. *Nature* 305: 140–142, 1983.
- GUILLERY RW. A study of Golgi preparations from the dorsal lateral geniculate nucleus of the adult cat. *J Comp Neurol* 128: 21–50, 1966.
- GUSTAFSON EL, DURKIN MM, BARD JA, ZGOMBICK J, AND BRANCHEK TA. A receptor autoradiographic and in situ hybridization analysis of the distribution of the 5-HT<sub>7</sub> receptor in rat brain. *Br J Pharmacol* 117: 657–666, 1996.
- HALL H, LUNDKVIST C, HALLDIN C, FARDE L, PIKE VW, MCCARRON JA, FLETCHER A, CLIFFE IA, BARF T, WIKSTROM H, AND SEDVALL G. Autoradiographic localization of 5-HT<sub>1A</sub> receptors in the post-mortem human brain using [<sup>3</sup>H]WAY-100635 and [<sup>11</sup>C]way-100635. *Brain Res* 745: 96–108, 1997.
- HORIKAWA K AND ARMSTRONG WE. A versatile means of intracellular labeling: injection of biocytin and its detection with avidin conjugates. *J Neurosci Methods* 25: 1–11, 1988.
- HUME SP, ASHWORTH S, OPACKA-JUFFRY J, AHIER RG, LAMMERTSMA AA, PIKE VW, CLIFFE IA, FLETCHER A, AND WHITE AC. Evaluation of [O-methyl-<sup>3</sup>H]WAY-100635 as an in vivo radioligand for 5-HT<sub>1A</sub> receptors in rat brain. *Eur J Pharmacol* 271: 515–523, 1994.
- ITO H, HALLDIN C, AND FARDE L. Localization of 5-HT<sub>1A</sub> receptors in the living human brain using [carbonyl-<sup>11</sup>C]WAY-100635: PET with anatomic standardization technique. *J Nucl Med* 40: 102–109, 1999.
- JACOBS BL AND FORNAL CA. Activity of serotonergic neurons in behaving animals. *Neuropsychopharmacology* 21, Suppl 2: 9S–15S, 1999.
- JACOBS BL AND FORNAL CAV. Activity of brain serotonergic neurons in the behaving animal. *Pharmacol Rev* 43: 563–578, 1991.
- JAHNSEN H AND LLINAS R. Electrophysiological properties of guinea-pig thalamic neurons, an in vitro study. *J Physiol (Lond)* 349: 205–226, 1984a.
- JAHNSEN H AND LLINAS R. Ionic basis for the electroresponsiveness and oscillatory properties of guinea-pig thalamic neurons in vitro. *J Physiol (Lond)* 349: 227–247, 1984b.
- KAYAMA Y, SHIMADA S, HISHIKAWA Y, AND OGAWA T. Effects of stimulating the dorsal raphe nucleus of the rat on neuronal activity in the dorsal lateral geniculate nucleus. *Brain Res* 489: 1–11, 1989.
- KHAWAJA X. Quantitative autoradiographic characterisation of the binding of [<sup>3</sup>H]WAY-100635, a selective 5-HT<sub>1A</sub> receptor antagonist. *Brain Res* 673: 217–225, 1995.
- KIA HK, MIQUEL MC, BRISORGUEIL MJ, DAVAL G, RIAD M, EL MESTIKAWY S, HAMON M, AND VERGE D. Immunocytochemical localization of serotonin<sub>1A</sub> receptors in the rat central nervous system. *J Comp Neurol* 365: 289–305, 1996.
- KOYAMA S, KUBO C, RHEE JS, AND AKAIKE N. Presynaptic serotonergic inhibition of GABAergic synaptic transmission in mechanically dissociated rat basolateral amygdala neurons. *J Physiol (Lond)* 518: 525–538, 1999.
- LEE K AND MCCORMICK DA. Abolition of spindle oscillations by serotonin and norepinephrine in the ferret lateral geniculate and perigeniculate nucleus in vitro. *Neuron* 17: 309–321, 1996.
- LI YW AND BAYLISS DA. Presynaptic inhibition by 5-HT<sub>1B</sub> receptors of glutamatergic synaptic inputs onto serotonergic caudal raphe neurones in rat. *J Physiol (Lond)* 510: 121–134, 1998.
- LOPEZ-GIMENEZ JF, VILARO MT, PALACIOS JM, AND MENGOD G. [<sup>3</sup>H]MDL 100,907 labels 5-HT<sub>2A</sub> serotonin receptors selectively in primate brain. *Neuropharmacology* 37: 1147–1158, 1998.
- LOPEZ-GIMENEZ JF, VILARO MT, PALACIOS JM, AND MENGOD G. Mapping of 5-HT<sub>2A</sub> receptors and their mRNA in monkey brain: [<sup>3</sup>H]MDL100,907 autoradiography and in situ hybridization studies. *J Comp Neurol* 429: 571–589, 2001.
- LUCAS JJ AND HEN R. New players in the 5-HT receptor field: genes and knockouts. *Trends Pharmacol Sci* 16: 246–252, 1995.
- LUSCHER C, JAN LY, STOFFEL M, MALENKA RC, AND NICOLL RA. G-protein-coupled inwardly rectifying K<sup>+</sup> channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron* 19: 687–695, 1997.
- MAREK GJ AND AGHAJANIAN GK. Excitation of interneurons in piriform cortex by 5-hydroxytryptamine: blockade by MDL 100 907, a highly selective 5-HT<sub>2A</sub> receptor antagonist. *Eur J Pharmacol* 259: 137–141, 1994.
- MARKS GA, SPECIALE SG, COBBEY K, AND ROFFWARG HP. Serotonergic inhibition of the dorsal lateral geniculate nucleus. *Brain Res* 18: 76–84, 1987.
- MCCORMICK DA. Neurotransmitter actions in the thalamus and cerebral cortex and their role in neuromodulation of thalamocortical activity. *Prog Neurobiol* 39: 337–388, 1992.
- MCCORMICK DA AND BAL T. Sleep and arousal: thalamocortical mechanisms. *Annu Rev Neurosci* 20: 185–215, 1997.
- MCCORMICK DA AND CONTRERAS D. Cellular and network mechanisms of epilepsy. *Annu Rev Physiol* 63: 815–846, 2001.
- MCCORMICK DA AND FEESER HR. Functional implications of burst firing and single spike activity on lateral geniculate relay neurons. *Neuroscience* 39: 103–113, 1990.
- MCCORMICK DA AND PAPE HC. Noradrenergic and serotonergic modulation of a hyperpolarization-activated cation current in thalamic relay neurons. *J Physiol (Lond)* 431: 319–342, 1990.
- MCCORMICK DA AND WANG Z. Serotonin and noradrenaline excite GABAergic neurones of the guinea-pig and cat nucleus reticularis thalami. *J Physiol (Lond)* 442: 235–255, 1991.

- McCORMICK DA AND WILLIAMSON A. Convergence and divergence of neurotransmitter action in the human cerebral cortex. *Proc Natl Acad Sci USA* 86: 8098–8102, 1989.
- MENGOD G, VILARO MT, RAURICH A, LOPEZ-GIMENEZ JF, CORTES R, AND PALACIOS JM. 5-HT receptors in mammalian brain: receptor autoradiography and in situ hybridization studies of new ligands and newly identified receptors. *Histochem J* 28: 747–758, 1996.
- MONCKTON JE AND McCORMICK DA. Comparative analysis of serotonin's action in cat, ferret, and monkey pulvinar. *Soc Neurosci Abstr* 25: 440, 1999.
- PAPE HC AND McCORMICK DA. Noradrenaline and serotonin selectively modulate thalamic burst firing by enhancing a hyperpolarization-activated cation current. *Nature* 340: 715–718, 1989.
- PAPE HC AND McCORMICK DA. Electrophysiological and pharmacological properties of interneurons in the cat dorsal lateral geniculate nucleus. *Neuroscience* 60: 1105–1125, 1995.
- PICKARD GE, SMITH BN, BELENKY M, REA MA, DUDEK FE, AND SOLLARS PJ. 5-HT<sub>1B</sub> receptor-mediated presynaptic inhibition of retinal input to the suprachiasmatic nucleus. *J Neurosci* 19: 4034–4045, 1999.
- PIGUET P AND GALVAN M. Transient and long-lasting actions of 5-HT on rat dentate gyrus neurones in vitro. *J Physiol (Lond)* 481: 629–639, 1994.
- PINAULT D AND DESCHENES M. Control of 40-Hz firing of reticular thalamic cells by neurotransmitters. *Neuroscience* 51: 259–268, 1992.
- POMPEIANO M, PALACIOS JM, AND MENGOD G. Distribution and cellular localization of mRNA coding for 5-HT<sub>1A</sub> receptor in the rat brain: correlation with receptor binding. *J Neurosci* 12: 440–453, 1992.
- POMPEIANO M, PALACIOS JM, AND MENGOD G. Distribution of serotonin 5-HT<sub>2</sub> receptor family mRNAs: comparison between 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors. *Mol Brain Res* 23: 163–178, 1994.
- ROBINSON DL AND COWIE RJ. The primate pulvinar: structural, functional, and behavioral components of visual salience. In: *Thalamus. Experimental and Clinical Aspects*, edited by Steriade M, Jones EG, and McCormick DA. New York: Elsevier, 1997, vol. II, p. 53–92.
- ROGAWSKI MA AND AGHAJANIAN GK. Norepinephrine and serotonin: opposite effects on the activity of lateral geniculate neurons evoked by optic pathway stimulation. *Exp Biol* 69: 678–694, 1980.
- SANCHEZ-VIVES MV, BAL T, KIM U, VON KROSIGK M, AND McCORMICK DA. Are the interlaminar zones of the ferret LGNd actually part of the perigeniculate nucleus? *J Neurosci* 16: 5923–5941, 1996.
- SCHMITZ D, EMPSON RM, AND HEINEMANN U. Serotonin reduces inhibition via 5-HT<sub>1A</sub> receptors in area CA1 of rat hippocampal slices in vitro. *J Neurosci* 15: 7217–7225, 1995.
- SHELDON PW AND AGHAJANIAN GK. Serotonin (5-HT) induces IPSPs in pyramidal layer cells of rat piriform cortex: evidence for the involvement of a 5-HT<sub>2</sub>-activated interneuron. *Brain Res* 506: 62–69, 1990.
- SPROUSE JS AND AGHAJANIAN GK. Electrophysiological responses of serotonergic dorsal raphe neurons to 5HT<sub>1A</sub> and 5-HT<sub>1B</sub> agonists. *Synapse* 1: 3–9, 1987.
- SPROUSE JS AND AGHAJANIAN GK. Responses of hippocampal pyramidal cells to putative serotonin 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> agonists: a comparative study with dorsal raphe neurons. *Neuropharmacology* 27: 707–715, 1988.
- STANHOPE KJ AND DOURISH CT. Effects of 5-HT<sub>1A</sub> receptor agonists, partial agonists and a silent antagonist on the performance of the conditioned emotional response test in the rat. *Psychopharmacology* 128: 293–303, 1996.
- STERIADE M, DOMICH L, AND OAKSON G. Reticularis thalami neurons revisited. Activity changes during shifts in states of vigilance. *J Neurosci* 6: 68–81, 1986.
- STERIADE M AND McCARLEY RW. *Brainstem Control of Wakefulness and Sleep*. New York: Plenum, 1990.
- STERIADE M, McCORMICK DA, AND SEJNOWSKI TJ. Thalamocortical oscillations in the sleeping and aroused brain. *Science* 262: 679–685, 1993.
- TO ZP, BONHAUS DW, EGLER RM, AND JAKEMAN LB. Characterization and distribution of putative 5-HT<sub>7</sub> receptors in guinea pig brain. *Br J Pharmacol* 115: 107–116, 1995.
- VERTES RP. A PHA-L analysis of ascending projections of the dorsal raphe nucleus in the rat. *J Comp Neurol* 313: 643–668, 1991.
- VON KROSIGK M, BAL T, AND McCORMICK DA. Cellular mechanisms of a synchronized oscillation in the thalamus. *Science* 261: 361–364, 1993.
- YOSHIDA M, SASA M, AND TAKAORI S. Serotonin-mediated inhibition from dorsal raphe nucleus of neurons in dorsal lateral geniculate and thalamic reticular nuclei. *Brain Res* 290: 95–105, 1984.