

Comparative physiological and serotonergic properties of pulvinar neurons in the monkey, cat and ferret

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Abstract

The basic electrophysiological properties and responses to serotonin of thalamocortical (TC) neurons in the ferret, cat, and monkey pulvinar were compared. Morphologically, thalamocortical neurons in these three species were similar, except for the presence of beaded dendrites in many monkey neurons. In all three species, the neurons exhibited two distinct firing modes: single spike activity and low threshold Ca^{2+} -spike mediated bursting. However, in monkeys, the low threshold Ca^{2+} spikes were followed by a prominent 50–100 ms afterhyperpolarization that could result in the generation of an additional rebound Ca^{2+} spike. The application of 5-HT to thalamocortical neurons in cat and monkey pulvinar resulted in a depolarization and an increase in membrane conductance through an enhancement of the hyperpolarization-activated cation current, I_h , apparently through the activation of 5-HT₇ receptors. In contrast, the application of serotonin to ferret pulvinar neurons resulted in a prominent hyperpolarization, owing to an increase in membrane potassium conductance. In monkey and ferret, application of serotonin could result in barrages of IPSPs in thalamocortical neurons. These results indicate that there are significant species-dependent differences in both the electrophysiological and pharmacological properties of pulvinar thalamocortical neurons. © 2003 Elsevier Ltd. All rights reserved.

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1. Introduction

Investigation of the post-synaptic electrophysiological actions of serotonin in the thalamus have revealed four distinct responses, which vary according to the neuron and species investigated. Application of serotonin (5-HT) to thalamocortical (TC) cells in many, but not all, thalamic nuclei of the ferret results in inhibition through an increase in membrane potassium conductance (Monckton and McCormick, 2002). In contrast, application of serotonin to the GABAergic inhibitory cells of the guinea-pig thalamic reticular nucleus and ferret perigeniculate nucleus results in a marked excitation through a decrease in potassium current (McCormick and Wang, 1991; Sanchez-Vives et al., 1996; Lee and McCormick, 1996). In the cat and ferret lateral geniculate nucleus (LGNd), and the rodent anterodorsal nucleus, serotonin causes an enhancement of the hyperpolarization-activated cation current, I_h (McCormick and Pape, 1990a; Chapin and Andrade, 2001a,b). The application of 5-HT to rat intralaminar and midline thalamocortical neurons results in a suppression of a slow afterhyperpolarization

that follows the repetitive generation of action potentials (Goaillard and Vincent, 2002). In addition to these post-synaptic actions, serotonin can also cause reduction of synaptic transmission through pre-synaptic actions, as has been shown at the retinogeniculate synapse in rodents (Chen and Regher, 2003). The finding that serotonin has a prominent post-synaptic enhancing effect on I_h in some species (cat and rat) while causing a prominent hyperpolarizing response in others (ferret), suggests that there may be significant species specific differences in the response to this neurotransmitter in the thalamus.

Serotonin is not alone in exhibiting species specific responses. The application of acetylcholine, for example, to lateral geniculate TC cells in the guinea-pig results in a large hyperpolarizing, followed by, depolarizing response through increases and decreases in membrane K^+ conductance. Application of this neurotransmitter to the same cells in cat LGNd results in the depolarizing response only (although both a hyperpolarizing and depolarizing response can be found in cat medial geniculate nucleus; McCormick and Prince, 1987). Together these results suggest that extrapolation of the post-synaptic actions of a neurotransmitter from one species to another should be done with considerable caution.

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Here we have examined the basic electrophysiological properties and post-synaptic response to serotonin of thalamocortical cells in the monkey, cat, and ferret pulvinar. We demonstrate that although the basic electrophysiological features of these neurons are similar between these three species there are important differences, namely the presence of a prominent post-burst AHP in monkey TC cells. In addition, we show that the main post-synaptic response of TC cells to serotonin in cat and monkey pulvinar is the enhancement of I_h , while in ferret TC neurons it is a prominent hyperpolarization. These results reinforce the notion that significant species-specific differences may exist in the electrophysiological and pharmacological properties of thalamocortical cells (Monckton and McCormick, 1999).

2. Methods

For the preparation of slices, ferrets of either gender between the ages of 6–35 weeks were deeply anesthetized with sodium pentobarbital (100 mg/kg) and sacrificed by decapitation. Additional experiments were performed on tissue obtained from adult cats (typically >1-year-old with one 39-day-old kitten) and adult monkeys (*Macaca fascicularis* and *Saimiri sciureus*) that were being sacrificed at the completion of other studies. These animals were sacrificed by a lethal dose of sodium pentobarbital (100 mg/kg) prior to tissue collection. This protocol was approved by the Yale University Institutional Animal Care and Use Committees and conforms to the guidelines recommended in "Preparation and Maintenance of Higher Mammals During Neuroscience Experiments", NIH Publication no. 91-3207.

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 in cold (4–8 °C) artificial cerebrospinal fluid (ACSF) with sodium replaced by sucrose while maintaining a constant osmolarity of 307 mOsm. After blocking, the tissue was affixed with cyanoacrylate in the appropriate plane and cut into 400 μ m sections using a DSK microslicer (model DTK-1000; Ted Pella Inc). 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): NaCl, 124; KCl, 2.5; MgSO₄, 2.0; NaH₂PO₄, 1.25; CaCl₂, 2; NaHCO₃, 26; dextrose, 10; and was aerated with 95% O₂–5% CO₂ 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 Inc.) from medium-walled glass (1BF100; World Precision Instrument). Micropipettes were filled with 2 M potassium acetate with 5 mM KCl and 2% biocytin

(ϵ -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 Ω . Biocytin filled neurons were visualized through standard avidin–biotin–horseradish peroxidase reaction (ABC Vectastain kit, Vectastain) processed with diaminobenzidine (Sigma) as described by Horikawa and Armstrong (1988).

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 ensure 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 Inc.) and analyzed with Clampan and Origin 5.0 (Microcal Software Inc.) 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).

Serotonin HCl, serotonin sulfate, SB-206553, risperidone, and SB-269970 were all obtained from Research Biochemicals International (Natick, MA).

3. Results

3.1. Monkey pulvinar thalamocortical neurons are morphologically distinct from those of the cat and ferret

Using atlases of the thalamus and basal telencephalon of the cat (Berman and Jones, 1982), Squirrel Monkey (Gergen and MacLean, 1962), and Macaca Nemestrina (Winters et al., 1969; Ogren and Hendrickson, 1979) we were able to readily identify and record from pulvinar neurons. As part of our experiments, we recovered the somatodendritic morphology of pulvinar thalamocortical neurons (filled with biocytin) in ferret, cat, and monkey. Upon examination of these neurons with a light microscope some differences in morphology became apparent. While the ferret and cat thalamocortical (TC) neurons are very similar, several of the monkey neurons exhibited beaded dendrites ($n = 5/9$ in Squirrel Monkey; $n = 1/1$ in *Macaca Fascicularis*; see Fig. 1). The ferret ($n = 74$) and cat ($n = 12$) TC neurons exhibited features typical of thalamocortical cells including fusiform somata with relatively smooth dendrites extending radially. These cells lacked the filiform appendages that are commonly found on interneurons (Guillery, 1966). Axons, when identifiable, always exited the plane of the section without collateralizing. Although the somatic and dendritic

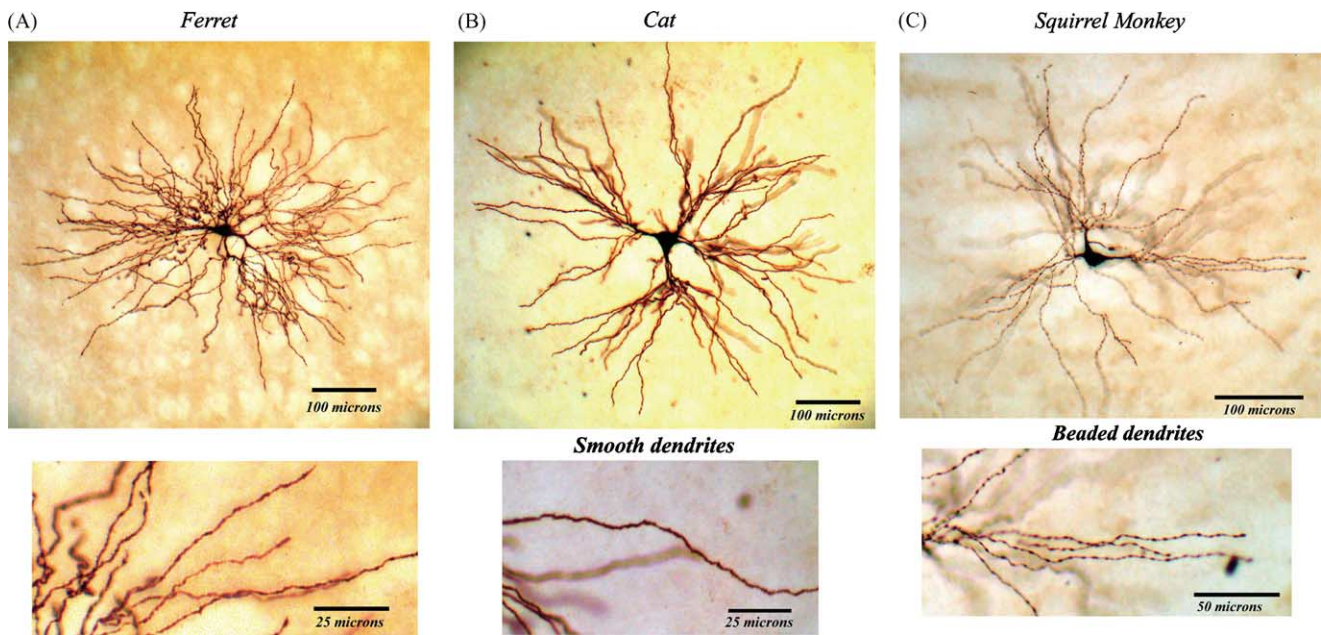


Fig. 1. Morphological features of ferret, cat and monkey pulvinal thalamocortical neurons. (A) Intracellularly filled thalamocortical neuron in the ferret pulvinal exhibits a radial dendritic arbor. The cell in (A) possessed relatively smooth dendrites. (B) Morphological features of a cat pulvinal thalamocortical neuron. This cell also exhibited relatively smooth dendrites. (C) Morphological features of a thalamocortical cell recorded in the Squirrel Monkey pulvinal. The dendrites of this monkey cell were beaded.

arbors of the monkey TC neurons were in general similar to those of the cat and ferret, 6 of 9 (7 in lateral pulvinal, 2 in medial pulvinal) of the filled monkey TC cells exhibited beaded dendrites (Fig. 1(C)). These beads appeared regularly throughout the length of the dendrites and no filiform appendages were found (Fig. 1(C)). Although it is possible that these beads were induced by damage caused by intracellular recording, their regularity and the lack of other signs of neuronal damage suggest that they are not an artifact. Dendritic beads have been reported in a relatively small subgroup of monkey pulvinal neurons (Ma et al., 1998). The dendritic beads observed here resemble those previously reported on interneurons in the neocortex (Thomson et al., 1996), thalamic nuclei (Saini and Garey, 1981; Garey and de Courten, 1983; Bickford et al., 1999), and visual cortex (Gonchar and Burkhalter, 1999). However, in none of the neurons recorded in the monkey possessing this feature did the axon ramify locally, suggesting these were indeed TC cells. Furthermore the electrophysiologic properties of these cells (broader action potentials and the existence of short duration rebound Ca^{2+} spikes) were typical of thalamocortical cells and not local interneurons (see Section 3.2; Pape and McCormick, 1995; Sanchez-Vives et al., 1996).

3.2. Intrinsic properties of ferret, cat, and monkey pulvinal thalamocortical neurons

Examination of the electrophysiological membrane properties of pulvinal thalamocortical neurons across species revealed very similar attributes. In all pulvinal, thalamocortical neurons examined in the ferret ($n = 250$), the cat

($n = 12$) and the monkey ($n = 11$; $n = 3$ for *Macaca fascicularis*; $n = 8$ for *Saimiri sciureus*) intracellular injection of a hyperpolarizing-current pulse resulted in the activation of a “depolarizing sag” (Fig. 2), which is generated from the activation of the hyperpolarization-activated cation current, I_h . Following removal of this hyperpolarization, TC cells in all three species generated rebound Ca^{2+} spikes which are mediated by the low threshold calcium current, I_T (Jahnsen and Llinás, 1984a,b). Since many of the cells recorded in the monkey pulvinal exhibited an unusual morphological feature (dendritic beads) and an unusual electrophysiological property (an afterhyperpolarization following rebound Ca^{2+} spikes—see below), we more closely examined the properties of their rebound bursts, since these are known to distinguish thalamocortical cells from local interneurons and thalamic reticular cells in other species (see Pape and McCormick, 1995; Zhu et al., 1999). Monkey neurons exhibited rebound Ca^{2+} spikes that averaged 41 (± 3 ; S.E.M. here and throughout; $n = 8$) ms in duration. These Ca^{2+} spikes were capped by 3–6 action potentials (mean = 4.5 ± 1.1 spikes) at an average peak frequency of 454 (± 55) Hz. Action potentials in monkey neurons were on average 0.47 (± 0.09) ms in duration at half height. All of these features are nearly identical to those previously reported for thalamocortical cells in the cat lateral geniculate nucleus (Pape and McCormick, 1995). They are markedly different from the properties of morphologically identified interneurons (Pape and McCormick, 1995; Zhu et al., 1999). Local interneurons in the cat LGNd exhibit thin spikes (mean duration at half amplitude = 0.34 ± 0.13 ms; $n = 16$), and may generate prolonged (100–300 ms) rebound

Intrinsic response properties of the pulvinar thalamocortical neurons

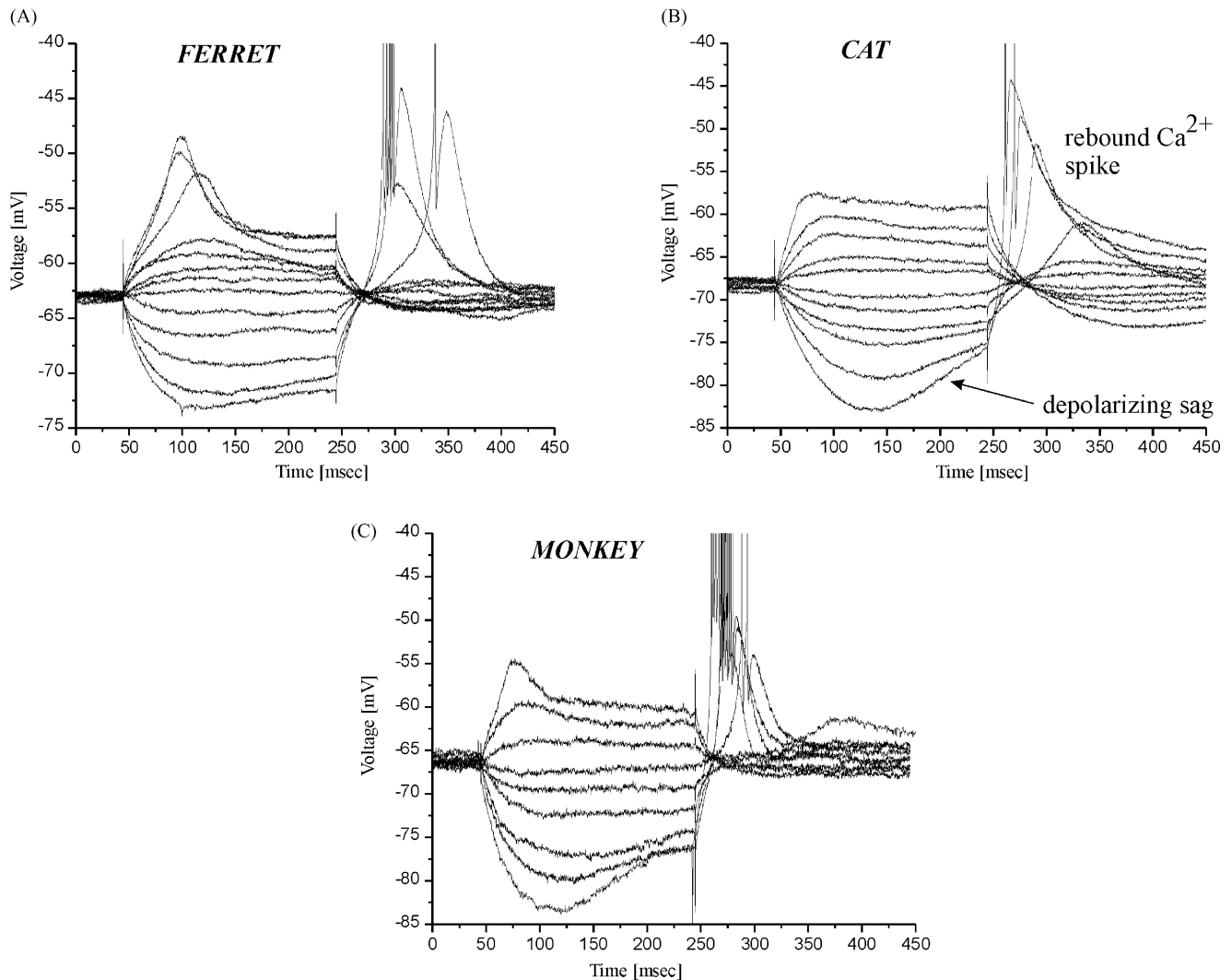


Fig. 2. Intrinsic electrophysiological properties of pulvinar thalamocortical neurons in the ferret, cat and monkey. Intracellular injection of hyperpolarizing and depolarizing current pulses reveal features typical for thalamocortical neurons including the activation of a depolarizing “sag” upon hyperpolarization, and the generation of rebound low threshold Ca^{2+} spike-mediate bursts of action potentials.

Ca^{2+} spike mediated bursts of 6–12 action potentials at lower frequencies (20–300 Hz). Therefore, we classify the neurons recorded in monkey pulvinar as thalamocortical cells based not only on their morphological features, but also on the nearly identical nature of their electrophysiological properties (except for the generation of an afterhyperpolarization following Ca^{2+} spikes) with morphologically identified thalamocortical cells in other species.

The current underlying rebound bursts in thalamocortical cells (T-current) and the current underlying the depolarizing sag upon hyperpolarization (h-current) have both been extensively studied in other thalamic nuclei and can endow TC neurons with the ability to rhythmically burst (McCormick and Pape, 1990a; Leresche et al., 1991; Soltesz et al., 1991). To examine the properties and modulation of the hyperpolarization-activated current, I_h , in TC cells, we performed single electrode voltage clamp experiments (see

Section 2). Hyperpolarizing-voltage clamp steps in pulvinar neurons from all three species resulted in the activation of a slowly activating inward current typical of I_h (Fig. 3). The block of this inward current by local application of ZD7288 (100 μM in micropipette placed <0.5 mm from the entry point of the recording electrode) confirmed its identification as I_h (Fig. 3(D)). The voltage dependence and time course of activation of I_h in pulvinar neurons from all three species (Fig. 3(A)–(C)) was similar to that previously reported for TC neurons in other thalamic nuclei (McCormick and Pape, 1990a).

3.3. Monkey pulvinar thalamocortical cells exhibit a post-burst AHP

In addition to the unique morphological features noted above, monkey TC cells also exhibits a distinct

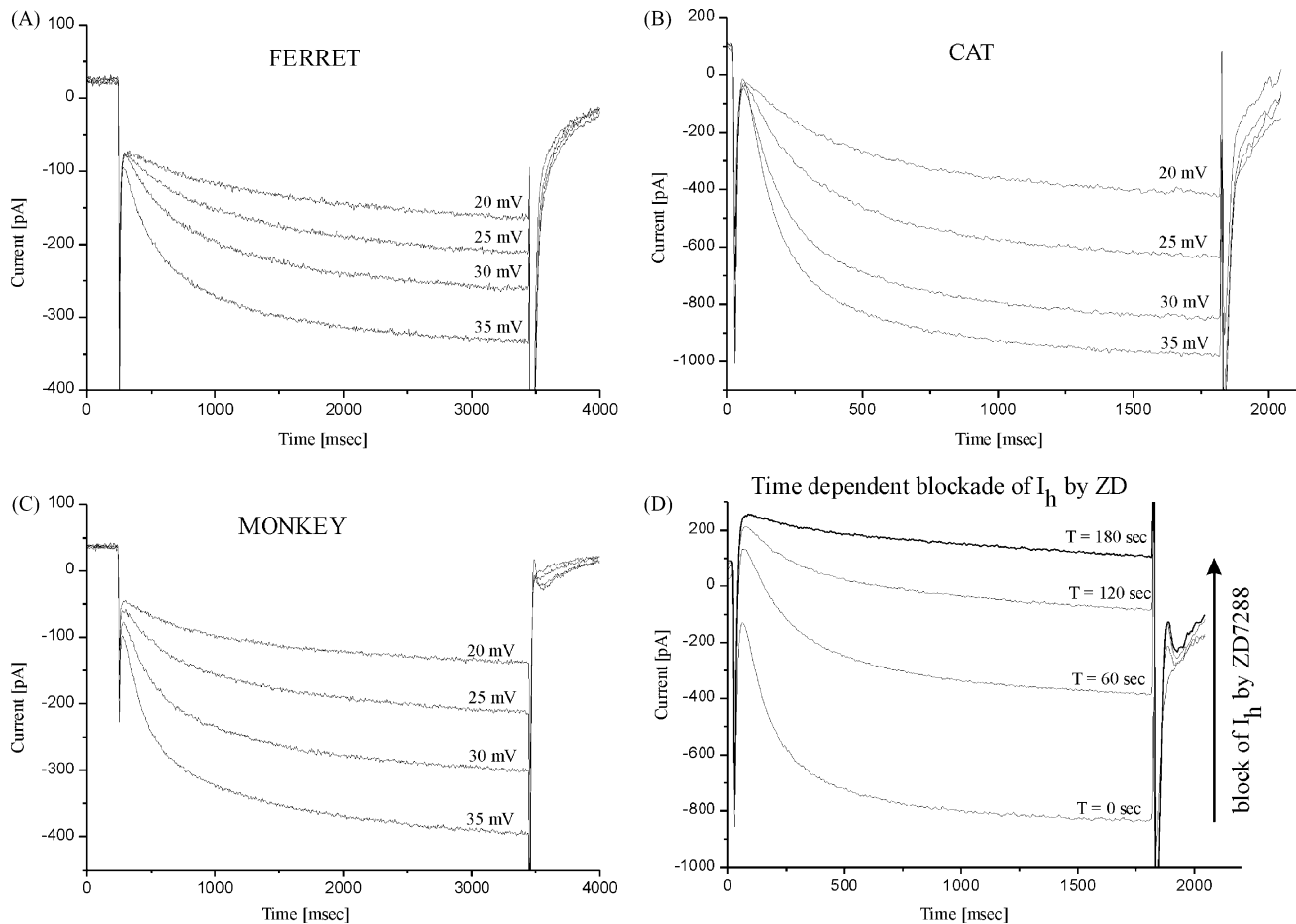


Fig. 3. Thalamocortical cells in all three species exhibit a robust h-current. Hyperpolarization in voltage clamp results in the activation of the h-current in: (A) ferret, (B) cat and (C) monkey pulvinar thalamocortical cells. (D) Local application of the h-channel blocker ZD7288 results in a gradual block of I_h . In the voltage clamp protocols, the cells were held between -50 and -60 mV in between injection of hyperpolarizing-voltage steps.

electrophysiological property not observed in the ferret or cat. The release of monkey TC neurons from hyperpolarizing potentials elicited not only a rebound low threshold Ca^{2+} spike and burst of action potentials but also a subsequent after-hyperpolarization potential (AHP; see Fig. 4, $n = 8/11$). When this AHP reached sufficient amplitude it was capable of eliciting an additional rebound burst with an inter-burst frequency of approximately 10 Hz (mean = 9.84 ± 0.46 Hz; S.E.M.; $n = 5$). Changing the steady state membrane potential (though the injection of dc) revealed the dependence of the AHP on the events that occurred during and following the hyperpolarizing-current pulse (Fig. 4). We hypothesized that this AHP was generated either through the activation of a K^+ current by the preceding Ca^{2+} spike and the associated burst of action potentials, or through the activation of I_h by the hyperpolarizing-current pulse (McCormick and Prince, 1988; McCormick and Pape, 1990a; Bal and McCormick, 1992). Moving the membrane potential of the cell to more hyperpolarized levels with the intracellular injection of dc revealed both an increase in the depolarizing sag as well as an increase in the rebound Ca^{2+} spike (Fig. 4). In addition, this hyperpolarization resulted

in a significant increase in the amplitude of the burst AHP (Fig. 4). Interestingly, the AHP could occur at a time when the activation of the depolarizing sag by the hyperpolarizing pulse was relatively minimal (Fig. 4(C), red trace). In the cell in Fig. 4(B), there was a correlation of 0.99 ($n = 10$ points) between the peak amplitude of the rebound Ca^{2+} spikes (as measured from the baseline to the envelope of spike threshold) and the amplitude of the AHP (relative to baseline). It should be noted that there was a prominent AHP following each burst at membrane potentials in which there was relatively little depolarizing sag in response to hyperpolarizing-current pulses (see overlays in Fig. 4(B) and (E)).

3.4. Serotonin's action in cat and monkey pulvinar neurons is different from its action in the ferret

Previously, we have shown that application of 5-HT to ferret TC neurons in pulvinar and midline/associative nuclei typically results in a hyperpolarization through the activation of a K^+ current mediated via 5-HT_{1A} receptors (Monckton and McCormick, 2002). In contrast, in the guinea-pig and cat

Monkey pulvinar thalamocortical neurons exhibit a prominent AHP

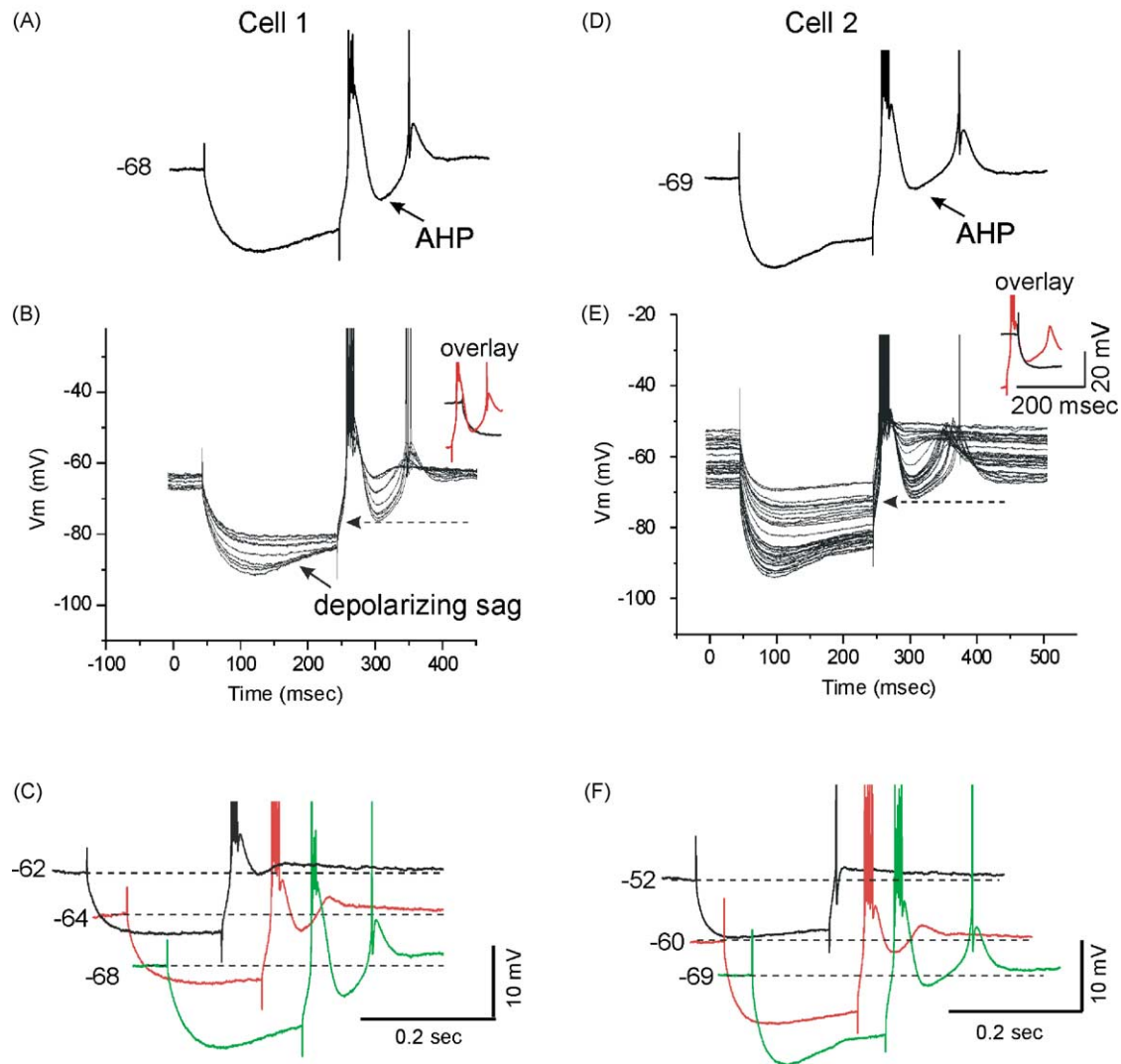


Fig. 4. Monkey pulvinar neurons may exhibit a robust AHP following rebound Ca^{2+} spikes. (A) Intracellular injection of a hyperpolarizing-current pulse results in a rebound low threshold Ca^{2+} spike that is followed by an afterhyperpolarization of between 50 and 100 ms duration. This AHP results in the generation of an additional low threshold Ca^{2+} spike. (B) Moving the membrane potential to more negative levels results in an increase activation of depolarizing “sag”, rebound Ca^{2+} spike, and the afterhyperpolarization. The dashed arrow illustrates that the peak of the hyperpolarization occurs at a membrane potential that shows relatively little depolarizing sag in response to the hyperpolarizing-current pulse. The overlay compares the amplitude–time course of the burst AHP with the response to hyperpolarization at the same membrane potential. (C) Expansion of three of the traces from (B) illustrating the relation between the amplitude of the rebound Ca^{2+} spike and the AHP. (D) The response of another neuron to the hyperpolarizing-current pulse. (E) Tonic hyperpolarizing the membrane potential to different levels results in an enhancement of the rebound low threshold Ca^{2+} spike and subsequently of the afterhyperpolarization. The horizontal dashed line illustrated the peak of the AHP occurs at a membrane potential where there is relatively little depolarizing sag to the hyperpolarizing-current pulse. (F) Expansion of three traces for detail. Traces are offset in voltage and time for clarity. Current pulse was 0.4 nA in (A)–(C) and 0.6 nA in (D)–(F).

LGND, application of 5-HT results in a small depolarization and increase in apparent membrane conductance through a shift in the voltage dependence of I_h (McCormick and Pape, 1990b).

Here, application of 5-HT (0.1 mM in micropipette) to ferret pulvinar TC neurons resulted in a large hyperpolarization and increase in membrane conductance (Fig. 5), as previously reported (Monckton and McCormick, 2002). In contrast to this response, application of 5-HT to cat ($n = 14$)

and monkey ($n = 15$) pulvinar neurons elicited a prominent depolarization that is accompanied by a reduction in the input resistance (see Fig. 5). This slow depolarization is similar to that reported in the LGND, which results from a shift in the voltage dependence of I_h (McCormick and Pape, 1990b).

We directly examined the effect of serotonin on I_h in cat and monkey pulvinar neurons through single electrode voltage clamp (Figs. 6 and 7). Application of 5-HT to these cells

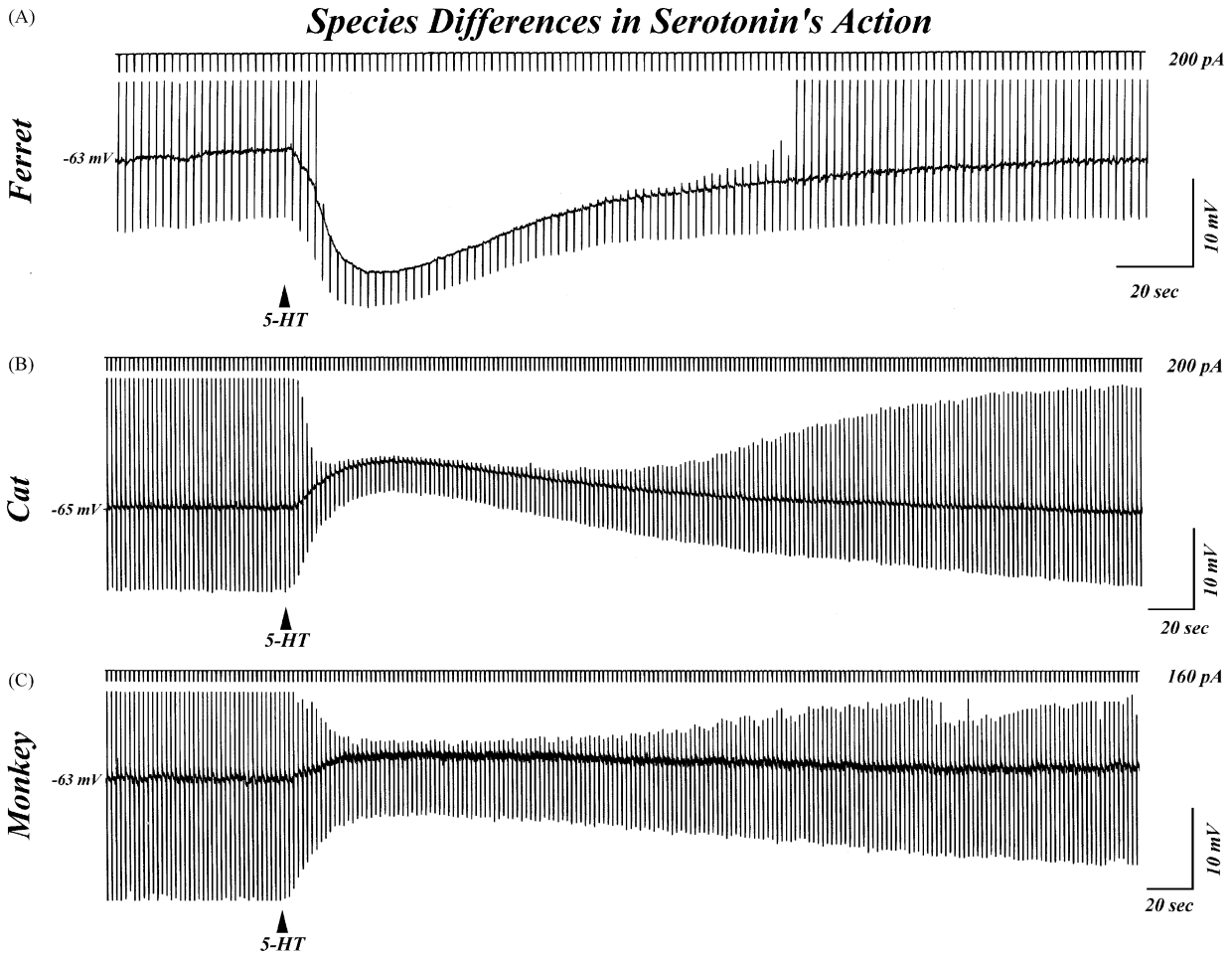


Fig. 5. Responses of ferret, cat and monkey pulvinal neurons to serotonin. (A) Application of serotonin to a ferret pulvinal neuron results in a robust hyperpolarization, owing to an increase in membrane K^+ conductance (Monckton and McCormick, 2002). In contrast, application of serotonin to either: (B) cat or (C) monkey pulvinal neurons results in a slow depolarization and an apparent increase in membrane conductance.

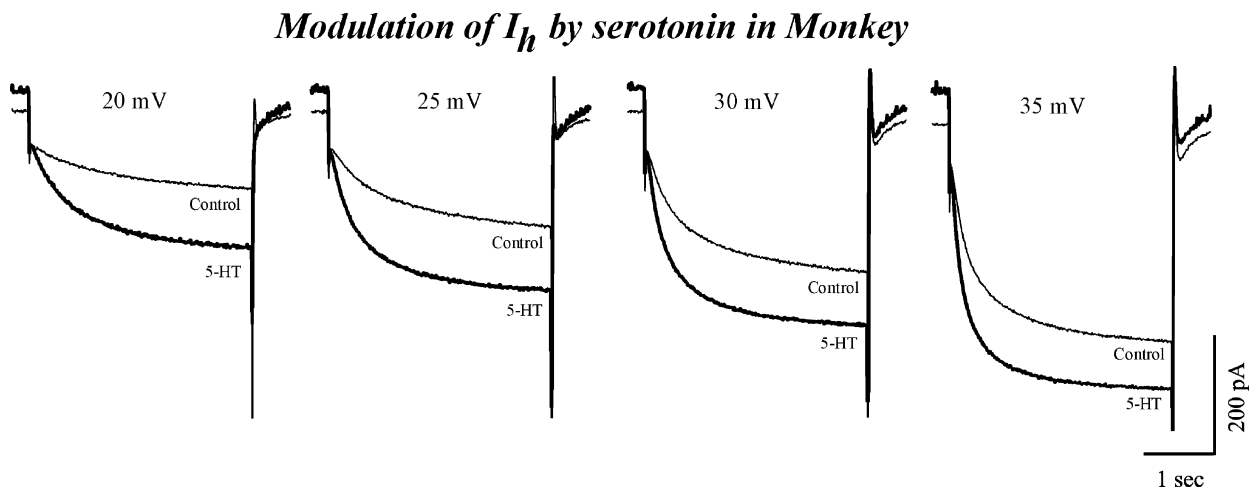


Fig. 6. Application of serotonin enhances the h-current in monkey thalamocortical neurons. Hyperpolarization of the membrane potential by 20, 25, 30, and 35 mV resulted in the slow activation of I_h . Application of 5-HT resulted in a marked enhancement of the h-current. The holding potential was -55 mV and current traces were aligned on the time of onset of I_h to better illustrate the effect of 5-HT on this current.

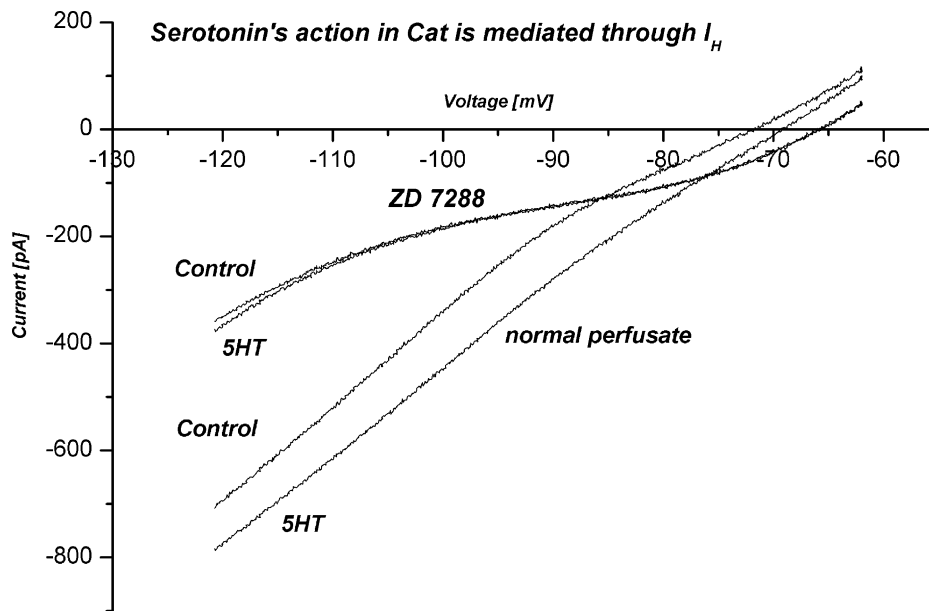


Fig. 7. Current–voltage plots before and after 5-HT confirm that this neuromodulator enhances I_h in cat pulvinal neurons. Application of the h-channel blocker ZD7288 (100 μ M in micropipette) blocks the action of 5-HT.

(cat, $n = 8$; monkey, $n = 2$) resulted in an enhancement of I_h (Figs. 6 and 7). The serotonin induced increase in this inward current is completely blocked by the local application of the I_h antagonist ZD7288 (250–500 μ M in micropipette) or cesium chloride (10 mM in micropipette) (see Fig. 7; $n = 4$). This provides strong evidence that the response to serotonin is mediated largely or entirely through the modulation of I_h .

3.5. Action of serotonin in the cat and monkey may be mediated through the 5-HT₇ receptor

In order to ascertain which 5-HT receptor mediates the depolarization in the cat and monkey, serotonin antagonists were employed. We hypothesized that the depolarizing and increase membrane conductance response to 5-HT may be mediated by either 5-HT_{1,2} or 5-HT₇ receptors. Immunohistochemical evidence supports the existence of 5-HT_{2C} receptors (Pompeiano et al., 1994) and low levels of 5-HT_{2A} receptors in the pulvinal (Mengod et al., 1996; Burnet et al., 1995). In addition, 5-HT₇ receptors are also prominent in the thalamus (To et al., 1995; Gustafson et al., 1996; Heidemann et al., 1998; Neumaier et al., 2001; Bonaventure et al., 2002).

To examine the pharmacological profile of the depolarizing response to 5-HT, we tested the ability of local application of several antagonists to block this response. Local application of the 5-HT_{2B/C} antagonist, SB-206553 (100 μ M in micropipette) appeared to have no effect on the response to 5-HT (see Fig. 8). However, when the non-selective 5-HT_{2/5-HT₇} antagonist risperidone (25 μ M in micropipette) was applied, the 5-HT-induced depolarization was abolished (see Fig. 8(C); $n = 5$; $n = 3$ for

monkey, $n = 2$ for cat). Taken together these data suggest that the receptor mediating the depolarization was mediated by either the 5-HT_{2A} or 5-HT₇ receptors (Roth et al., 1994). Indeed, the 5-HT-induced enhancement of I_h was blocked by the local application of the specific 5-HT₇ antagonist SB-269970 (100 μ M in micropipette; $n = 3$ cat; $n = 1$ monkey; Fig. 9; Bacon and Beck, 2000; Lovell et al., 2000). These results corroborate recent findings indicating that serotonin enhances I_h in thalamocortical neurons through 5-HT₇ receptors (Chapin and Andrade, 2001a,b).

3.6. Serotonin increases GABAergic tone in ferret and monkey pulvinal

Although the direct post-synaptic action of serotonin differs between the three species studied, the indirect action mediated through interneurons (through local interneurons or by activation of terminals of thalamic reticular neurons) is conserved between ferret and monkey. Consistent with the action of serotonin in the ferret (Monckton and McCormick, 2002), application of serotonin in the monkey elicits an increase in the inhibitory post-synaptic potentials (IPSPs) in the thalamocortical cells (see Fig. 10). Increases in IPSPs were found in 33% (5/15) of neurons in monkey and 61% (55/90) of the ferret. In the monkey neurons, IPSPs are superimposed upon a slower excitatory depolarizing potential mediated by the enhancement in I_h . The increases in the background IPSPs outlast the depolarization in all of the neurons where IPSPs were elicited by 5-HT. This prolonged activation of interneurons is consistent with the long time frame of the depolarization of interneurons by 5-HT in the ferret (see Monckton and McCormick, 2002). It is unlikely

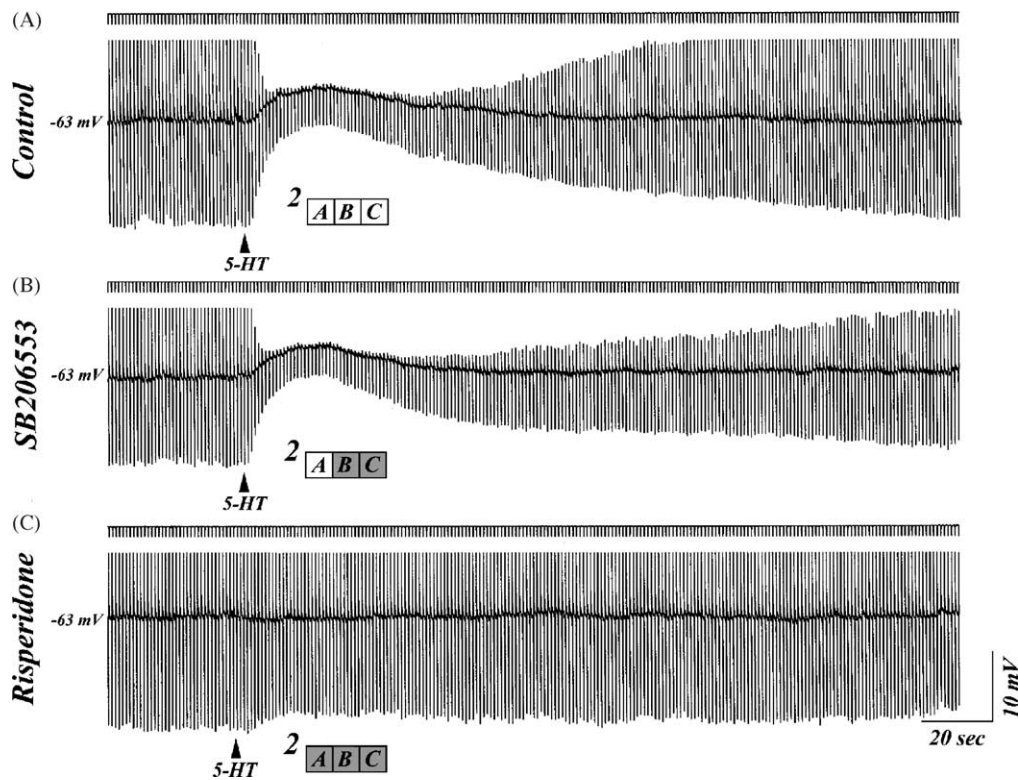


Fig. 8. Pharmacological properties of the depolarizing response to serotonin in cat thalamocortical cells. (A) Response to serotonin prior to application of antagonists. (B) Block for 5-HT_{2B,C} receptors with SB206553 (100 μ M in micropipette) does not block the serotonin response. (C) Local application of risperidone (25 μ M in micropipette), which blocks 5-HT_{2A,B,C} and 5-HT₇ receptors blocks the depolarizing response.

that the excitation of interneurons was a secondary consequence of the depolarizing action of serotonin on thalamocortical cells in the monkey, since we did not observe serotonin to be capable of causing these cells to discharge action potentials.

3.7. Age dependent decrease in the hyperpolarizing action of serotonin on pulvinar thalamocortical neurons from the ferret

The ferret is born altricial or developmentally pre-mature in comparison to cats and monkeys. One should take developmental age into consideration when comparing 5-HT responses across species. To determine whether or not developmental differences could play a role in the response disparity between the ferret and the other species studied we examined the amplitude of the 5-HT induced hyperpolarization across ferrets of differing ages. A scatter-plot of the amplitude of the hyperpolarizing response to serotonin application with respect to age illustrates a larger magnitude response with a high degree of variability early in development. This hyperpolarizing response decreases with postnatal age and becomes less variable (see Fig. 11). Using a Spearman rank test a highly significant correlation between age and amplitude of hyperpolarization was found ($z = 5.278$, $P < 0.001$). Although the amplitude of the hy-

perpolarizing response decreases with age, in no case did the application of serotonin elicit a depolarization in ferret pulvinar neurons even in nearly 9-month-old animals. Finally, the application of 5-HT to two pulvinar thalamocortical neurons recorded in tissue obtained from the kitten (39-day-old) resulted in a depolarizing response to serotonin (not shown).

4. Discussion

Our results indicate that while thalamocortical neurons across different species exhibit a number of similar properties, they also exhibit significant differences. All three species exhibited electrophysiological features consistent with a strong hyperpolarization-activated cation current, I_h , and a low threshold Ca^{2+} current, I_T (Jahnsen and Llinás, 1984a,b; McCormick and Pape, 1990a; Soltesz et al., 1991; Curro Dossi et al., 1992). The interaction of these two currents can result in the generation of rhythmic burst firing at low (0.5–4 Hz) frequencies. The observation of this type of activity in human thalamus suggests that these mechanisms have been conserved through evolution (Lenz et al., 1989, 1998). One prominent difference between species studied here was that the monkey neurons often exhibited a robust afterhyperpolarization following the generation of rebound Ca^{2+} spikes and associated bursts of action

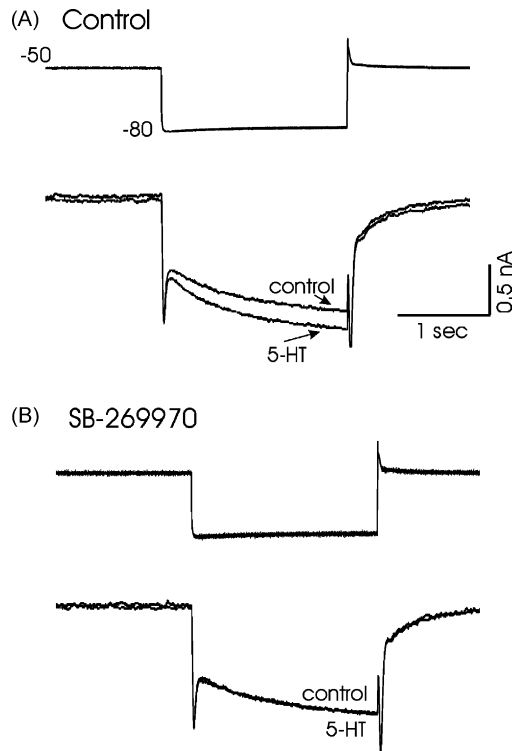


Fig. 9. The 5-HT₇ antagonist SB-269970 blocks the enhancement of I_h by serotonin. (A) Hyperpolarizing-voltage steps from -50 to -80 mV result in the activation of I_h in this cat pulvinal neuron. The application of serotonin enhances this current. (B) Following the local application of the 5-HT₇ receptor antagonist SB-269970 ($10 \mu\text{M}$ in micropipette), the application of 5-HT fails to enhance I_h .

potentials. This afterhyperpolarization interacted with the low threshold Ca^{2+} spike to give these cells the propensity to oscillate at about 10 Hz, which is within the frequency range of spindle waves and alpha waves in vivo (Andersen and Andersson, 1968; Steriade et al., 1993). We propose that this afterhyperpolarization results from the activation of a Ca^{2+} -activated and/or voltage dependent K^+ current. Although the de-activation and reactivation of I_h may contribute (McCormick and Pape, 1990a), several observations argue against an important role of I_h in the generation of the AHP. First, increasing the activation of rebound Ca^{2+} spikes and associated bursts of action potentials through the steady hyperpolarization of the neuron resulted in a significant increase in the amplitude of the afterhyperpolarization following each burst. Second, the amplitude and time course of the rising phase of the afterhyperpolarization was considerably larger and faster than the time course of activation of depolarizing sags at the same membrane potentials (see Fig. 4). Finally, although thalamocortical cells from all three species exhibited h-currents of similar time course and magnitude, only those in the monkey exhibited a robust afterhyperpolarization. Thalamocortical neurons typically exhibit prominent afterhyperpolarizations following the generation of high threshold Ca^{2+} spikes, presumably

through the activation of either Ca^{2+} -activated K^+ currents or through the activation of voltage dependent K^+ channels (McCormick, unpublished observations; see Fig. 9 in Jahnsen and Llinás, 1984b). The lack of generation of AHPs following low threshold Ca^{2+} spikes in ferret and cat suggests that in these species Ca^{2+} -activated K^+ channels may be located in a way that allows them to sense Ca^{2+} entry through high threshold, but not low threshold, Ca^{2+} channels. If the burst AHP in monkey pulvinal neurons is generated through a Ca^{2+} -activated K^+ current, then one possible explanation is that in the monkey (but not cat and ferret), these channels are located adjacent to low threshold, as well as high threshold, Ca^{2+} channels. Another possible difference may be that rebound Ca^{2+} spikes activate high threshold Ca^{2+} channels in monkey neurons, but fail to do so in either the cat or ferret. These and other possibilities remain to be explored.

4.1. Action of serotonin on thalamocortical neurons in the cat and monkey pulvinal

The post-synaptic actions of 5-HT on ferret, cat and monkey pulvinal neurons are quite distinct. At no time did a thalamocortical neuron of the cat or monkey hyperpolarize, nor did a ferret pulvinal thalamocortical neuron depolarize, to serotonin. The absence of a hyperpolarizing response to 5-HT of the cat and monkey neurons (following blockade of the depolarizing response) argues against any substantial expression of the 5-HT_{1A} receptors on pulvinal TC cells in these species.

The pharmacological profile found here of the depolarizing response is consistent with it being mediated by the activation of 5-HT₇ receptors (Chapin and Andrade, 2001a,b). The 5-HT_{2B,C} antagonist, SB-206553, was ineffective at blocking the depolarizing response to serotonin in cat and monkey neurons, whereas the 5-HT_{2/5-HT₇} antagonist risperidone was effective. Furthermore, the application of the 5-HT₇ receptor antagonist SB-269970 was effective in blocking the enhancement of I_h by serotonin. Receptor localization studies have shown that the thalamus contains a high level of 5-HT₇ receptors (To et al., 1995; Gustafson et al., 1996; Bonaventure et al., 2002; Neumaier et al., 2001), and these receptors are positively coupled to adenylyl cyclase, resulting in increases in intracellular cAMP levels (Vanhoenacker et al., 2000; Goillard and Vincent, 2002). The h-current is strongly enhanced by cAMP, perhaps through direct binding of this second messenger to h-channels (Pape, 1996; Luthi and McCormick, 1999; Wang et al., 2002). An additional possible pathway for the modulation of I_h is through increases in intracellular Ca^{2+} and the activation of Ca^{2+} -sensitive adenylyl cyclases (see Luthi and McCormick, 1998, 1999). The possibility that part of the 5-HT response is mediated through the activation of the 5-HT_{2A} receptor, which can increase intracellular Ca^{2+} concentration (through increases in 1,4,5-inositol triphosphate-IP₃) remains to be explored.

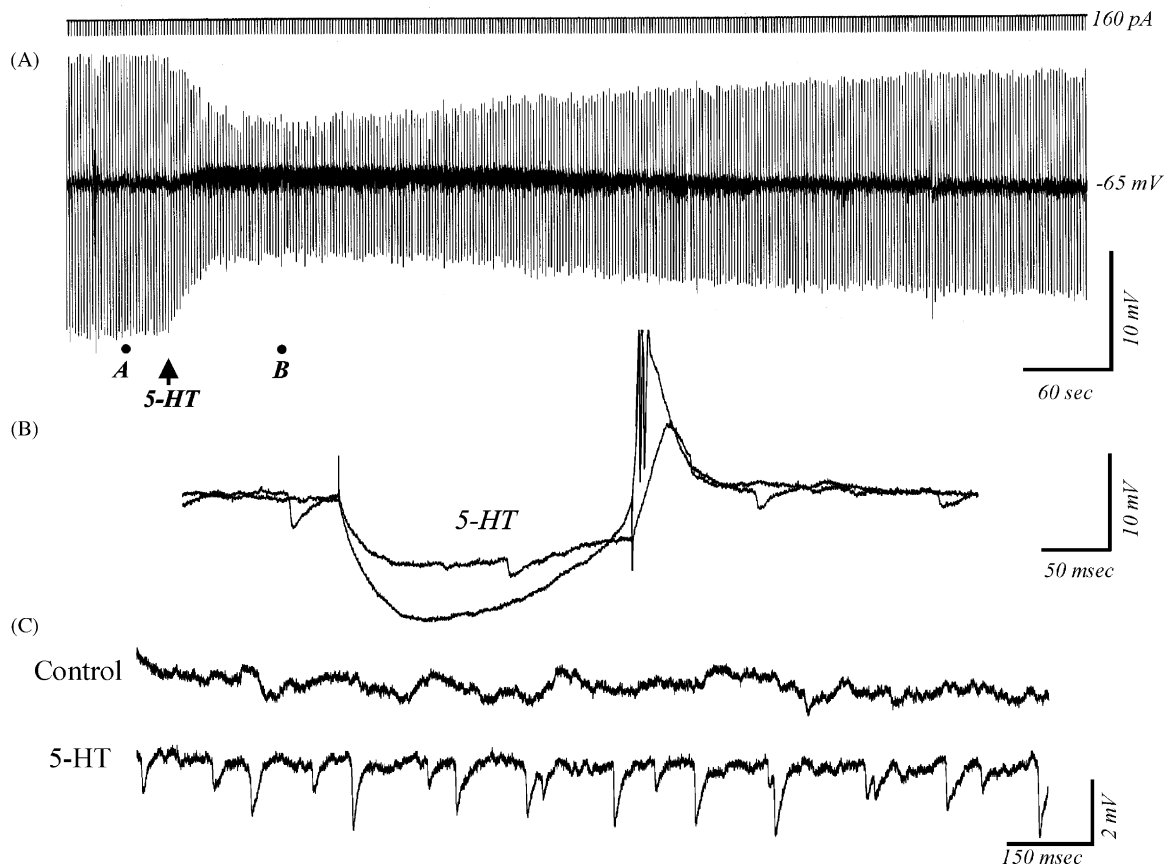
Serotonin Application Results in Barrages of IPSPs

Fig. 10. Application of 5-HT can also result in the activation of inhibitory post-synaptic potentials in thalamocortical cells in monkey thalamocortical cells. (A) Application of 5-HT to this thalamocortical cell in the monkey (Squirrel Monkey (*saimiri sciureus*)) pulvinar results in a small depolarization and a marked decrease in response to hyperpolarizing-current pulses, and consequently a decrease in rebound burst firing (expanded in (B) for detail). In addition to these effects, the application of serotonin results in a marked increase in the arrival of IPSPs in the thalamocortical cell (expanded in (C) for detail).

The expected functional consequences of serotonin's action in ferret and cat or monkey neurons are quite distinct. Hyperpolarization due to the activation of a potassium current results in an enhancement of burst firing in thalamic neurons (reviewed in McCormick, 1992). If the TC cells are in the single spike firing mode, then activation of this response will decrease single spike activity and increase the probability of generating rebound burst discharges, such as those involved in the generation of intrinsic or network delta (0.5–4 Hz) oscillations (McCormick and Pape, 1990a), sleep spindles (Steriade et al., 1993; Bal et al., 1995; McCormick and Bal, 1997), and at least some forms of generalized spike-wave seizures (reviewed in McCormick and Contreras, 2001). In comparison, release of serotonin onto thalamocortical cells in the cat and monkey pulvinar may result in a block of rhythmic burst firing, owing to depolarization through an enhancement of I_h (McCormick and Pape, 1990b; Lee and McCormick, 1996).

The action of serotonin in the thalamus is even more complicated, when considering its effects on interneurons. In the ferret and guinea-pig, application of serotonin to

the GABAergic neurons of the thalamic reticular or perigeniculate nuclei (or intralaminar interneurons in the ferret LGNd) results in a strong depolarization and promotion of single spike activity through the reduction of a K^+ current (McCormick and Wang, 1991; Funke and Eysel, 1993, 1995; Sanchez-Vives et al., 1996). Application of serotonin to local interneurons can result in a modest excitation (Pape and McCormick, 1995; Monckton and McCormick, 2002). Together, these results indicate that the release of serotonin in the cat and monkey pulvinar may promote an abolition of rhythmic burst activity, such as that occurring during slow wave sleep. These actions of serotonin may also help to move the network into a state that is more typical of the waking and attentive brain, and indeed, the firing rate of dorsal raphe serotonergic neurons is known to increase in relation to arousal and to decrease in anticipation of sleep (Trulsson and Jacobs, 1979; Jacobs et al., 1990). In the ferret pulvinar, in contrast, the release of serotonin may result in a more complicated situation in which burst firing is promoted in thalamocortical cells, but inhibited in thalamic reticular neurons. It is yet unclear how these diverse actions may contribute to

Age Related Decline in Hyperpolarization to Serotonin in Ferret

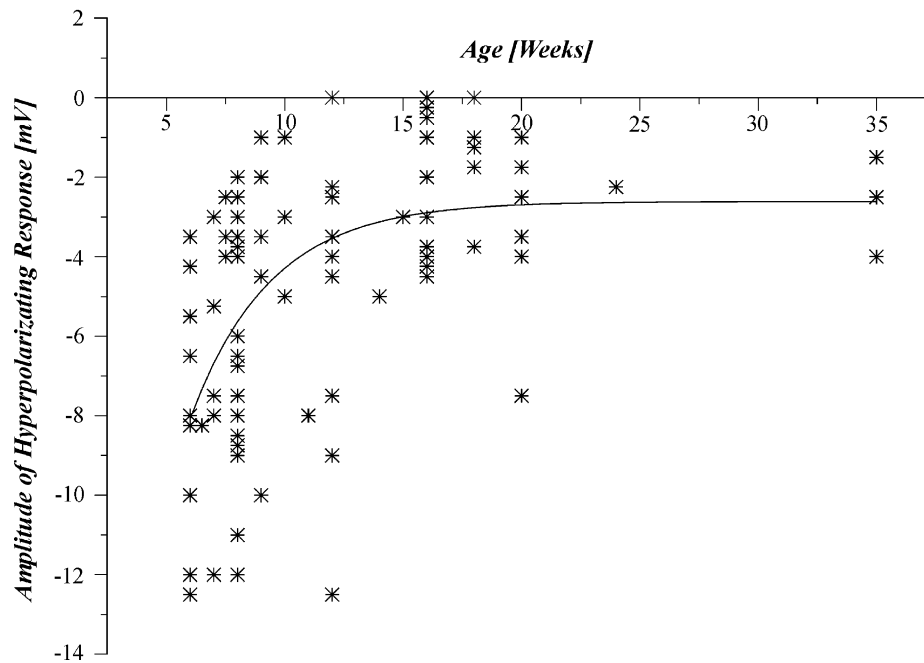


Fig. 11. The hyperpolarizing response to 5-HT in the ferret pulvinar is age dependent, but does not disappear completely by 35 weeks (approximately 9 months) of age. All cells were from -55 to -65 mV prior to application of serotonin.

the overall level of arousal associated with the waking state in the ferret.

4.2. Species differences in response to serotonin are not likely to be entirely due to developmental differences

Developmental differences between the ferret and the other species examined are not likely to be entirely responsible for the observed differences in 5-HT's action. Although developmental decrements in the expression of the 5-HT_{1A} receptor has been reported, for example, in rats, lamb, and humans (Daval et al., 1987; Richards et al., 1990a,b; Bar-Peled et al., 1991; Dillon et al., 1991; del Olmo et al., 1998), expression reaches its mature level relatively early in postnatal life. The present results demonstrate that serotonin application evoked hyperpolarizing responses even in 35-week-old ferrets, although the magnitude of this response was smaller than observed in younger animals. Ferrets attain their adult size by 4 months of age, and are sexually mature at 9–12 months. Furthermore, preliminary examination of the kitten (39-day-old) pulvinar revealed a depolarizing, increased conductance, response to serotonin ($n = 2$). Ferret LGNd is considered to be fully mature (base on anatomical analysis) by the fourth post-natal week (Linden et al., 1981), an age that is younger than the youngest ferret used in this study. These results suggest that, although there are developmental decreases in the hyperpolarizing action of serotonin in the ferret pulvinar, they do not completely account for the species differences

outlined here. Further investigation, however, into actions of serotonin in older ferrets is warranted.

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