Cortical and Collicular Inputs to Cells in the Rat Paralaminar Thalamic Nuclei Adjacent to the Medial Geniculate Body

Philip H. Smith,1 Edward L. Bartlett,2 and Anna Kowalkowski1

1Department of Anatomy, University of Wisconsin Medical School–Madison, Madison, Wisconsin; and 2Departments of Biological Sciences and Biomedical Engineering, Purdue University, West Lafayette, Indiana

Submitted 4 March 2007; accepted in final form 25 May 2007


INTRODUCTION

Inputs from other sensory modalities have been shown to affect our perception of auditory events. For example, in the classic demonstration of the McGurk effect (McGurk and McDonald 1976) when a visual image of a speaker saying “ga” is paired with a sound recording of “ba” the listener hears the phoneme “da.” This phenomenon occurs rapidly and preattentively. Such observations would indicate that somewhere along the auditory pathway, visual inputs are capable of influencing the responses of neurons and that these effects have a very short latency. There are very few areas of the auditory pathway where visual inputs are known to impinge. One such region is the auditory thalamus or medial geniculate body (MGB), in particular the medial division of the MGB (MGM) and adjacent nuclei collectively known as paralaminar nuclei (Herkenham 1980). Komura et al. (2005) showed that MGB cells located primarily in the paralaminar nuclei responded directly to either visual or auditory stimuli presented alone or had auditory responses that could be significantly enhanced by simultaneously presented visual stimuli. McEchron et al. (1996) stimulated the superior colliculus (SC), a probable source of this visual input, and was able to generate evoked spikes in MGB cells but not from cells elsewhere in the MGB. Anatomical studies have also verified the multimodal nature of the paralaminar nuclei showing major auditory inputs primarily from the external and dorsal nuclei of the inferior colliculus (IC) (Kudo and Niimi 1980; LeDoux et al. 1985; Linke et al. 1999; Oliver and Hall 1978) as well as inputs from cells in all layers of the SC (Altman and Carpenter 1961; Graham 1977; Hicks et al. 1986; Holstege and Collewijn 1982; Linke et al. 1999; Martin 1969; Tarlov and Moore 1966).

In addition to an unusual repertoire of inputs, we recently reported that cells in the MGM display an unusual combination of anatomical and physiological features when compared with the “standard” thalamic (TC) neurons seen elsewhere in auditory and other areas of the sensory thalamus (Smith et al. 2006). Some of these unusual features may be important in determining how synaptic inputs influence the cell’s output. Anatomically, paralaminar cell dendrites can be long, branch sparingly, and encompass a large area. These dendrites can have well-defined spines. In addition, the axons of MGM cells can have collaterals that branch locally within the same or nearby paralaminar nuclei. Physiologically, MGM cell spikes are larger in amplitude than standard TC neurons, can be shorter in duration, and often show dual afterhyperpolarizations (AHPs) with a fast component and a slow, apamin-sensitive component. These cells can also have a reduction or complete absence of the low-threshold, voltage-sensitive calcium conductance that reduces or eliminates the voltage-dependent burst response (LTS).

Given the unusual physiological properties of paralaminar neurons, one of our goals was to examine whether the synaptic responses of paralaminar neurons fit within the typical framework of sensory thalamic neurons generally and auditory thalamic neurons specifically. For TC neurons in primary sensory thalamus, ascending inputs terminate in large boutons and generate large excitatory postsynaptic potentials (EPSPs) in postsynaptic TC neurons. These inputs are considered “driver” inputs because the receptive fields of TC neurons are largely determined by a small number of ascending inputs.
(Guillery 1995; Sherman and Guillery 1998). Conversely, descending layer 6 corticothalamic inputs terminate in small boutons and generate small individual EPSPs. Furthermore, because inactivation of cortex causes little shift in the receptive fields of TC neurons, the corticothalamic inputs are considered “modulator” inputs (Guillery 1995; Sherman and Guillery 1998). However, in nonprimary somatosensory and visual thalamus, corticothalamic inputs from layer 5 have all of the characteristics of “driver” inputs (Reichova and Sherman 2005).

To obtain a basic understanding of the influence of the auditory and visual inputs on cells in the paralaminar nuclei and to evaluate their status as drivers or modulators we utilized the slice preparation to record from MGM cells while stimulating inputs from the inferior or superior colliculi. We also did light (LM) and electron microscopy (EM) to look at the characteristics of labeled axon terminals formed by these inputs. A similar evaluation was made on the third major inputs to paralaminar nuclei—the corticothalamic terminals arising from auditory cortex.

Moreover, we examined whether the collicular inputs could produce GABAergic inhibition in paralaminar neurons and, if so, determined potential functional roles of the inputs. Whereas the “driver” and “modulator” distinction can be applied to most or all of the thalamic regions that have been studied, one connectivity pattern seems to be unique to the auditory thalamus. Previous studies from this lab have demonstrated a robust, feedforward inhibitory input from IC to primary (MGV) and nonprimary (MGD) auditory thalamus (Bartlett and Smith 1999; Peruzzi et al. 1997). These IC axons generate short-latency γ-aminobutyric acid type A (GABAA) inhibitory postsynaptic potentials (IPSPs) in MGV and MGD neurons that often precede IC EPSPs, as well as generating GABAβ IPSPs. Given these previous results, we sought to determine whether collicular GABAergic inputs, if present, 1) were short-latency, 2) generated GABAα and GABAβ IPSPs, and 3) were effective in suppressing excitation from the same source.

**Methods**

**Brain slice experiments**

**INTRACELLULAR RECORDING.** All methods were approved by the University of Wisconsin Institutional Animal Care and Use Committee. Animals were maintained in an American Associations for Accreditation of Laboratory Animal Care–approved facility. Our experimental methods are similar to those described previously (Bartlett and Smith 1999, 2002; Smith et al. 2006). Brain slices from 3- to 6-wk-old Long–Evans hooded rats were used. Rats were given an anesthetic overdose then perfused with chilled, oxygenated sucrose saline (subsequently described). The portion of the brain containing the thalamus was removed and 400- to 500-μm sections were cut and collected in 0.1 M phosphate buffer (pH 7.4). The sections were then incubated in H2O2, rinsed in phosphate buffer, then refrigerated and incubated overnight in the avidin–biotin–HRP complex (ABC Kit, Vector Labs). The following day, the sections were rinsed in phosphate buffer and the Neurobiotin reacted using the diaminobenzidine (DAB)–nickel/cobalt intensification method, mounted, counterstained with cresyl violet, and coverslipped.

To determine a cell’s location, a low-power ×40 camera lucida drawing of the MGB and surrounding structures was made and the location of the labeled cell noted. The location of the cell body relative to the divisions of the rat MGB was made by comparing our sections/drawings with sections in the atlas of Paxinos and Watson (1986), illustrations in the cytoarchitectural study of Clerici and Coleman (1990), and the illustrations and descriptions of paralaminar subdivisions by LeDoux et al. (1985) and Doron and LeDoux (2000). A high-power ×1,250 drawing of the cell body, dendritic tree, and initial portion of the axon was also made. A description of MGB cell morphology has been provided in a previous publication (Smith et al. 2006).

**DATA ANALYSIS.** **Physiology.** Several variables of the basic intrinsic cell physiology were measured using Clampfit software (Axon Instruments) so the data could be compared with our previous data from cells in the MGV and MGD (Bartlett and Smith 1999). These measures have been recently reported (Smith et al. 2006).

**Anatomy.** Using the camera lucida drawings of the labeled MGB cells, we measured the dendrites of a population of multipolar and elongate cells at 50, 100, 200, and 300 μm from the cell body. These diameters were compared with the diameters of dendrites in the paralaminar nuclei, evaluated at the EM level (see following text), that were postsynaptic to terminals labeled by extracellular gross injection of Neurobiotin into either the IC, SC, or auditory cortex.

Statistical analysis was performed using Origin Pro7 (OriginLab, Northampton, MA) and Microsoft Excel (Microsoft, Redmond, WA).
data presented as means ± SD. A value of $P < 0.05$ or less was considered to represent a significant difference.

Extracellular injection experiments

SC and IC injections. Injections were made unilaterally into the SCs of three rats and the ICs of three rats. Some of the injection sites have been illustrated in a previous publication (Bartlett and Smith 2000). The rat was anesthetized with sodium pentobarbital (35 mg/kg). An incision was made in the scalp, exposing the skull, and a hole drilled just rostral to the lambdoid suture and lateral to the midline. The posterior aspect of the cerebral cortex was carefully aspirated, exposing the superior and inferior colliculi on one side. A 1-μl Hamilton syringe (Hamilton, Reno, NV) was visually guided into either the inferior or superior colliculus. A 10% solution of Neurobiotin (0.3–0.4 μl) in distilled water was slowly injected into the structure over a 15- to 20-min period. The syringe was left in place for an additional 20–30 min and then withdrawn. A second penetration was made at a different location of the same colliculus and the procedure repeated. Occasionally a third penetration would be made in the same colliculus and the procedure again repeated. The IC injections distributed label in the central nucleus of the IC as well as the external and dorsal cortical areas of the IC. The SC injections distributed label in superficial, intermediate, and deep layers of the structure.

Auditory cortex injections. Injections were made unilaterally into the cortex of three rats. Some of the injection sites have been illustrated in a previous publication (Bartlett and Smith 2000). The rat was anesthetized as earlier. An incision was made in the scalp, exposing the lateral aspect of the skull immediately over auditory cortex. A small hole was drilled in the skull and a cut made in the dura. A 1-μl Hamilton syringe was visually guided to the surface of the cortex and then advanced approximately 1 mm. The extracellular injection methods were the same as those described earlier. The injections distributed label through all layers of portions of primary and secondary auditory cortical areas.

Postinjection processing. Section preparation. After the gross injections the syringe was removed and the wound margins were closed by suture or surgical staples and treated with a broad-spectrum antibiotic and lidocaine. After 24 h the rat was given an overdose of sodium pentobarbital and perfused through the heart with 100–200 ml of 0.9% phosphate-buffered saline followed by 1,000 ml of a 3% paraformaldehyde/1% gluteraldehyde solution in 0.1 M sodium phosphate buffer (pH 7.4). The tissue was refrigerated for 1 h and the brain removed and placed in the same fixative overnight. Coronal sections (70–80 μm thick) were cut with a vibratome and the Neurobiotin tracer was visualized using the DAB–nickel/cobalt intensification method as described earlier (Adams 1981). Sections were rinsed in phosphate buffer and these free-floating sections inspected with a light microscope to determine whether the gross injection had successfully labeled axons and axon terminals in the medial geniculate. Some of the sections containing the axon terminals in the MGB were selected to be processed for electron microscopy. Sections not selected for electron microscopy were mounted on slides, dehydrated, stained with cresyl violet, and coverslipped.

Those sections selected for EM analysis were fixed in 0.5% osmium tetroxide for 30 min, rinsed in buffer, and dehydrated through a series of graded alcohols and propylene oxide. Sections were then placed in unaccelerated Epon-Araldite resin for 1 h at room temperature and then transferred into a fresh batch of unaccelerated resin overnight. The sections were then embedded and flat mounted in accelerated resin between weighted, coated slides at 65°C overnight. The portion of the embedded section containing the MGB was isolated from the rest of the section and mounted on a beam capsule. A camera lucida drawing of the flat-mounted tissue was made, noting the location(s) of the labeled axons and terminals in MGB. Thin sections (70–80 nm) were then cut and mounted on coated nickel grids.

GABA postembedding immunogold labeling. An affinity-isolated GABA antibody conjugated to bovine serum albumin (BSA, Sigma–Aldrich, catalog no. A2052) was used to evaluate the GABAergic nature of the terminals. The thin section, mounted on a nickel grid, was carefully immersed in solution A (0.745 g Tris buffer, 0.9 g NaCl, 0.1 ml Triton-X in 100 ml dH2O, pH 7.6) and 5% BSA for 30 min, then in solution A and 1% BSA for 5 min, then in solution A and 1% BSA containing the GABA antibody at 1:250 dilution at room temperature overnight. The following day the section was rinsed in solution A and 1% BSA twice for 5 min and once for 30 min, then rinsed in solution B (0.688g Tris buffer, 0.9g NaCl, 0.1 ml Triton-X in 100 ml dH2O, pH 8.2) for an additional 5 min. The section was then immersed in the secondary antibody (goat anti-rabbit IgG with attached 15-nm gold particles) diluted 1:25 in solution B (pH 8.2) for 1 h and rinsed twice for 5 min each in solution A and twice for 5 min in distilled water. After that they were fixed in 8% EM-grade gluteraldehyde, rinsed in distilled water, and then counterstained with uranyl acetate and lead citrate and rinsed again. Sections were examined using a Philips CM120 electron microscope. For control sections the primary antibody step was eliminated.

Analysis of axon terminals and their postsynaptic targets Light microscopy. Labeled terminal swellings from axons arising in the SC, IC, and cortex were measured at the light microscopic level. For labeled SC terminals ×1,000 camera lucida drawings were made of 100 to 200 terminal swellings in each of the paralaminar regions medial to the MGB (SG, MGM, and PP/PIN). These drawings were enlarged 200% and terminal measurements made using National Institutes of Health (NIH) Image/J 1.60 software. The same procedure was used for terminals from the IC and cortex.

Electron microscopy. For analyzing Neurobiotin-labeled axon terminals in the MGB arising from the IC, SC, and cortex and synapsing on dendrites, the area of the axon terminal profile and the postsynaptic dendrite least diameter (length of minor axis) were determined for each synapse using The NIH Image/J 1.60 software. Other terminal features, such as synapse location (on spines, dendrite, somata, etc.), were also noted.

In GABA immunogold–labeled material, the “background” gold particle density was calculated for each thin section to account for possible processing differences between sections. Aggregates of two or more particles were counted as a single particle. Axon terminals were considered GABAergic if their particle density exceeded the mean gold-particle density of the background by fivefold. Background density was measured over non-GABAergic structures such as blood vessel lumens, glial cells, and/or dendritic profiles (there are very few GABAergic neurons in the rat medial geniculate). Statistical comparisons of measurements were made using Minitab software (Minitab). For all results, ANOVA tests were used to determine whether groups differed significantly with $P < 0.05$ used as the minimum criterion for statistical significance. Results are reported as means ± SD.

Results

General

Figure 1 illustrates the basic differences that we previously reported (Smith et al. 2006) in the anatomy and physiology of cells in the paralaminar nuclei when compared with those in MGV and MGD and elsewhere in the sensory thalamus. Tufted cells (Fig. 1, top middle) are the only main cell type in MGV and stellate/multipolar cells are the main cell type in MGD (Fig. 1, top right). The dendrites of both these cell types do not display spines and their axons never give off local collaterals. In the MGD/paralaminar nuclei there are also stellate/multi-
and no rebound burst is seen when the cell is recovering from a hyperpolarizing current pulse (Fig. 1, middle column, arrow in fourth trace). MGM cell sodium spikes are larger in amplitude, can be shorter in duration, and can show dual AHPs with fast and slow components (Fig. 1, middle column, arrows in third trace). Similar physiological differences were reported in the rat LGN (Crunelli et al. 1987a) where cells in ventral LGN lacked the low-threshold calcium conductance.

**Synaptic physiology**

**GENERAL.** In horizontal slices, we recorded from 44 cells in the paralaminar nuclei. Synaptic events were generated in 40/44 of these cells by electrically shocking (stimulating) the brachium of the inferior colliculus. Stimulating the thalamic radiations, which contain corticothalamic axons, in these same slices elicited synaptic events in 28/44 of these cells. In coronal slices, we recorded synaptic events generated by stimulating the superior collicular input from 34 cells in paralaminar nuclei (34/34 cells). Recorded cells were labeled with Neurobiotin and recovered. We were unable to distinguish any obvious differences in the collicular or cortical synaptic inputs between cell types categorized by anatomy or physiology in the paralaminar nuclei (stellate vs. elongate). Cells with spiny dendrites vs. those with smooth dendrites, cells with the low-threshold calcium burst vs. those with no calcium burst) so the data will be reported without reference to these features.

**EXCITATION.** In horizontal slices, activation of IC inputs by stimulation of the BIC typically (35/44 cells) elicited short-latency EPSPs that gradually increased in amplitude with increasing stimulus strength (Fig. 2A, second panel). In rare instances (two of 44) this gradual increase would suddenly change to a large jump at one stimulus level indicative of the activation of a larger synaptic input (not shown). We presume that these rare large synaptic inputs arise from tectothalamic axons but it is remotely possible that they arise from the activation of backfired corticotectal axons that are running in the BIC and also send a collateral to the thalamus. Activation of cortical inputs in these slices by stimulation of the thalamic radiations also typically (28/44) generated short-latency EPSPs with increasing amplitude (Fig. 2A, top). There was never a sudden jump in EPSP amplitude when we increased stimulus intensity, which would indicate the activation of a larger terminal. In coronal slices stimulation of the SC also elicited EPSPs (34/34 cells) in cells in the MGM and SG that would gradually increase in amplitude with increasing stimulus strength (Fig. 2A, third panel). In some of these same coronal slice experiments we recorded from cells in the MGV and MGD while stimulating the SC output and were never able to activate synaptic events in these cells. We also noted in 28% of recorded neurons (22/78) that a second or multiple, longer-latency EPSP(s) (Fig. 2A, asterisk in bottom panel; Fig. 2B, asterisk in top panel) could be elicited when stimulating the IC, SC, or cortical inputs to paralaminar cells. Experiments using the glutamate antagonists APV (n = 7) or DNQX and CNQX (n = 6) showed that EPSPs from all three sources had both N-methyl-d-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) components (Fig. 2B), with considerable variation in the contribution of each component among cells.
For cells in other parts of the sensory thalamus that possess the low-threshold calcium conductance the same excitatory synaptic inputs can often generate burst and tonic mode spiking whose timing is depending on the cell’s membrane potential. This was also the case for those paralaminar cells that displayed this calcium conductance (Fig. 2C, top). When depolarized, stimulus-elicited EPSPs would typically generate a short-latency “tonic” spike. As the cell was moved to more hyperpolarized levels the EPSP became subthreshold. At still more hyperpolarized levels the subthreshold EPSP was able to activate the low-threshold calcium conductance and burst spikes were generated (burst). At a still more hyperpolarized level the EPSP was again subthreshold. In other cells the EPSP was large enough that a well-timed onset spike would occur regardless of the membrane potential (not shown).

We also looked at the paired-pulse behavior of these excitatory inputs. When two stimuli were applied to the cortical input at varying intervals the cortical EPSP showed strong paired-pulse facilitation (PPF) that lasted hundreds of milliseconds (Fig. 3, A, top and B). In two representative cells, similar paired stimuli with varying delays elicited slight paired-pulse depression or slight PPF in both the SC and IC inputs (Fig. 3, A, bottom two panels and B).

FIG. 2. Excitatory inputs to paralaminar cells. A: synaptic responses of different paralaminar cells to increasing stimuli applied to their cortical (top panel), inferior colliculus (IC, 2nd panel), or superior colliculus (SC, 3rd panel) inputs. Bottom: increasing shock stimuli applied to the SC input, illustrating that some MGM cells also display a later excitatory postsynaptic potential (EPSP; asterisks) that can be activated by collicular or cortical inputs. B, top: responses of an MGM cell to 2 shock stimuli applied to its SC input in normal saline (normal) and saline containing 25 µM 6,7-dinitroquinoxaline-2,3-dione (DNQX). Asterisk highlights a second longer-latency EPSP generated by the input. Bottom: responses of a MGM cell to a single stimulus applied to its IC input in normal saline (normal) and saline containing 25 µM 2-amino-5-phosphonovaleric acid (APV). C, top: responses of MGM cell that had a low-threshold calcium conductance, to the same shock stimulus applied to its IC input with the cell held at different membrane potentials. When depolarized the EPSP elicited a short-latency tonic spike (tonic). As the cell was moved to more hyperpolarized levels the EPSP became subthreshold. At still more hyperpolarized levels the subthreshold EPSP was able to activate the low-threshold calcium conductance and burst spikes were generated (burst). At a still more hyperpolarized level the EPSP was again subthreshold. Bottom: responses of MGM cell that did not had the low-threshold calcium conductance to the same shock stimulus applied to its IC input at different membrane potentials. At depolarized levels the EPSP was suprathreshold and generates a single short-latency spike (tonic). As the cell was hyperpolarized the EPSP remained subthreshold. Scale bars in middle panel of A apply to all traces in top 3 panels. sa, stimulus artifact in this and subsequent figures.

For cells in other parts of the sensory thalamus that possess the low-threshold calcium conductance the same excitatory synaptic inputs can often generate burst and tonic mode spiking whose timing is depending on the cell’s membrane potential. This was also the case for those paralaminar cells that displayed this calcium conductance (Fig. 2C, top). When depolarized, stimulus-elicited EPSPs would typically generate a single short-latency “tonic” spike. When the same cell was hyperpolarized into the calcium burst mode the same EPSP might not reach spike threshold but would exceed the threshold of the calcium conductance, causing the cell to fire one or more spikes with a longer latency (burst). This membrane-potential-dependent response was different for MGM cells without the low-threshold calcium conductance. For these cells, EPSPs that could generate a short-latency spike at depolarized levels could be subthreshold at more hyperpolarized levels because there was no calcium conductance to activate (Fig. 2C, bottom). In other cells the EPSP was large enough that a well-timed onset spike would occur regardless of the membrane potential (not shown).

We also looked at the paired-pulse behavior of these excitatory inputs. When two stimuli were applied to the cortical input at varying intervals the cortical EPSP showed strong paired-pulse facilitation (PPF) that lasted hundreds of milliseconds (Fig. 3, A, top and B). In two representative cells, similar paired stimuli with varying delays elicited slight paired-pulse depression or slight PPF in both the SC and IC inputs (Fig. 3, A, bottom two panels and B).

INHIBITION. We have previously shown (Bartlett and Smith 1999; Peruzzi et al. 1997) that cells in the dorsal and ventral divisions of MGB can receive ascending inhibitory inputs from the inferior colliculus. These IPSPs are GABAergic and typi-
cally display both GABA$_A$ and GABA$_B$ components whose amplitudes increase as more axons are recruited with increasing stimulation strengths. Paralaminar cells also showed GABAergic IC inputs (Fig. 4A). Simulation of the BIC in horizontal slices often (22 of the 35 cells that displayed an EPSP as well, two of the remaining nine displayed only an IPSP) generated a GABAergic IPSP that was eliminated with either picrotoxin ($n=4$) or bicuculline ($n=6$). As Fig. 4A illustrates this IPSP could have a shorter onset latency than the EPSP. Unlike IC inhibitory inputs to MGV or MGD cells, stimulation of IC inputs to paralaminar cells rarely (two of 24 with GABA$_A$ IPSPs) activated GABA$_B$ receptors.

We were surprised to find that stimulation of the SC input in coronal slices also frequently (19 of 34) generated an IPSP in paralaminar cells as well (Fig. 4B). As with the IPSPs originating from IC inputs those from the SC: 1) could have a latency similar to or even shorter than the EPSP latency, 2) could be blocked by GABA$_A$ antagonists ($n=6$), and 3) were only rarely (two of 19) accompanied by longer-latency, longer-duration GABA$_B$-mediated inhibitory events. Although the anatomical data subsequently described confirm that there is an inhibitory SC input to paralaminar cells it is possible that some of these IPSPs might have been generated by the activation of non-SC axons running in the brachium.

**EFFECTS OF INHIBITION ON CELL RESPONSES.** Activation of the inhibitory input from either the IC or the SC could affect the spike output properties of MGM cells to their excitatory inputs in several ways. Figure 4 illustrates the effect of the IPSP on spike output while the cell remains at the same membrane potential but the stimulus strength applied to the input is increased. In Fig. 4A the cell does not fire a spike at low (top trace), medium (middle trace), or high (bottom trace) stimulus strengths but when the inhibition is removed the EPSP becomes suprathreshold at high stimulus strengths (Fig. 4A, bottom trace). In Fig. 4B EPSPs elicited at low, medium, and high stimulus strengths are all suprathreshold despite the presence of an early IPSP. However, removal of the IPSP alters the timing (Fig. 4B, top and bottom) and the number (Fig. 4B, bottom) of spikes generated. Figure 4C graphically illustrates this for another cell. In normal saline the cell fired a single spike (triangle) at the onset, whereas at higher stimulus strengths the IPSP prevented this early spike. Bath application of picrotoxin blocked the IPSP and the cell was able to fire an onset spike (circle) at all stimulus strengths as well as one or more longer-latency spikes (smaller circles). The longer-latency spikes did not arise from activation of a calcium burst.

The IPSP could also dramatically alter the EPSP-generated spike output of the cell at different membrane potentials (Fig. 5). The plot at the right of Fig. 5A illustrates the spike output of a cell when its IC input is stimulated with the same stimulus intensity as the cell’s membrane potential is changed in normal saline (triangles) and in saline containing bicuculline (circles). The two sets of intracellular traces to the left in Fig. 5A illustrate the responses at two of the membrane potentials. The lighter trace is the response in bicuculline. In normal saline with the cell held at around $-40$ mV (Fig. 5A, top left) the short-latency IPSP prevents any EPSP from generating a spike until later in the trace. In bicuculline the same stimulus strength at the same membrane potential elicits two short-latency spikes the timing of which is illustrated in the graph. When the membrane potential was moved to around $-65$ mV (Fig. 5A, bottom two traces) the IPSP prevented the cell from generating an early spike, although the early EPSP was able to activate the calcium burst, which in turn activated a longer-latency spike. In bicuculline at this membrane potential the same stimulus was now able to elicit three spikes in rapid succession.

Figure 5, B and C illustrates how the inhibitory input can have an effect on the spike output when both stimulus strength and membrane potential are changed. At depolarized levels around $-57$ mV (top traces in B and C) both low (B) and high (C) stimulus strengths elicited an inhibitory event in normal saline (dark traces) that was strong enough to delay the spike response. When GABA was blocked (lighter traces) the same excitatory input was able to elicit two short-latency spikes at low stimulus strengths and a barrage of five spikes at the higher stimulus level. If the cell was hyperpolarized (bottom traces in B and C) the excitatory input was unable to generate spikes in normal saline (dark traces), but when GABA was blocked the excitatory events

**FIG. 4.** Inhibitory inputs from the colliculi. A: synaptic responses of a paralaminar cell to stimulation of bicuculline (BIC) at increasing stimulus strengths (top to bottom) in normal saline (angled arrows) and saline containing 25 µM picrotoxin (picro). B: synaptic responses of a paralaminar cell to stimulation of the brachium of the SC (BSC) at increasing stimulus strengths (top to bottom) in normal saline (small arrows) and saline containing 25 µM picrotoxin (picro). In the bottom panels of A and B the inhibitory postsynaptic potential (IPSP) is labeled. C: spike response of another cell as the stimulus strength was increased in saline (triangles) and saline containing 20 µM picrotoxin (circles). Larger symbols represent the first spike in each trial; smaller symbols represent subsequent spikes. Scale bar in A applies to all traces in A and B.
The increased number of spikes seen when GABA receptors are blocked may in some cases be explained by the enhanced activation of local excitatory inputs to the cell being recorded from. This is illustrated in Fig. 5D. In normal saline (left) stimulating the IC input at three different membrane potentials elicited an early IPSP (arrow) followed by an EPSP that was able to generate a spike at two of the membrane potential levels. Occasionally, stimulating the input would also activate the discrete longer-latency EPSPs (asterisks) that are often seen in these cells. When GABA<sub>A</sub> receptors were blocked (right) the probability and number of these later EPSPs generated in this cell by the same stimulus was greatly increased (asterisks) and they were sometimes capable of eliciting spikes (arrow). Thus our data indicate that these late EPSPs, seen when either the collicular or cortical input is stimulated, may be generated by the activation of other paralaminar cells that have local axon collaterals synapsing on the cell from which we are recording.

**THE LATE EPSP.** The data presented in Fig. 6 are consistent with the idea that local collaterals give rise to the longer-latency EPSPs often seen when stimulating a collicular or cortical input. Figure 6A illustrates the proposed circuit. Cell 1 receives a stimulated (lightning bolt) collicular or cortical input. In addition it also receives an axon collateral input from a second paralaminar cell (cell 2) that is also being driven by the same stimulated input. We have previously demonstrated (Smith et al. 2006) that paralaminar cells can have local axon collaterals. Figure 6B illustrates the response of a paralaminar cell to activation of its IC inputs and would represent cell 1 in A. This cell displayed a short-latency EPSP and a second consistent longer-latency EPSP (Fig. 6B, top, asterisk) as the stimulus strength was increased. When the NMDA-receptor antagonist APV was added to the bath the early stimulus-induced EPSP was reduced in amplitude, whereas the longer-latency EPSP disappeared (Fig. 6, middle). AMPA-receptor antagonist DNQX can also have the same effect of eliminating the late EPSP (see Fig. 2B). Figure 6B, bottom illustrates a possible response of cell 2 in the circuit illustrated in A that could explain the APV-induced loss of the late EPSP in cell 1. Addition of the NMDA antagonist made an EPSP that generated a spike in normal saline (normal) subthreshold (APV), thus removing any spike-generated synaptic influence that cell 1 would have on cell 1. Figure 6C illustrates another scenario that is consistent with the circuit diagram in Fig. 6A. The top panel shows the response of another MGM cell that could represent cell 1, to activation of its IC input as the membrane potential is changed. This MGM cell had no low-threshold calcium conductance. In normal saline (top) the cell received an early excitatory input from the IC that could be suprathreshold as well as a consistent EPSP with a longer latency (asterisk). In the presence of the GABA antagonist picrotoxin (middle) the same shock stimulus generated essentially the same onset response, but the latency of the longer-latency EPSP became significantly shorter (asterisk). The bottom panel in Fig. 6C illustrates a response of an MGM cell that could represent cell 2 in the diagram in A and could account for the latency reduction of the second EPSP seen in cell 1 in picrotoxin. In normal saline stimulating the input to the cell in the bottom panel (cell 2) elicits an IPSP that blocked the ability of the early EPSP to generate a spike. Instead the cell responded with a long-latency spike (normal) as the membrane potential.
IC, SC, and Cortical Terminals. Light microscopy. We measured the areas of several hundred labeled terminals in the paralaminar nuclei after making Neurobiotin injections into the IC, SC, or auditory cortex. Figure 7 illustrates representative samples of these terminals. Although the size of these three populations of terminals overlaps considerably (Fig. 8, A and B) one-way ANOVA of the areas of these IC (n = 418, mean area = 1.1 ± 0.83 μm²), SC (n = 463, mean area = 0.90 ± 0.74 μm²), and cortical (n = 354, mean area = 0.75 ± 0.465 μm²) terminals showed that there were significant differences in their means [F(2,1232) = 23.04, P < 0.0001]. Post hoc t-test showed that the IC terminals were significantly larger than the SC (P < 0.0005) and cortical (P < 0.0001) terminals and the SC terminals significantly larger (P < 0.001) than the cortical terminals.

We also compared the terminals from a single input source that were located in three different regions occupied by the suprageniculate (SG), MGM, and peripeduncular/posterior intralaminar (PP/PIL) paralaminar nuclei (Fig. 8C). For all three terminal types the mean areas of terminals located in MGM were the largest. The results of a one-way ANOVA showed significant differences among the means of the IC [F(2,415) = 22.06, P < 0.0001], SC [F(2,460) = 10.5, P < 0.0001], and cortical terminals [F(2,351) = 31.78, P < 0.0001] in these three subregions. Post hoc t-test revealed that the mean area of the SC terminals (1.145 ± 1.13 μm², n = 145) in the MGM area returned to rest after the IPSP. When the IPSP was blocked with picrotoxin the early EPSP was then able to elicit an earlier spike. Such a change in the spike output of cell 2 would lead to a decrease in the latency of the synaptic event it generated in cell 1. Thus it would appear that local paralaminar axon collaterals provide an additional excitatory input to neighboring paralaminar cells. We cannot, however, rule out the possibility that in rare cases this late EPSP might be generated by SC cells that are activated by shock-induced antidromic activation.

Synaptic anatomy

IC, SC, and Cortical Terminals. Light microscopy. We measured the areas of several hundred labeled terminals in the paralaminar nuclei after making Neurobiotin injections into the IC, SC, or auditory cortex. Figure 7 illustrates representative samples of these terminals. Although the size of these three populations of terminals overlaps considerably (Fig. 8, A and B) one-way ANOVA of the areas of these IC (n = 418, mean area = 1.1 ± 0.83 μm²), SC (n = 463, mean area = 0.90 ± 0.74 μm²), and cortical (n = 354, mean area = 0.75 ± 0.465 μm²) terminals showed that there were significant differences in their means [F(2,1232) = 23.04, P < 0.0001]. Post hoc t-test showed that the IC terminals were significantly larger than the SC (P < 0.0005) and cortical (P < 0.0001) terminals and the SC terminals significantly larger (P < 0.001) than the cortical terminals.

We also compared the terminals from a single input source that were located in three different regions occupied by the suprageniculate (SG), MGM, and peripeduncular/posterior intralaminar (PP/PIL) paralaminar nuclei (Fig. 8C). For all three terminal types the mean areas of terminals located in MGM were the largest. The results of a one-way ANOVA showed significant differences among the means of the IC [F(2,415) = 22.06, P < 0.0001], SC [F(2,460) = 10.5, P < 0.0001], and cortical terminals [F(2,351) = 31.78, P < 0.0001] in these three subregions. Post hoc t-test revealed that the mean area of the SC terminals (1.145 ± 1.13 μm², n = 145) in the MGM

![Image](http://jn.physiology.org/Downloaded_from/http://jn.physiology.org/10.220351.on.August.6.2016)
was larger than that in the SG (0.77 ± 0.52 μm², n = 191, P < 0.0001) and the PP/PIL (0.86 ± 0.44 μm², n = 145, P < 0.01). This was also the case for the cortical terminals where the mean for those in the MGM (1.03 ± 0.45 μm², n = 106) was larger than both the SG (0.67 ± 0.45 μm², n = 122, P < 0.0001) and PP/PIL (0.605 ± 0.39 μm², n = 126, P < 0.0001).

For IC terminals the mean area was also largest for those in the MGM (MGM cortical: 1.36 ± 1.08 μm², n = 118; SG: 1.19 ± 0.76 μm², n = 170; PP/PIL: 0.73 ± 0.42 μm², n = 130) but the difference was significant only for comparisons of the MGM and PP/PIL nuclei.

The larger size of the cortical terminals in the MGM compared with those in SG and PP/PIL made us wonder whether these terminals were at all comparable in size to the giant corticothalamic terminals that are known to arise from layer 5 pyramidal cells in auditory cortex. In the rat these giant terminals arising from primary auditory cortex have been shown to terminate in the deep MGD and marginal zone (Rouiller and Welker 1991). We measured a population of these giant terminals in the deep MGD and marginal zones that we had labeled for comparison with the labeled cortical terminals in the MGM nuclei. As Fig. 7 visually illustrates and Fig. 8D graphically illustrates there is virtually no overlap in the size of the giant terminals (mean area = 6.53 ± 2.53 μm², n = 145) with the MGM cortical terminals (1.03 ± 0.45 μm², n = 106). Thus although the corticothalamic terminals that we labeled in the paralaminar nuclei vary in size between subnuclei all of them are the small variety that presumably arise from cortical layer 6 pyramidal cells.

Electron microscopy. We evaluated 173 IC, 75 SC, and 79 cortical Neurobiotin-labeled terminals in the paralaminar nuclei at the EM level. Examples of labeled terminals are shown in Figs. 9 (IC), 10 (SC), and 11 (cortex). Of the terminals evaluated 150/173 IC, 39/75 SC, and all 79 of the cortical terminals were in tissue that was also processed for the GABA antibody. In this tissue we noted that 29 of the 150 IC terminals (Fig. 9), seven of the 39 SC terminals (Fig. 10), and none of the 75 cortical terminals (Fig. 11) labeled with the GABA antibody. The results of a one-way ANOVA showed significant differences [F(2,229) = 16.73, P < 0.001] among the mean areas of the excitatory terminals from IC (0.9 ± 0.69 μm²), SC (0.63 ± 0.69 μm²), and cortex (0.43 ± 0.18 μm²). Post hoc t-test revealed that the excitatory SC terminals were significantly larger than the cortical terminals (P < 0.02), whereas the excitatory IC terminals were significantly larger than both the excitatory SC (P < 0.05) and the cortical (P < 0.001) terminals. The mean area of the GABAergic IC terminals (1.26 ± 0.74 μm²) was significantly larger than any of the IC terminals.

FIG. 8. A–C: light microscopic measurements of labeled collicular and cortical terminals found in the paralaminar nuclei adjacent to the MGB. A: plot of the areas of labeled IC, SC, and cortical terminals. B: mean ± SD of the areas of labeled IC, SC, and cortical terminals in the paralaminar nuclei. C: mean ± SD of the area of labeled IC (top), SC (middle), and cortical (bottom) terminals in the PP/PIL, MGM, and SG subdivisions of the paralaminar nuclei adjacent to the MGB. D: plot comparing the distribution of the areas of cortical terminals in the SG paralaminar nucleus (MGM cortical) with areas of giant cortical terminals in the deep subnucleus of the MGD (giant).

FIG. 9. Inputs from the inferior colliculus. A: Neurobiotin-labeled IC terminal synapsing on what appears to be a spine (asterisk) emanating from a dendrite (d). B: Neurobiotin-labeled IC terminal synapsing on what appears to be a spine (asterisk) in the vicinity of a Neurobiotin-labeled IC axon (a). C: Neurobiotin-labeled terminals (arrows) synapsing on dendrites (d) in the vicinity of a GABAergic terminal (asterisk). D: GABAergic Neurobiotin-labeled IC terminal synapsing on a dendrite (d) next to a GABAergic axon (asterisk). E: GABAergic Neurobiotin-labeled IC terminal in the vicinity of a Neurobiotin-labeled axon (asterisk). F: GABAergic Neurobiotin-labeled IC axon (white asterisk) near a GABAergic axon (black asterisk). Scale bar in F = 1 μM and applies to all electron micrographs.
excitatory terminal means but not significantly larger than the SC GABAergic terminals (1 ± 1.25 μm²).

When a structure postsynaptic to an IC terminal could be positively identified (43/150) both the non-GABAergic, presumably glutamatergic (Fig. 9, B and C), and the GABAergic (Fig. 9D) IC terminals almost always synapsed on dendrites (Fig. 9D). In three instances the non-GABAergic IC terminals instead synapsed on what might be dendritic spines (Fig. 9, A and B, asterisks). When a postsynaptic structure could be identified opposite an SC terminal (13/39) both the non-GABAergic IC terminals and the GABAergic SC terminal synapsed on dendrites (Fig. 10). In these instances the non-GABAergic IC terminals synapsed on what might be dendritic spines (Fig. 9, A and B, asterisks). When a postsynaptic structure could be identified opposite an SC terminal (13/39) both the non-GABAergic IC terminals and the GABAergic SC terminal synapsed on dendrites (Fig. 10). In these instances the non-GABAergic IC terminals synapsed on what might be dendritic spines (Fig. 9, A and B, asterisks).

We were interested in trying to determine where on the dendritic tree of paralaminar cells these terminals might be synapsing. Figure 12 illustrates measurements we made of dendrite diameters from camera lucida drawings of Neurobiotin-labeled multipolar and elongate cells that were recorded from in brain slices as well as the diameters of dendrites postsynaptic to Neurobiotin-labeled IC, SC, and cortical terminals at the EM level from the gross injection experiments. For both cell types the dendrites of labeled cells in slices tapered abruptly between 50 (stellate diameters = 2.78 ± 1.24 μm, elongate = 2.90 ± 1.81 μm) and 100 μm (stellate diameters = 1.57 ± 0.69 μm, elongate = 1.71 ± 1.02 μm) then gradually tapered over the next 200 μm (Fig. 12). At a given distance from the cell body the multipolar and elongate dendrite diameters were not significantly different until 300 μm from the cell body, where the multipolar cell dendrites were slightly larger (P < 0.05). Measurements of the dendrites postsynaptic to our labeled collicular and cortical terminals at the EM level showed that the collicular and cortical terminals synapsed on dendrites with similar diameters. All of our GABAergic collicular terminals that could be seen synapsing on a postsynaptic structure did so on small dendrites, although we had only a small number of these for each of the colliculi (n = 3 for SC, n = 6 for IC), so it was possible to qualitatively evaluate only where they synapsed on the dendritic tree. For the non-GABAergic terminals the results of a one-way ANOVA showed no significant differences [F(2,98) = 2.79, P = 0.07] among the diameters of dendrites postsynaptic to each input (IC mean postsynaptic diameter = 1.23 ± 0.5 μm, SC = 1.02 ± 0.64 μm, cortical = 1.4 ± 0.45 μm). Thus based on the small mean value of the dendritic diameters measured postsynaptic to excitatory collicular and cortical terminals it would appear that many of the terminals synapse on dendrites that are some distance from the cell soma. Qualitatively that would seem to be the case for the GABAergic collicular terminals as well.
Excitation dendritic tree. Dendritic terminals also appear to be terminating out on the cells generated by their excitatory inputs. The inhibitory can dramatically affect the spike output of these paralaminar nuclei that receive excitatory inputs from the cortex. Our physiological data showed that cells in the paralaminar nuclei receive excitatory inputs from the SC, IC, and cortical terminals labeled in the gross injection experiments and viewed at the electron microscopic level. Means (closed square) ± SD are shown.

DISCUSSION

General

Using both physiological and anatomical criteria, we have shown that both stellate and elongate cells in the paralaminar nuclei receive synaptic inputs from the SC, IC, and the cortex. Physiologically the individual EPSPs from all three sources are usually small, although multiple inputs can sum together to generate a large synaptic event with NMDA and AMPA components. Cortical inputs show paired-pulse facilitation, whereas both collicular EPSPs show weak paired-pulse facilitation or depression. In addition to the direct excitatory input, our evidence indicates that axon collaterals of paralaminar cells activated by cortical or collicular inputs generate longer-latency EPSPs in neighboring paralaminar cells. Anatomically, the terminals from all three sources are typically small and synapse on smaller dendrites. The SC and IC also appear to contain GABAergic cells that send inhibitory axons to synapse on paralaminar cells. These GABAergic IPSPs can arrive before the EPSP. Unlike the GABAergic IC inputs to the MGV and MGD, which activate both GABA_A and GABA_B receptors, those from the IC and SC to paralaminar cells usually activate only GABA_A receptors. The presence of these inhibitory inputs can dramatically affect the spike output of these paralaminar cells generated by their excitatory inputs. The inhibitory collicular terminals also appear to be terminating out on the dendritic tree.

Excitation cortex. Our physiological data showed that cells in the paralaminar nuclei receive excitatory inputs from the cortex that 1) generate small synaptic events that are additive as stimulus intensities are increased, 2) show paired-pulse facilitation to repeated stimuli, and 3) have both NMDA and AMPA components. Our anatomical experiments indicate that these synaptic events are generated by small synaptic terminals that terminate on distal dendrites. These features are identical to those generated by cortical inputs to cells in MGV/MGD (Bartlett and Smith 1999), V1 inputs to dLGN, and S1 inputs to ventrobasal complex (see Sherman and Guillery 2001), indicating that the small-terminal cortical input is similar across primary and nonprimary sensory cortical regions. Hazama et al. (2004) showed that the rat primary auditory cortex (TE1) provides a major input to MGV and MGD and only a minor input to paralaminar nuclei, primarily to SG. Kimura et al. (2004) provided evidence that some cortical terminals arising from a cortical area posteroventral to TE1, designated PD, project to SG as well. Most were of the small-terminal variety but a few were large terminals arising from layer 5 pyramidal cells. We never saw any physiological evidence of large-terminal inputs in paralaminar cells. However, these terminals are few in number and we may have simply not recorded from SG cells receiving this large terminal input. Arnault and Roger (1990) provided anatomical evidence that the majority of the cortical input to MGM and other paralaminar nuclei arises from secondary auditory areas designated Te2 and Te3 but did not specify terminal size.

IC and SC. We also noted excitatory inputs from both inferior and superior colliculi. In most cases, stimulating the IC or SC input generated small EPSPs that would summate as stimulus strength was increased. These small collicular EPSPs showed weak paired-pulse facilitation or depression and had both NMDA and AMPA components. The proportions of IC (two of 44) and SC inputs (zero of 34) that generated large-amplitude IC EPSPs were significantly smaller compared with our previous data (Bartlett and Smith 1999), where we observed large-amplitude IC EPSPs in 41% (17 of 41) of MGV cells and in 29% (nine of 31) of MGD cells. This is consistent with the interpretation that much of the IC input to MGV and some of the IC input to MGD terminate in large “driver” inputs, whereas the IC and SC inputs to paralaminar nuclei are not typically “driver” inputs. Several studies (Arnault and Roger 1987; LeDoux et al. 1985; Linke 1999) indicate that the IC inputs to the paralaminar nuclei arise primarily from the external and dorsal cortices, whereas the MGV input arises from the central nucleus. Thus it may be that the makeup of the excitatory inputs from cortical areas of the IC to paralaminar nuclei differ somewhat from that of the IC central nucleus input to the MGV in that they are more likely to generate smaller excitatory events.

Very little is known about the excitatory tectothalamic output of the SC to any area of the thalamus. Tectothalamic projections to the bush baby LGN are relatively small and synapse on small dendrites (Feig and Harting 1994). In contrast, tectothalamic terminals in the cat lateral posterior nucleus are large and synapse on large proximal dendrites of thalamocortical neurons (Kelly et al. 2003). Even less is known about SC projections to paralaminar nuclei. Linke (1999) has shown that the SG receives SC inputs primarily from superficial layers, whereas other paralaminar nuclei get more of their input from deeper layers. These SC terminals can end on the dendrites of paralaminar cells projecting to the amygdala (Linke et
Local activation of paralaminar neurons

Activation of the cortical or collicular inputs was often able to elicit distinct EPSPs with longer latencies in paralaminar cells that appeared to be arising by axon collaterals of other paralaminar cells (Fig. 6). Cortical and collicular inputs to MGV and MGD cells never activate such events, which correlates with the fact that MGV and MGD cells never show local axon collaterals (Bartlett and Smith 1999) and that paralaminar cell collaterals typically remain confined to paralaminar nuclei (Smith et al. 2006). There are no data available to suggest why paralaminar cells might need such local, feedforward excitation whereas MGV and MGD cells do not. However, as Fig. 5D (right) illustrates, when only excitatory inputs are activated this collateral input can provide a strong postonset excitatory drive to paralaminar cells. Paralaminar neurons can project to cortex, amygdala, and striatum and are thought to be involved in several important functions such as fear conditioning (e.g., LeDoux 2000), the modulation of the cortical high-frequency gamma oscillation (Barth and McDonald 1996; Sukov and Barth 2001), and cortical information binding (see Jones 1998). With local collaterals, activation of a small number of paralaminar cells could potentially generate or enhance firing in a much larger number of cells by local excitation. Therefore local collaterals might serve as an efficient means to distribute behaviorally relevant information from the IC and SC to a wide array of behaviorally relevant brain circuits.

Comparison of synaptic responses in paralaminar neurons with and without LTS

The lack of low-threshold calcium conductance in some of the paralaminar cells meant that the excitatory cortical and collicular inputs to these cells generated different spike responses when compared with their counterparts that had this conductance. As Fig. 2C (top) illustrates, a cell with the low-threshold Ca conductance receiving an EPSP would generate a short-latency tonic spike when depolarized and a longer-latency burst LTS when hyperpolarized. In contrast, a similar EPSP in a cell with little or no observable Ca conductance (Fig. 2C, bottom) might be capable of generating an onset response when depolarized but is incapable when hyperpolarized. A similar phenomenon was described by Crunelli et al. (1987b) in comparisons of the responses of rat dorsal and ventral LGN (dLGN and vLGN) cells to their optic nerve inputs. Cells in the dLGN have the low-threshold calcium conductance, whereas vLGN cells do not. As a result optic nerve inputs were capable of generating spikes in dLGN cells when they were either depolarized or hyperpolarized, whereas no spikes were generated in vLGN when they were hyperpolarized because there was no Ca conductance to activate. It has been shown in both the primary visual system (see Sherman and Guillery 2001) and the primary auditory system (Massaux et al. 2004) that the responses of thalamocortical neurons to sensory inputs in awake animals contain both tonic and burst modes of firing. This suggests that cells without the burst mode would send a different pattern of spike information to the cortex. However, some paralaminar neurons appear capable of high-frequency firing in the absence of LTS (Fig. 5C).

In addition to alterations in stimulus-evoked firing patterns, the participation of non-LTS paralaminar neurons in thalamocortical rhythms such as delta waves and sleep spindles would also be altered because both thalamocortical rhythms depend on LTS activity to sustain them (e.g., Kim and McCormick 1998; Kim et al. 1997). The lack of LTS and the lack of GABAergic IPSPs effectively uncouples non-LTS paralaminar neurons from thalamocortical rhythms. Such neurons could potentially allow for rapid response to behaviorally relevant, arousing stimuli (e.g., fear pathway, baby crying), even during sleep.

Inhibition

Until recently, GABAergic inputs arising from axons of cells in the thalamic reticular nucleus and/or local thalamic interneurons were thought to be the sole sources of inhibitory restraint on thalamocortical neurons. Recent evidence indicates that this is not the whole picture in the auditory thalamus or in higher-order thalamic nuclei in other sensory thalamic nuclei. Bartho et al. (2002) showed that the zona incerta (ZI) provides a large-terminal GABAergic input to thalamocortical neurons in the posterior thalamic nuclear group (Po). These ZI inhibitory synaptic events can be activated by whisker deflections, could precede EPSPs from the trigeminal complex (Lavallee et al. 2005), and could suppress whisker-evoked excitatory responses (Trangeser and Keller 2004). This indicated that ZI acts as a selective gating mechanism for ascending somatosensory information. Axons from the anterior pretectal (APT) nucleus provide a similar inhibitory input to Po and other higher-order nuclei, which could also act as a sensory gate (Bokor et al. 2005). The large size and proximal dendritic location of both ZI and APT terminals and their activation of only GABA_A receptors had led to speculation that these GABAergic inputs to extralemniscal nuclei perform a function different from that of inputs from the thalamic reticular nucleus.

The IC inhibitory input to paralaminar neurons appears to be unique compared with either the ZI and APT inputs or the feedforward IC inhibitory inputs to MGV/MGD. Our previous data from cells in the MGV (Bartlett and Smith 1999; Peruzzi et al. 1997) have shown that, at least in the auditory system, a nontalamic reticular nucleus (non-TRN) GABAergic input arising from cells in the inferior colliculus impinges on cells in this first-order relay nucleus. The GABAergic IC inputs to MGV/MGD are in the form of small terminals that synapse on distal dendrites and activate both GABA_A and GABA_B receptors (Bartlett and Smith 1999; Bartlett et al. 2000). We now show that the higher-order paralaminar nuclei, including the MGM, adjacent to the auditory thalamus also receive this dendritic GABAergic input from the IC but on these cells it typically activates only GABA_A receptors. Thus the termination sites of IC inhibitory inputs to paralaminar neurons are similar to TRN termination sites, whereas the receptors activated by synaptic stimulation are similar to ZI and APT inputs. Our observation of only GABA_A IPSPs is consistent with the
study of Munoz et al. (1998), who showed a clear decrement in GABA<sub>B</sub>-receptor density in paralaminar auditory nuclei versus MGV and MGD.

We were surprised to find that our anatomical and physiological data also provided evidence that the SC sends an inhibitory input to the paralaminar nuclei as well. This input activates only GABA<sub>A</sub> receptors and our preliminary data indicate that the terminals also end on small dendrites. Most reports of tectal projections to the thalamus have indicated that such projections are excitatory and presumably glutamatergic (Jeon et al. 1997; Kelly et al. 2003; Kobayashi and Nakamura 2003; May 2005; Taylor and Lieberman 1987). Apart from the commissural SC projection, which is partly GABAergic (Apel and Behan 1990; Behan 1985; Oliver et al. 2000), we could find only one study (Dauvergne et al. 2004) indicating that long-range SC efferents could be inhibitory. In that anatomical study some SC cells that were retrogradely labeled by injections in the rat facial motor nucleus or the sensory trigeminal complex were also immunoreactive for glutamic acid decarboxylase (GAD). Thus it would seem that the network of inhibitory influences on the thalamus is complex, especially in higher-order thalamic nuclei. Given the role of the superior colliculus in directing eyes and the head movements the SC inhibition project to paralaminar neurons might act to suppress other sensory signals to maintain gaze.

**Effect of GABA input on cell output**

In slice preparations where both inhibitory and excitatory axons use the same pathway to access a given area, electrical stimulation of that pathway may not represent the sequence of synaptic events that are activated by actual auditory stimuli. For example, auditory frequencies that activate inhibitory inputs may not perfectly overlap with those activating excitatory inputs or excitatory inputs to a given cell might respond to the onset of a stimulus, whereas the inhibitory inputs might have a sustained input to the same stimulus. Thus, despite this, electrical stimulation can demonstrate important features of the excitatory and inhibitory inputs that are relevant to the in vivo situation. In vivo intracellular responses to noise bursts from nonlemniscal MGB cells in anesthetized guinea pigs (Yu et al. 2004) show that both EPSPs and IPSPs can be elicited by the same stimulus and that the IPSPs can occur with short latencies similar to EPSPs. The use of a broadband auditory stimulus in this study does not permit an evaluation of what frequency ranges the inhibitory and excitatory inputs were driven by. It does show that inhibitory inputs, presumably arising from the IC, are activated with a short enough latency that they could provide a gating control of sound-evoked responses elicited by EPSPs. Our results exemplify such gating. Figures 4 and 5 show that the presence of an early inhibitory event can prevent EPSPs from generating spikes (Fig. 4A) or can change the latency of EPSP-evoked spikes (Fig. 4B). Figures 4C and 5 illustrate that artificially removing the IPSP using antagonists can drastically alter the latency and number of spikes generated by a cell to its excitatory input. One interesting possibility is that IC inhibition can veto SC excitation and vice versa to dynamically respond to competing sensory inputs. It is hoped that further in vivo intracellular MGB recordings will be made to determine the extent of receptive field overlap of the excitatory and inhibitory inputs to a given cell and their temporal activation patterns.

**Incorporation of IC and SC excitatory inputs into driver versus modulator framework**

Guillery and Sherman (Guillery 1995; Sherman and Guillery 1998) developed a framework for understanding the flow of information between the thalamus and cortex, especially in sensory pathways. According to this scheme, driver inputs are large-terminal synapses that produce large postsynaptic potentials and strongly influence the postsynaptic cell’s receptive field, whereas modulator inputs are small-terminal synapses that produce small postsynaptic potentials and weakly influence the postsynaptic cell’s receptive field. In the auditory system, large-terminal IC inputs primarily to MGV and occasionally to MGD and layer 5 cortical inputs to MGD and the marginal zone are considered drivers (e.g., Bartlett et al. 2000; Rouiller and Welker 1991), whereas layer 6 cortical inputs to MGV and MGD are considered modulators. Can this scheme of modulator and driver be made to fit the inputs to paralaminar nuclei? Our anatomical and physiological data and the anatomical data of others (Arnault and Roger 1990; Kimura et al. 2004) indicate that the vast majority of the cortical terminals to this area are small and are acting like modulator inputs to cells in this region of the thalamus. What then are the driver inputs to this region? Oddly, our data indicate that two other major paralaminar inputs, those from the SC and IC, do not seem to provide driverlike inputs either. Two possible options could explain this. First, it is possible that there is a separate driver input that we have not accounted for. MGM has been shown to receive some minor auditory inputs from the ventral nucleus of the lateral lemniscus (Kudo 1981; Whitley and Henkel 1984) and the cochlear nucleus (Malmeier et al. 2002). It has also been shown that this area receives somatosensory input from the spinal cord (Gauriau and Bernard 2004; Zhang and Geisler 1995). Thus it is plausible that these inputs could be acting as the drivers for some paralaminar cells. It is also plausible that there is a driver input from cortical layer 5 that we simply did not stimulate. However, this still does not account for the SC and IC inputs. A second alternative is that there are no (or only few) driver inputs to this area and that the receptive fields of many cells in this area are not being determined by a small number of these potent inputs. Instead, the IC and SC inputs, despite having the synaptic characteristics of modulators (Fig. 2), are collectively generating the receptive fields. Rather than a small number of “driver” inputs determining a cell’s receptive field the numerous small IC and SC inputs to paralaminar neurons might be considered a new class of thalamic inputs called “integrators,” defined as small-axon terminals that generate small EPSPs and have little or no paired-pulse depression. What separates integrators from modulators is that the collective activities of integrator inputs are critical for the formation of the thalamic neuron’s receptive field. Similar arguments could potentially be made for neurons in MGV and MGD that receive small-terminal excitatory IC inputs that exhibit weak depression or paired-pulse facilitation (Bartlett and Smith 2002).

In conclusion, neurons in the paralaminar auditory nuclei integrate multisensory excitatory and inhibitory inputs from IC and SC, among other sources, and distribute their outputs to
sensory cortex, association cortex, amygdala, and striatum. The small EPSPs produced by individual integrator inputs suggest that paralaminar neurons do not respond only to a small group of stimulus features, but rather take into account many external and internal factors. Through their local excitatory collaterals, paralaminar neurons can then amplify their responses and influence neural computations in disparate brain regions involved in perception, evaluation, and action.

ACKNOWLEDGMENTS

We thank B. August and R. Massey for help with the electron microscopy.

GRANTS

This research was supported by National Institute on Deafness and Other Communication Disorders Grant R01 DC-006212 to P. Smith.

REFERENCES


Sherman SM, Guillery RW. On the actions that one nerve cell can have on another: distinguishing “drivers” from “modulators.” *Proc Natl Acad Sci USA* 95: 7121–7126, 1998.


