Published in final edited form as:

J Comp Neurol. 2019 May 15; 527(9): 1478–1494. doi:10.1002/cne.24644.

Evaluation of medial division of the medial geniculate (MGM) and posterior intralaminar nucleus (PIN) inputs to the rat auditory cortex, amygdala and striatum

Philip H. Smith, Daniel J. Uhrich, Karen A. Manning

Department of Neuroscience, University of Wisconsin School of Medicine and Public Health, Madison, WI 53705, USA.

Abstract

The medial division of the medial geniculate (MGM) and the posterior intralaminar nucleus (PIN) are association nuclei of the auditory thalamus. We made tracer injections in these nuclei to evaluate/compare their presynaptic terminal and post-synaptic target features in auditory cortex, amygdala and striatum, at the light and electron microscopic levels. Cortical labeling was concentrated in layer 1 but in other layers distribution was location-dependent. In cortical areas designated dorsal, primary and ventral (AuD, Au1, AuV) terminals deep to layer 1 were concentrated in infragranular layers and sparser in the supragranular and middle layers. In ectorhinal cortex (Ect), distributions below layer 1 changed with concentrations in supragranular and middle layers. In temporal association cortex (TeA) terminal distributions below layer 1 was intermediate between AuV/1/D and Ect. In amygdala and striatum, terminal concentrations were higher in striatum but not as dense as in cortical layer 1. Ultrastructurally, presynaptic terminal size was similar in amygdala, striatum or cortex and in all cortical layers. Post-synaptically MGM/PIN terminals everywhere synapsed on spines or small distal dendrites but as a population the post-synaptic structures in cortex were larger than those in the striatum. In addition, primary cortical targets of terminals were larger in primary cortex than in area Ect. Thus, although post-synaptic size may play some role in changes in synaptic influence between areas it appears that terminal size is not a variable used for that purpose. In auditory cortex, cortical subdivision-dependent changes in the terminal distribution between cortical layers may also play a role.

Graphical Abstract

The medial division of the medial geniculate (MGM) and posterior interlaminar nucleus (PIN) of auditory association thalamus innervate auditory cortex, striatum and amygdala. At all locations, axon terminals end primarily on spines and small dendrites. In auditory cortex terminal concentration is highest in layer one. Concentration in other layers depends on the cortical

Corresponding author Philip Smith, Department of Neuroscience, 1300 University Avenue, 141 Bardeen Labs, Madison, WI 53706, Phone: 608-262-0291, FAX: 608-262-7306, Phsmith@wisc.edu.

Authors Contributions: All authors were involved in experimental design and data collection; PHS carried out quantitative analysis; PHS wrote and DJU and KAM edited the manuscript and all authors participated in the figure preparation.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical Approval: All procedures were in accordance with the ethical standards of the University of Wisconsin School of Medicine and Public health and with the US National Institutes of Health Guidelines, and were approved by the University of Wisconsin's Institutional Animal Care and Use Committee.

subdivision. Terminal concentrations were higher in striatum than amygdala. Change in post-synaptic terminal size and distribution suggest shifts in function of this thalamic input across target regions.

Keywords

Electron microscopy; RRID: AB_2336606; RRID: RGD_5508398; RRID: SCR_014199; RRID: SCR_014483; RRID: SCR002815; RRID: SCR_016299; RRID: SCR_001935; RRID_SCR_006369; thalamocortical; thalamoamygdalar; thalamostriatal

Introduction

The medial division of the medial geniculate (MGM) and the posterior intralaminar nucleus (PIN) also known as PIL (Paxinos and Watson, 2007) are situated immediately medial to the ventral division of the medial geniculate (MGV) and ventral to the boundary between MGV and the dorsal division of the medial geniculate (MGD). Although there are disagreements as to their exact borders relative to one another in the rat (see Linke 1999, Linke and Schwegler, 2000; Bordi and LeDoux, 1994a,b; Lanuza et al., 2008; Marquez-Legorreta et al., 2016) both MGM and PIN are classified as association nuclei of the acoustic thalamus (Winer and Morest, 1983; LeDoux et al., 1987; Winer et al., 1988). Regardless of these boundary disagreements, there are several common anatomical and physiological features linking cells in this region.

A minority of MGM/PIN cells are anatomically similar to the tufted and stellate cells common to the two major subdivisions of the auditory thalamus, MGv and MGD. The majority have a much more variable somata size and display elongate dendritic trees that can extend across their nuclear borders (Morest, 1964; Clerici and Coleman, 1990; Clerici et al., 1990; Winer et al., 1988). In addition, axons of MGM/PIN cells can give rise to local collaterals a feature never displayed by MGv and MGD cells. These presumed excitatory collaterals do not venture into MGv or MGD (Smith et al., 2006). Physiologically, in vitro recordings have revealed that, unlike MGv and MGD cells, some MGM/PIN cells can show a reduction or absence of the low threshold voltage sensitive calcium conductance (Smith et al., 2006) which acts to reduce or eliminate the voltage dependent burst response (LTS).

Unlike cells in MGv/MGD, cells in MGM/PIN receive not only auditory inputs but inputs from other sensory modalities as well. Auditory inputs arise not only from the lemniscal pathway running through the central nucleus of the inferior colliculus (ICC) but a large percentage arises from the non-lemniscal auditory pathway primarily from the external and dorsal cortex of the inferior colliculus (IC; LeDoux et al., 1985, 1987; Linke, 1999; Mellott et al., 2014). Synapses from these inputs are both excitatory and inhibitory, terminating primarily on distal dendrites and generating glutamatergic epsps and GABAergic ipsp's respectively (Peruzzi et al., 1997; Smith et al., 2006, 2007). Ryugo and Weinberger (1978) indicated that when the IC was repetitively stimulated electrically, presumably activating this input, cell populations in areas medial to MGv/MGD displayed long lasting potentiated evoked responses while cells in MGv and MGD did not.

Cells here also receive somatosensory inputs from spinal cord and/or trigeminal nucleus. Although no synaptic physiology is available from these inputs, reports indicate that MGM and PIN cells respond in vivo not only to auditory stimuli but to somatosensory stimuli or both (Bordi and LeDoux, 1994a,b). Both areas also receive inputs from the superior colliculus (SC), primarily the SC deep layers (Linke, 1999). These inputs terminate primarily on distal dendrites, can be either glutamatergic or GABAergic and in vitro recordings show they can generate excitatory or inhibitory postsynaptic events (Smith et al., 2007).

The outputs of rat MGM and PIN cells are diverse (see Linke, 1999; Linke and Schwegler, 2000; Linke et al., 2000) but have several common features. Axons of cells from both areas can project to the amygdala and terminate primarily in the lateral nucleus of the amygdala (AL, LeDoux et al., 1990, 1991; Turner and Herkenham, 1991; Linke et al., 2000). At the electron microscopic (EM) level, these terminals end primarily on dendritic spines containing the glutamate receptor subunits for both AMPA and NMDA glutamatergic receptors (Radley et al. 2007). In a review article Weinberger (Weinberger, 2011) indicated that MGM and PIN cells and their projections to amygdala are critical components of auditory fear conditioning pathway and may in fact be the site of memory acquisition and storage for this process. MGM/PIN of rats trained using an auditory fear conditioning paradigm show increased levels of the activated cAMP response element-binding protein (CREB) a transcription factor with a well documented role in neural plasticity/long term memory formation. In addition, exogenous increases of CREB levels exclusively in these areas enhanced the formation of an auditory conditioned fear memory (Han et al., 2008).

MGM/PIN outputs also project to the caudal striatum (CPU) (Veening et al., 1980; Takada et al., 1985; LeDoux et al., 1991). A brief report of the ultrastructure of this MGM/PIN thalamo-striatal projection is available (Moriizumi and Hattori, 1992) as well as results from a more rostrally situated paralamina thalamic nuclei that have been functionally linked to MGM and PIN (Herkenham, 1986; Linke et al., 2000). Most of the CPU terminals from these other paralamina nuclei ended on spines and small dendrites of the GABAergic medium spiny stellate cells that are part of the direct and indirect basal ganglia pathways (Lacey et al., 2007; Ellander et al., 2013; Huerta-Ocampo et al., 2014). Some innervate other interneurons as well (Dube et al., 1988; Lapper and Bolum, 1992; Assous et al., 2017). A recent report (Chavez and Zaborsky, 2017) also indicates that a few MGM/PIN cells may also project directly to cholinergic cells in the basal forebrain that in turn project to auditory cortex.

In auditory cortex both PIN and MGM have been reported to send their primary input to layer 1 and 6 within various cortical subdivisions (Huang and Winer, 2000; Linke and Schwegler, 2000; Niimi and Naito, 1974; Niimi et al., 1984; Ryugo and Killackey, 1974). Some of these cortically projecting MGM/PIN axons apparently branch and additionally contribute to the amygdala projection (Namura et al., 1997). In areas immediately dorsal to the rhinal fissure MGM/PIN also innervate layers 3 and 4 (Linke and Schwegler, 2000). Barth and McDonald (1996) showed that electrical stimulation of the MGM/PIN area generates gamma oscillations in auditory cortex while MGv or MGD stimulation inhibits them. As with the projection of this system to the striatum no evaluation of these terminals has been made in cortical layer 1 or any other layer.

We have labeled MGM/PIN cells and their projections to cerebral cortex, striatum and amygdala. Our goals are 1) to look in more detail at the distribution of these synapses in areas in and around auditory cortex, 2) to evaluate the features of MGM/PIN terminals in cortex, basal ganglion and amygdala looking for similarities or differences in features including their size and what the post-synaptic structure is, 3) to compare features of these terminals with those arising from cells in other areas of the auditory thalamus that we (Smith et al., 2010, 2012) have previously reported.

Methods

All methods were approved by the University of Wisconsin School of Medicine and Public Health Animal Care and Use Committee. Animals were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care-approved facility. Experiments were carried out in accordance with the guidelines published in the National Institute of Health *Guide for the Care and Use of Laboratory Animals*. Adult male Long Evans rats (>300 g, Harlan, RRID: RGD_5508398) were maintained on a 12 hour light/dark cycle in standard University of Wisconsin animal housing with food and water ad libitum. All necessary steps were used to minimize suffering and the number of animals used.

Parcellation of the auditory thalamus:

Several cytoarchitectural schemes have been proposed to the rat medial geniculate and surrounding areas (e.g. Konig and Klippel, 1963; Clerici et al., 1990; Pelligrino et al., 1979;

Paxinos and Watson, 1986; Winer and Larue, 1987) that were unified in a series of papers by LeDoux and colleagues (LeDoux et al. 1985, 1987, 1990). Linke and colleagues (Linke, 1999; Link and Schwegler, 2000; Linke et al., 2000) agreed with this scheme with the exception of the exact positions of MGM relative to PIN. Subsequent additional cytoarchitecture, myeloarchitecture and molecular marker evidence (Marquez-Legorreta et al, 2016) further modified these positions. Figure 1a illustrates these differences. However, all schemes are in agreement that, at most rostrocaudal levels, the suprageniculate nucleus (SG) lies medial to and forms the medial boundary of MGD while the MGM and PIN lies medial to the MGv and the boundary between SG and MGM/PIN occurs at the same dorsoventral location as the MGv/MGD boundary.

Extracellular MGM/PIN injections:

Animals were anesthetized with sodium pentobarbital (35 mg/kg, I.P.) or with ketamine (70–90 mg/kg I.P.) plus xylazine (8–10 mg/kg I.P.), with supplemental doses of 1/3 the initial doses and xylazine supplements administered only one third as often as ketamine supplements, as needed to maintain surgical level anesthesia. The deeply anesthetized rat was prepared for aseptic surgery and placed in the stereotaxic apparatus. Body temperature was maintained at 36°C using a heating blanket with feedback control via a rectal thermometer.

To label thalamocortical, thalamoamygdalar and thalamostriatal terminals, Neurobiotin (Vector Labs, Cat. No. SP1120–20, RRID_AB2336606:) or Biotinylated dextran amine (BDA, Sigma) microinjections were made into the region immediately medial to MGv. Following anesthesia, the dorsal surface of the skull was exposed. A hole was drilled from 5.0 to 6.4 mm caudal to bregma and 2.8 to 3.4 mm lateral to the midline (the stereotaxic coordinates of MGM/PIN). A glass electrode (outer tip diameter of 20–30 µm, filled with 6% Neurobiotin in 0.9% NaCl or 8% biotinylated dextran amine (BDA) was guided to the exposed dorsal surface of the cortex and manually advanced 5 mm into the brain. According to the Paxinos and Watson (2007) brain atlas (RRID: SCR_006369) this would position the electrode a few hundred microns above the MGM/PIN complex. The electrode was then advanced in 5–10 µm steps while wide band noise bursts from a free field speaker positioned 15 cm from the contralateral ear were presented (1 stimulus/second). The electrode was advanced until an evoked auditory response was seen or before a depth of 6.5–7.0 mm was reached. Neurobiotin or BDA was injected iontophoretically (Neurobiotin, 25–30 µA anodal current - 7 seconds on, 7 seconds off; BDA 5–10 µA anodal current - 7 seconds on, 7 seconds off) for either 20 (BDA) or 25 (Neurobiotin) minutes. The electrode was left in place for an additional 20 minutes and then withdrawn.

Post-injection Processing

Section preparation: Tissue preparation has been described in detail elsewhere (Smith et al., 2010) and is summarized here. The electrode was slowly withdrawn and the wound margins were closed and treated with a broad spectrum antibiotic and lidocaine. 24 hours (Neurobiotin injections) or 7 days (BDA injections) later the rat was given a lethal dose of sodium pentobarbital and perfused with 0.9% phosphate buffered saline followed by 1000

ml of a 3% paraformaldehyde/1% glutaraldehyde solution in 0.1M sodium phosphate buffer (pH 7.4).

The cadaver was refrigerated and the brain removed and placed in the same fixative overnight. 70–80 μ m thick sections were then cut with a vibratome and the tracer visualized using the DAB-nickel/cobalt intensification method (Adams, 1981). Sections were rinsed in phosphate buffer and these free floating sections inspected to determine whether the gross injection had successfully labeled axons and axon terminals in auditory cortex, amygdala and basal ganglion. Some of the sections containing the thalamocortical, thalamoamygdalar or thalamostriatal terminals were selected to be processed for electron microscopy (E.M.). Sections not selected for E.M. were mounted on slides and dehydrated. Half of these sections were stained with cresyl violet and coverslipped while the other half were coverslipped unstained.

Those sections selected for E.M. analysis were fixed in 0.5% osmium tetroxide for 30 minutes, rinsed, and dehydrated through a series of graded alcohols and propylene oxide. Sections were placed in unaccelerated Epon-Araldite resin then transferred into a fresh batch of unaccelerated resin overnight. Next, the sections were then embedded and flat mounted in accelerated resin at 65° C. The portion of the embedded sections containing either the auditory cortex, striatum or amygdala was isolated 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/terminals and nearby fiduciary structures. Seventy to eighty nanometer thin sections were then cut and mounted on formvar-coated nickel grids and stained with lead citrate and uranyl acetate.

Parcellation of the auditory cortex:

The terminology and atlas of Paxinos and Watson (2007) was used to identify auditory cortical regions. In our figures that contain photomicrographs or camera lucida representations of sections we first noted the locations of the cortical boundaries from Paxinos and Watson's atlas at that particular rostrocaudal level and inserted our own boundary locations of auditory cortical subdivisions based on changes in the laminar features in our tissue. Our cortical boundary locations were in reasonable agreement with those shown by Paxinos and Watson at each rostrocaudal level. Areas designated Au1, AuV and AuD correspond approximately to the area designated as area 41 by Krieg (1946) and includes the area designated Te1 by Zilles (Zilles et al., 1990). Au1 and AuV receive their primary thalamic inputs from MGv and are characterized cytoarchitectonically in previous descriptions of rat primary auditory cortex (Zilles and Wree, 1985; Roger and Arnault, 1989). Au1 and AuV are distinguished from one another by anatomical and physiological features (Zilles et al., 1990; Romanski and LeDoux, 1993a, b; Polley et al., 2007; Storace et al., 2010a, b).

Regarding cortical layers in rat primary auditory cortical areas, our evaluation of layer boundaries from cresyl violet-stained sections (Fig. 1b) concurs with the general agreement on layer boundaries within area 41 (Au1, AuD, AuV) by other authors (Zilles and Wree, 1985; Vaughan, 1983; Roger and Arnault, 1989; Games and Winer, 1988). In cell-stained tissue the boundary between layers 1 and 2 is well defined at all cortical locations as the

transition between cell sparse and cell dense regions. The bottom of layer 4 is at approximately 50% of cortical (gray matter) depth and the bottom of layer 2 is at the 25% depth. Although layers 3 and 4 thus occupy from 25% to 50% of cortical depth there is disagreement among these authors on the exact size/boundaries of layers 3 and 4. Our approach is as follows. We define layers 3 as occupying half of the 25–50% region i.e. from 25–37.5% of cortical depth and layer 4 the lower ½ from 37.5 to 50% of cortical depth. We have shown that thalamocortical projections from rat MGv and MGD occupy all of layer 4 and the “lower half” of layer 3 (Smith et al., 2012). So, for Au1, AuV and AuD in this paper we will define 1) supragranular layers as those layers above the lower half of layer 3 that ends at 31.25% of the total cortical depth 2) the lower half of layer 3 and layer 4, that occupies 31.25 – 50% of cortical depth as the middle layers and 3) infragranular layers (5 and 6) at 50–100% of cortical depth. There is also disagreement on how much of this lower ½ of the cortex is occupied by layers 5 and 6 respectively. Because ¾ of these reports agree in general we have designated layer 5 as running from 50–71% of cortical depth and layer 6 from 71–100%.

The ventral border of AuV is adjacent to a region where cortex begins to thin considerably (Fig. 1b), the cell density in layer 4 is lower, and the cortical layers are more poorly differentiated. Moving ventrally from the ventral border of AuV one encounters regions designated temporal association area (TeA), entorhinal cortex (Ect) just dorsal to the rhinal fissure, perirhinal fissure (PRh) surrounding the fissure. Despite thinning, our evaluation indicates that the general proportions of cortical thickness assigned to each layer in these areas is similar to those seen more dorsally.

Analysis of axon projections and terminals, light microscopy:

Labeled axons distributed in cerebral cortex were plotted under dark field optics by means of a camera lucida attachment on a Leitz Orthoplan microscope (x 78.75 –200). All labeled axonal processes were incorporated. Bright field optics were used for evaluation of Nissl-stained tissue to determine regional boundaries. Reconstructions were scanned digitally and final figures prepared using Adobe Photoshop.

To evaluate the distribution of labeled terminals in auditory cortex high magnification (1000X) camera lucida drawings of narrow (260 µm) strips of cortex containing labeled terminals were made from the cortical surface to the white matter below layer 6. This was done at multiple locations in auditory cortex to evaluate the differences in terminal distribution through the layers. All en passant and en terminaux swellings (boutons) on labeled axons in these cortical strips were drawn. Camera lucida drawings of labeled terminals were also made of small areas of amygdala and striatum (see Fig. 7) to compare terminal concentrations between areas. As they course through the neuropil labeled axon collaterals of MGM/PIN cells typically maintain a fairly constant diameter that is interrupted by a noticeable increase in diameter after which the diameter rapidly returns to its original level. If the diameter change at one of these points was greater than 3 times the axon's standard diameter as it coursed through the neuropil it was considered a bouton.

Analysis of axon terminals and their postsynaptic targets, electron microscopy:

For analyzing neurobiotin/BDA-labeled axon terminals arising from MGM/PIN the following procedure was established. A given thin section was manually scanned at 10,000X. After identifying a labeled synaptic terminal, based on the presence of vesicles pre-synaptically and a post-synaptic thickening of the membrane apposed to the terminal, a photo of the terminal was taken at 29,000X and its location noted on the camera drawing that had been made of the block face prior to sectioning (see above). Using the 29,000X image measurements of the labeled terminal and its postsynaptic target were made for each synapse using NIH Image/J 1.60 software (RRID: SCR_001935). Other terminal features were also noted.

Statistical tests:

Statistical comparisons of electron microscopic measurements were made on profiles quantified via Minitab software (Minitab, RRID: SCR_014483). For all results statistical analysis was done using either Origin Pro7 (RRID: SCR_002815) or Microsoft Excel software (RRID: SCR_016299). Data was presented as mean $\pm$ S.D. A p value of 0.05 was considered to represent a significant difference.

Digital processing of images:

Digitized light level photomicrographs were acquired in black and white with a Spot camera (Diagnostic Instruments) mounted on a Nikon Eclipse E600 microscope using a 40X oil objective, brightfield illumination, and Nomarski optics. The final figures were prepared using Adobe Photoshop (RRID: SCR_014199), and tonal adjustments were applied across the entire image (levels and curves functions) with no further manipulations.

Results

We made a total of 12 injections that targeted the subdivisions of the medial geniculate nucleus and/or adjacent thalamic nuclei. 3 of these labeled cells primarily confined to areas designated MGM and PIN. The data presented here were collected from these experiments.

Light Microscopy:

Figure 2 illustrates data from one of these 3 injections showing the injection site and labeled cells in thalamus (a) and backfilled cells in the inferior (IC, panel b) and superior (SC, panel c) colliculus resulting from this injection. Sections of the auditory thalamus and surrounding areas are seen at various rostrocaudal locations. Labeled cells are indicated by black dots and the center of the injection site as a larger gray dot seen in the 4th panel. The gray bar illustrates the approximate medial border and the dorsoventral extent of MGv in each section that also corresponds to the dorsoventral extent of MGM and PIN. The large majority of the thalamic cells (88%) labeled fall in the area medial to MGv and ventral to the MGv/MGD border. Although not primarily a retrograde label, Neurobiotin and BDA lightly labeled/backfilled cells in inferior and superior colliculi projecting to the MGM/PIN area of the thalamus as well. Figure 2b illustrates labeled cells in IC regions backfilled from the injection shown in a. As previously described (Linke, 1999) much of the IC projection (88.5%) to this thalamic area arises from non-lemniscal IC i.e. external cortex, that in the rat

has been subdivided into a lateral (LCIC) and rostral (RCIC) areas (Malmierca et al., 2011) and dorsal cortical (DCIC) IC with a minor projection from central nucleus cells (CIC). Fig. 2c illustrates labeled cells in SC projecting here. Again, as previously described (Linke, 1999), MGM and PIN receive a SC projection from cells in deeper SC layers (97%). Similar results were seen in the other cases and these observations help to confirm that the injections were localized to the MGM/PIN thalamic region.

Terminal labeling from MGM/PIN axons was seen in the ipsilateral cerebral cortex, the amygdala and the striatum. In cerebral cortex label was evident throughout the rostrocaudal extent of auditory cortical areas and dorsally and ventrally adjacent cortical areas from 2 to 3 mm caudal to Bregma to the caudal boundaries of auditory areas (around 8.5 mm caudal) and beyond (8.5–9.25 mm caudal) where labeling was confined to ectorhinal (Ect) and perirhinal (PRh) areas. Figure 3 shows camera lucida drawings from dark-field images of sections illustrating the labeling configurations across layers in major auditory cortical areas, from approximately 4.5 mm to 6 mm caudal to Bregma. Although these types of illustrations tend to emphasize axons and not terminals, at every location layer 1 seems to be consistently labeled while in other layers in a given section the label seems to vary as one moves from dorsal to ventral auditory cortical areas. Robust axonal labeling was also observed in portions of posterior parietal cortex (PtPar), lateral secondary visual cortex (V2L), temporal association cortex (TeA), ectorhinal cortex (Ect), and occasionally in perirhinal cortex (PRh). Axons in layer 1 displaying en terminaux and en passant swellings can vary in size considerably and are occasionally quite large (Fig. 4).

To generate a more quantitative measure of the layer distribution of terminals we drew all axonal swellings in 260 μ m wide strips of cortex from the cortical surface to the white matter. Our previous published results showed that these swellings represent synaptic terminals (Smith et al., 2010, 2012). Figure 5 illustrates 5 strips from various dorsoventral locations in a single section (Fig. 5f) immediately adjacent to the section seen in Figure 3d. Each strip is located within an anatomically defined region in temporal cortex. A comparison of the plots (Fig. 5a–e) and percentage of swellings within layers (Fig. 5g) indicate the following: 1) For those areas designated AuD, AuI and AuV (a–c), the distribution of terminals is very similar. Quantification of the terminal distributions verified this, so in the plot shown in Fig. 5g the data from these three areas are combined. As Fig. 5g illustrates, in these 3 areas, layer 1 (L1) and infragranular (Igr) layers contain the bulk of the terminals while the supragranular (Sgr) and middle (Mid) layers are more sparsely innervated. This was a consistent observation across sections (Fig. 3). 2) The terminal distribution changes markedly in Ect (Fig. 5e). Although layer 1 is still the most densely labeled and contains from 20–25% of the swellings (Fig. 5g, Ect) the distribution in other layers changes with a large percentage now found in the supragranular and middle layers and a much reduced percentage in infragranular layers. 3) In the area lying between regions designated AuI/V/D and Ect (Fig. 5d, TeA) layer 1 is again densely labeled but at other depths the terminal distribution is intermediate between the more dorsally situated AuI areas and the more ventrally situated Ect (Fig. 5g).

We were interested to see if these features of terminal distributions were consistent in different rostrocaudal locations. Figure 6 illustrates terminal strips from Te1, TeA and Ect

regions at other rostrocaudal locations. The distributions in these areas are consistent with the data from the single section in Fig. 5. Terminals are consistently concentrated in layer 1. In Te1 the bulk of the remaining terminals are in infragranular layers and in Ect this shifts to supragranular and middle layers. TeA again appears to be a transition zone with a sizable proportion of terminals in supragranular and middle layers and in infragranular layers.

En passant and en terminaux swellings were seen in the caudal striatum and lateral nucleus of the amygdala as well (Figure 7). The gray areas in the upper panel of Figure 7a shows the extent of terminal labeling in one section and the panels in 7a labeled 1, 2 and 3 represent camera lucida drawings of the terminals seen in the small gray squares in the upper left figure in amygdala (1) striatum (2) and cortical layer I (3). Casual inspection as well as counts of swellings in each panel (N at upper right) indicated that there were twice as many in striatum and 3 times more in cortical layer I when compared to amygdala.

Electron Microscopy:

We have selected tissue containing labeled terminal swellings from striatum, amygdala and three auditory cortical regions and evaluated the size of synaptic terminals and the structure they synapsed on. Cortical regions Ect, TeA and combined areas AU1/AuV/AuD cortex were selected for evaluation because of the variations in their layer distributions of thalamic terminals (see Figs. 5 and 6).

Auditory cortex: We evaluated MGM/PIN terminals and their targets in 3 regions of the cortex, Ect, TeA, and combined areas Au1/AuD/AuV. These 3 regions have different layer distributions of terminals so we wanted to see if there were also differences in terminal and post-synaptic size between areas.

Ect: Measurements of 110 MGM/PIN terminals and 111 post-synaptic structures were made. As seen in Fig. 8a (white bars) and Fig. 9 the large majority (97.3%) of the pre-synaptic terminals were small (area $< 1 \mu\text{m}^2$) with a mean terminal area of $0.48 \pm 0.31 \mu\text{m}^2$. Post-synaptically (Fig. 8c) most (109/111) of these terminals synapsed on small structures (area $< 1 \mu\text{m}^2$, mean area = $0.34 \pm 0.22 \mu\text{m}^2$) with 86.1% of those on structures $< 0.5 \mu\text{m}^2$ indicating that spines and small dendrites were the preferred targets.

TeA: Measurements of 109 MGM/PIN terminals and 112 post-synaptic structures were also made in the cortical area designated TeA (Fig. 8a, black bars). As with Ect, TeA terminals were small (105/109 with areas $< 1 \mu\text{m}^2$) with a mean terminal area of $0.44 \pm 0.38 \mu\text{m}^2$. Again, similar to Ect, post-synaptically (Fig. 8c) most (93.8% $< 1 \mu\text{m}^2$, 81.9% of those $< 0.5 \mu\text{m}^2$) of these terminals synapsed on what are likely spines or small dendrites (mean area = $0.419 \pm 0.45 \mu\text{m}^2$).

Au1/AuD/AuV: Finally, 104 MGM/PIN terminals (Fig. 8a, gray bars) as well as 106 post-synaptic structures (Fig. 8c) were measured in areas designated Au1/AuD/AuV. 93.3% of these terminals had areas smaller than $1 \mu\text{m}^2$ (mean area = $0.5 \pm 0.32 \mu\text{m}^2$) and synapsed on small structures (mean area = $0.53 \pm 0.24 \mu\text{m}^2$), 86.8% on structures $< 1 \mu\text{m}^2$, 70.7% of those $< 0.5 \mu\text{m}^2$.

Striatum: A total of 164 labeled MGM/PIN terminals and 171 of their post-synaptic targets in the striatum were evaluated (Figs. 8, 10). As with the cortical terminals the target number is larger because a few terminals synapsed on two structures. Figure 8b (gray bars) illustrates the size distribution of labeled terminals in striatum as well as amygdala (white bars) and the combined populations of all 3 cortical areas (black bars). As seen in the cortex, almost all (93%) of the striatal terminals were small (area $< 1 \mu\text{m}^2$) with a mean terminal area of $0.47 \pm 0.24 \mu\text{m}^2$ (Fig. 8, 10). The large majority of all these terminals (96%) synapsed on structures with areas $< 1 \mu\text{m}^2$ and the majority of those (88%) on structures smaller than $0.5 \mu\text{m}^2$ (mean area $0.32 \pm 0.25 \mu\text{m}^2$) indicating again that the main targets of MGM/PIN terminals were spines or small dendrites (Fig. 10a–f). In a few cases what appeared to be a multivesicular body (Fig. 10e, arrow) or a spine apparatus (Fig. 10f, arrow) were seen. While some terminals were seen in close proximity to cell bodies (not shown) closer inspection revealed that they were not synapsing on somata, rather on smaller neighboring spines/small dendrites.

Amygdala: We also evaluated the MGM/PIN innervation of the amygdala at the E.M. level (Fig. 8, 11). As they approach and enter the lateral amygdale MGM/PIN axons are myelinated and upon entering the amygdala they demyelinate to form synaptic terminals (Fig. 11a–f). A total of 121 terminals and 124 of their post-synaptic targets were evaluated. As in the striatum, target number is larger because a few terminals synapsed on two structures. The large majority (95%) of the terminals were small (area $< 1 \mu\text{m}^2$) with a mean terminal area of $0.43 \pm 0.24 \mu\text{m}^2$ (Fig. 8b, white bars). As in the striatum most (94%) of these terminals synapsed on small post-synaptic structures (area $< 1 \mu\text{m}^2$, 80.3% of those $< 0.5 \mu\text{m}^2$, mean area $0.41 \pm 0.30 \mu\text{m}^2$) dendrites (Fig. 11a–b, d–e). A few synapsed on larger dendrites (Fig. 11f) and in one case a terminal was seen ending on a cell body (Fig. 11c). This terminal and the cell body area is not included in the graph in Figs. 8b and d. Our observations are similar to the MGM/PIN post-synaptic targets of terminal distribution in amygdala reported by Farb and LeDoux (1997) and Radley et al., (2007).

Comparisons

Pre-synaptic: Despite the observations that all MGM/PIN terminals, regardless of terminal location, were small we made statistical comparisons of these terminals between target locations. ANOVA comparisons between the MGM/PIN terminals from striatum, amygdala and the combined populations of all cortical areas showed no significant differences ($F(2, 605) = 0.96$, $P = 0.39$). Likewise ANOVA of the terminals in the 3 cortical areas, Ect, TeA and Au1/AuD/AuV, showed no significant differences ($F(2, 320) = 1.44$, $P = 0.23$). Finally, in TeA we were able to find a large enough number of synaptic terminals in the supragranular, middle and infragranular layers to make statistical comparisons between them and again found no significant difference in terminal size between areas/layers ($F(2, 107) = 2.34$, $P = 0.1$). Thus it appears that terminals made by MGM/PIN axons are similar in size regardless of the termination site in cortex or elsewhere.

Post-synaptic: Again, despite the observation that MGM/PIN terminals synapsed on small structures regardless of terminal location, we made statistical comparisons these targets between target locations. Some statistical differences were noted. ANOVA comparing

areas of post-synaptic structures in amygdala ($0.41\mu\text{m}^2$), striatum ($0.32\mu\text{m}^2$) and cortex ($0.43\mu\text{m}^2$) showed a significant difference ($F(2, 619) = 5.82$, $P = 0.003$) and when T tests were run incorporating Bonferroni correction both amygdalar and cortical post-synaptic structures were significantly larger than those in striatum (amygdala, $P = 0.0075$), cortex, $P = 0.001$). ANOVA comparisons between cortical areas also indicated significant differences. ($F(2, 325) = 7.43$, $P = 0.0007$). When t tests were done utilizing Bonferroni correction for type 1 errors it appears that post-synaptic structures in Au1/AuD/AuV are significantly larger than those in Ect ($P = 0.00016$). Although the average size of TeA post-synaptic structures is also smaller than those in Au1/AuD/AuV (0.42 vs $0.53\mu\text{m}^2$) a t-test with Bonferroni correction indicates that the differences are not significant ($P = 0.054$).

Discussion

We have evaluated the synaptic inputs of MGM/PIN cells to cortical and non-cortical regions. The locations of backfilled cells and inputs from deep SC layers and non-lemniscal IC areas verified that the majority of these labeled cells were in MGM/PIN. Light microscopy of the projections of axons of labeled MGM/PIN cells showed labeling in cerebral cortex, amygdala and striatum with terminal concentrations higher in striatum than amygdala. In cortex, still higher terminal concentrations were seen in layer 1 but terminal distribution in other layers varied depending on cortical location. For cortical areas AuD, Au1 and AuV most terminals were in layer 1 and infragranular layers with the supragranular and middle layers more sparsely innervated. In cortical area Ect, layer 1 was still densely labeled but the distribution in other layers changed with a large percentage in supragranular and middle layers and fewer in infragranular layers. In cortical area TeA, layer 1 is again densely labeled and the terminal distribution in other layers is intermediate between Au1/AuV/AuD and the more ventrally situated Ect. At the E.M. level MGM/PIN terminals were shown to be the same size in all areas of cortex as well as in amygdala and striatum and are also similar regardless of cortical layer. Post-synaptically MGM/PIN terminals in all locations are synapsing primarily on spines or small distal dendrites but there are significant differences in their sizes, larger in the cerebral cortex compared to striatum and larger in primary cortex compared to cortical area Ect.

Terminal and post-synaptic size:

We previously showed (Smith et al., 2012) that, like the thalamocortical terminals of paralamina origin described here, the TC terminals arising from cells in primary auditory thalamus (MGV) are also small and synapse preferentially on spines/small dendrites. Synaptic preference for such small structures would seem to be in conflict with physiological observations that these same inputs generate strong, reliable, temporally precise responses in neurons in layers 3 and 4 (Rose and Metherate, 2005). However, using 2 photo imaging of spines activated by this input, Richardson et al. (2009) demonstrated that the spines excited by MGV inputs were preferentially located close ($< 100\mu\text{m}$) to the cell body and generated large post-synaptic potentials while intracortical synaptic connections are made throughout the dendritic tree of layer 3/4 neurons. These results indicate the importance of synaptic positioning of afferent inputs. Similar experiments have not been done on the auditory paralamina inputs to cortical recipient neurons. However, in rat barrel

cortex Castejon et al. (2016) electrically stimulated posteromedial nucleus (POm), the paralemniscal somatosensory nucleus that projects primarily to layers 1 and 5, and showed that they could drive both supra and infragranular pyramidal cells to spike threshold directly. However, stimulation of this POm input just before whisker stimulation, that activated the lemniscal pathway through VPM thalamic nucleus, could suppress the late responses of cortical neurons to the whisker stimulation. The effect was blocked by inactivation of layer 1 inhibitory neurons indicating that, in the somatosensory system, the paralemniscal input can directly activate pyramidal neurons in various layers but by additionally activating layer 1 inhibitory neurons it can modify the responses of pyramidal cells to their primary input.

Layer distribution of terminals:

We and others (Linke, 1999, Linke and Schwegler, 2000; Linke et al., 2000) have shown that MGM/PIN consistently projects to layer 1 regardless of the auditory cortical subdivision but in deeper layers there is a shift in layer distribution between primary and more ventrally situated non-primary areas. As one moves from primary areas toward the rhinal sulcus into TeA and Ect (Figs. 5, 6) the terminal concentration shifts from infragranular to middle layers. In 1998 Jones (Jones, 1998) proposed the core and matrix model of thalamocortical connections in sensory thalamic nuclei including the medial geniculate nucleus. Matrix neurons were classified as widely- and superficially-projecting calbindin-positive cells that received primarily non-lemniscal input and were spread throughout the thalamus but concentrated in nonlemniscal thalamic nuclei. “Superimposed” upon the matrix are the core-neurons, distinguished by their parvalbumin immunoreactivity, innervation by ascending lemniscal inputs and highly ordered projection to middle layers of the cortex in an area-specific manner. In monkeys (Jones, 1998, 2001) the MGM/PIN area is populated primarily with matrix neurons but these were interspersed with “islands” of parvalbumin positive core neurons. Data from rodents indicates a mix of core and matrix in MGM/PIN (Rubio-Garrido et al., 2007; Cruikshank et al., 2001) but the relative concentrations are not known. The question then becomes, are the MGM/PIN matrix cells providing the layer 1 inputs to all auditory cortical regions and the infragranular layer inputs to primary cortical areas while the minority of MGM/PIN core cells provide the enhanced granular layer inputs to the TeA/Ect cortical regions? Single fiber labeling will be required to answer this question.

A related question is whether there is a physiological difference between the core and matrix cells in the MGM/PIN cells giving rise to these cortical inputs. Our previous data from slices (Smith et al., 2006) has shown that MGM/PIN cells that are likely to be matrix cells can have an unusual combination of anatomical and physiological features when compared to the “standard” “core” thalamic neurons seen in the laminar portion of auditory thalamus (MGV/MGD) and infrequently in MGM/PIN. Many MGM/PIN cells distinguished by long sparingly branching sometimes spiny dendrites, physiologically showed a reduction/absence of the low threshold, voltage-sensitive calcium conductance. In core cells this voltage sensitive conductance causes the burst response. Thalamic burst firing has been shown to be more reliable than tonic firing for generating spikes in the cortex (Swadlow and Gusev, 2001). These more tonically firing MGM/PIN neurons could thus have a distinctly different effect on cortical (or striatal or amygdalar) neurons. In the lateral geniculate nucleus, relay

cells firing in tonic mode show better linear summation, but have poorer detectability for visual stimuli (Sherman, 2001).

Finally it is interesting that the MGM/PIN inputs to areas TeA and Ect go to both layer 1, where they are thought to perform a modulatory function, and to granular layers that are where inputs are usually thought to act as driving input. These areas immediately above the rhinal fissure form the major auditory cortical projection to the amygdala (Shi and Cassell 1997). It has been shown that lesions of these areas ventral to primary cortical areas does not abolish recent fear memories or the new acquisition of fear memories but dramatically attenuated remote (months after training) memory recall of fear associated stimuli. This would indicate that these areas may act as a substrate for remote associative memory formation and storage. In addition the genes required for synaptic plasticity that are used as an index of neuronal activation are recruited by recent and/or remote fear memories in the granular layers. All of this indicates that the granular layer inputs to TeA/Ect provided by MGM/PIN might be involved in this long term memory formation.

Striatal input:

The striatum is a major component in a series of loops running from the cortex to the striatum and back to the cortex via the thalamus. GABAergic medium spiny stellate neurons (MSNs) in the striatum are the main recipients of the cortical excitatory afferents to this area. These MSNs initiate “direct” and “indirect” pathways through the striatum. The direct pathway MSNs directly innervate the output nuclei of the basal ganglia (internal segment of the globus pallidus and substantia nigra pars reticulata), while indirect pathway MSNs form an indirect path to the output nuclei that includes the external segment of the globus pallidus and the subthalamic nucleus. In addition to their cortical input MSNs also receive inputs from the intralaminar nuclei of the thalamus and are thought to provide information on sensory events of behavioral significance and are likely to be important for arousal, attention, orienting, and action selection (Kimura et al., 2004; McHaffie et al., 2005; Rodriguez-Sabate et al., 2015). Several labs have demonstrated that these intralaminar inputs provide excitatory vGLUT2 positive contacts to MSNs belonging to both the direct and indirect pathway MSNs, preferentially synapsing on spines and small dendrites (Lacey et al., 2007; Lei et al., 2013; Doig et al., 2010; Huerta-Ocampo et al., 2014). Optic activation of these inputs (Ellander et al., 2013) generated small amplitude responses. In addition these synapses showed short-term depression and predominantly expressed postsynaptic NMDA receptors, suggesting a modulatory role for this input. Our data showing most terminal endings primarily on small dendrites and spines is consistent with these results from other intralaminar nuclei. We also have shown that the structures post-synaptic to MGM/PIN terminals in striatum are smaller than at terminal sites in cortex and amygdala. This could be for a variety of reasons including 1) the striatal terminals more frequently synapse on spines rather than dendrites 2) the terminals are synapsing further out on the dendritic tree of striatal neurons. Unfortunately, it is not always possible to identify where a post-synaptic structure is a spine or a dendrite and comparisons of the sizes of distal dendrites in these 3 areas are not available.

Amygdalar inputs:

The amygdala is a key brain structure involved in processing emotions, motivation and positive and negative stimuli. (Namburi et al., 2015; Kim et al., 2016, 2017; Beyeler et al., 2018). One of the most heavily studied components of the amygdala's repertoire is the conditioned fear response where an auditory stimulus paired with a negative, typically foot-shock stimulus teaches the animal to fear the auditory stimulus. Amygdalar cells are a critical convergence point in the fear conditioning circuitry (LeDoux, 2000; Maren, 2001) and the auditory information reaches the lateral nucleus of the amygdala (LA) from both the MGM/PIN thalamic nuclei (LeDoux et al., 1984, 1990a,b; Turner and Herkenham, 1991; Doron and LeDoux, 2000; Linke et al., 2000) and the non-lemniscal cortical areas we have studied here (Mascagni et al., 1993; Romanski and LeDoux, 1993a; Shi and Cassell, 1997; McDonald, 1998). We and others have shown that the MGM/PIN axons form synapses that are almost exclusively on small dendrites or spines of amygdalar cells. The majority of these synapses are on those structures belonging to excitatory amygdalar cells but some also act on GABA-labeled small dendrites as well (Woodson et al., 2000). The action of these thalamic inputs onto GABAergic cell dendrites may be critical in the process of fear learning (Wolff et al., 2014). During the auditory cue in a fear conditioning paradigm parvalbumin-expressing GABAergic interneurons are activated and shut off somatostatin-expressing interneurons whose inhibition is situated on distal dendrites of excitatory amygdalar neurons. This dendritic disinhibition enhances the effects of the excitatory thalamic inputs to these same distal dendrites thus providing favorable conditions for fear learning. The foot-shock that follows inhibits both interneuron subtypes strongly increasing excitatory cell activity, which is required for fear learning. These thalamic inputs also seem to be intimately involved in fear generalization, a failure to discriminate dangerous from safe stimuli, commonly seen in anxiety disorders such as PTSD (Ghosh and Chattarji, 2015). In vivo recordings from excitatory amygdalar cells after fear conditioning using a weak shock paired with a tone showed a high percentage of neurons that exhibit a selective increase in firing to the tone paired with the shock but not to an unpaired tone and a low percentage that responded just as well to both stimuli. Doubling the shock strength significantly increased the percentage of the units responding to both auditory stimuli, shifting the balance toward a greater proportion of generalizing over cue-specific cells. These changes that translate to an elevated freezing response to both the paired and unpaired auditory stimulus appears to be dependent on protein synthesis-dependent plasticity in medial geniculate and subsequent changes in the expressions of AMPA receptor and synaptic scaffolding proteins at their amygdala synapses (Ferrara et al., 2017). Clearly, a thorough understanding of the cells giving rise to these thalamoamygdalar projection from the MGM/PIN is vital to our understanding and treating such debilitating conditions.

Acknowledgements:

Grant support: NIH NIDCD R01 grant # DC012782 to P.H. Smith, and funding from the Wisconsin Alumni Research Foundation (University of Wisconsin, Madison, USA; PRJ-63SU) and the University of Wisconsin Foundation (University of Wisconsin, Madison, USA; AAC4523) to D.J. Uhlrich. We are most grateful to Anna Kowalkowski, Anna Thiessen and Jay Campbell for the excellent technical support.

References

- Adams JC (1981). Heavy metal intensification of DAB-based HRP reaction product. *Journal of Histochemistry and Cytochemistry* 29(6), 775. [PubMed: 7252134]
- Allon N, & Yeshurun Y (1985). Functional organization of the medial geniculate body's subdivisions of the awake squirrel monkey. *Brain Research*, 360(1–2), 75–82. [PubMed: 4075184]
- Assous M, Kaminer J, Shah F, Garg A, Koós T, & Tepper JM (2017). Differential processing of thalamic information via distinct striatal interneuron circuits. *Nature Communications*. 8:1–14. doi: 10.1038/ncomms15860.
- Bartlett EL, & Smith PH (1999). Anatomic, intrinsic, and synaptic properties of dorsal and ventral division neurons in rat medial geniculate body. *Journal of Neurophysiology*, 81(5), 1999–2016. [PubMed: 10322042]
- Bartlett EL, Stark JM, Guillery RW, & Smith PH (2000). Comparison of the fine structure of cortical and collicular terminals in the rat medial geniculate body. *Neuroscience*, 100(4), 811–828. [PubMed: 11036215]
- Barth DS, & MacDonald KD (1996). Thalamic modulation of high-frequency oscillating potentials in auditory cortex. *Nature*, 383(6595), 78–81. [PubMed: 8779725]
- Beyeler AC, Chang CJ, Silvestre M, Lévêque C, Namburi P, Wildes CP, & Tye KM (2018). Organization of valence-encoding and projection-defined neurons in the basolateral amygdala. *Cell Reports*, 22(4), 905–918. 10.1016/j.celrep.2017.12.097 [PubMed: 29386133]
- Bordi F, & LeDoux JE (1994). Response properties of single units in areas of rat auditory thalamus that project to the amygdala. I. Acoustic discharge patterns and frequency receptive fields. *Experimental Brain Research*, 98(2), 362–274.
- Bordi F, & LeDoux JE. (1994). Response properties of single units in areas of rat auditory thalamus that project to the amygdala. II Cells receiving convergent auditory and somatosensory inputs and cells antidromically activated by amygdala stimulation. *Experimental Brain Research*, 98(2), 275–286.
- Brett B, Di S, Watkins L, & Barth DS (1994). A horseradish peroxidase study of parallel thalamocortical projections responsible for the generation of mid-latency auditory-evoked potentials. *Brain Research*, 647(1), 65–95. [PubMed: 8069706]
- Castejon C, Barros-Zulaica N, & Nuñez A (2016). Control of Somatosensory Cortical Processing by Thalamic Posterior Medial Nucleus: A New Role of Thalamus in Cortical Function. *PLoS One*. 11(1). 1–33. doi: 10.1371/journal.pone.0148169. eCollection 2016.
- Chavez C, & Zaborszky L (2017). Basal forebrain cholinergic-auditory cortical network: Primary versus nonprimary auditory cortical areas. *Cerebral Cortex*, 27(3), 2335–2347. doi: 10.1093/cercor/bhw091 [PubMed: 27073229]
- Clerici WJ & Coleman JR (1990). Anatomy of the rat medial geniculate body: I. Cytoarchitecture, myeloarchitecture, and neocortical connectivity. *Journal of Comparative Neurology*, 297(1), 14–31. [PubMed: 2376630]
- Clerici WJ, McDonald AJ, Thompson R, & Coleman JR (1990). Anatomy of the rat medial geniculate body: II. Dendritic morphology. *Journal of Comparative Neurology*, 297(1), 32–54. [PubMed: 2376632]
- Cruikshank SJ, Killackey HP, & Metherate R (2001). Parvalbumin and calbindin are differentially distributed within primary and secondary subregions of the mouse auditory forebrain. *Neuroscience*. 105(3), 553–69. [PubMed: 11516823]
- Cruikshank SJ, Rose HJ, & Metherate R (2002). Auditory thalamocortical synaptic transmission in vitro. *Journal of Neurophysiology*, 87(1), 361–384. [PubMed: 11784756]
- Di S, & Barth DS (1992). The functional anatomy of middle-latency auditory evoked potentials: Thalamocortical connections. *Journal of Neurophysiology*, 68(2), 425–431. [PubMed: 1382119]
- Doig NM, Moss J & Bolam JP (2010). Cortical and thalamic innervation of direct and indirect pathway medium-sized spiny neurons in mouse striatum. *Journal of Neuroscience*, 30(44), 14610–14618. doi: 10.1523/JNEUROSCI.1623-10.2010 [PubMed: 21048118]

- Donishi D, Kimura A, Okamoto K, & Tamai Y (2006). “Ventral” area in the rat auditory cortex: A major auditory field connected with the dorsal division of the medial geniculate body. *Neuroscience*, 141(3):1553–1567. [PubMed: 16750887]
- Doron NN, & LeDoux JE (1999). Organization of projections to the lateral amygdala from auditory and visual areas of the thalamus. *Journal of Comparative Neurology*, 417(3), 385–386.
- Doron NN, & LeDoux JE (2000). Cells in the posterior thalamus project to both amygdala and temporal cortex: a quantitative retrograde double-labeling study in the rat. *Journal of Comparative Neurology*, 425(2), 257–274. [PubMed: 10954844]
- Doron NN, LeDoux JE, & Semple MN (2002). Redefining the tonotopic core of rat auditory cortex; physiological evidence for a posterior field. *Journal of Comparative Neurology*, 453(4), 345–360. [PubMed: 12389207]
- Dube L, Smith AD, & Bolam JP (1988). Identification of synaptic terminals of thalamic or cortical origin in contact with distinct medium-sized spiny neurons in the rat neostriatum. *Journal of Comparative Neurology*, 267(4), 455–471. [PubMed: 3346370]
- Ellender TJ, Harwood J, Kosillo P, Capogna M, & Bolam JP (2013). Heterogeneous properties of central lateral and parafascicular thalamic synapses in the striatum. *Journal of Physiology*. 591(1), 257–72. doi: 10.1113/jphysiol.2012.245233. Epub 2012 Oct 29. [PubMed: 23109111]
- Farb CR, & LeDoux JE (1997). NMDA and AMPA receptors in the lateral nucleus of the amygdala are postsynaptic to auditory thalamic afferents. *Synapse*, 27(2), 106–121. [PubMed: 9266772]
- Ferrara NC, Cullen PK, Pullins SP, Rotondo EK & Helmstetter FJ (2017). Input from the medial geniculate nucleus modulates amygdala encoding of fear memory discrimination. *Learning and Memory*, 24(9), 414–421. doi: 10.1101/lm.044131.116. Print 2017 Sep. [PubMed: 28814467]
- Games KD, & Winer JA (1988). Layer V in rat auditory cortex: projections to the inferior colliculus and contralateral cortex. *Hearing Research*, 34(1), 1–25. [PubMed: 3403382]
- Ghosh S, & Chattarji S (2015) Neuronal encoding of the switch from specific to generalized fear. *Nature Neuroscience*, 18(1), 112–130. doi: 10.1038/nn.3888. Epub 2014 Dec 1. [PubMed: 25436666]
- Han JH, Yiu AP, Cole CJ, Hsiang HL, Neve RL & Josselyn SA (2008). Increasing CREB in the auditory thalamus enhances memory and generalization of auditory conditioned fear. *Learning and Memory*, 15(6), 443–453. doi: 10.1101/lm.993608. Print 2008 Jun. [PubMed: 18519545]
- Herkenham M (1986). New perspectives on the organization and evolution of nonspecific thalamocortical projections In: Jones EG, Peters A (Eds) *Cerebral cortex*, vol 5. Sensory-motor areas and aspects of cortical connectivity. (pp 403–445). Plenum Press, New York.
- Huang CL, & Winer JA (2000). Auditory thalamocortical projections in the cat: laminar and areal patterns of input. *Journal of Comparative Neurology*, 427(2), 302–331. [PubMed: 11054695]
- Huerta-Ocampo I, Mena-Segovia J, & Bolam JP (2014). Convergence of cortical and thalamic input to direct and indirect pathway medium spiny neurons in the striatum. *Brain Structure and Function*, 219(5), 1787–1800. doi: 10.1007/s00429-013-0601-z. Epub 2013 Jul 6. [PubMed: 23832596]
- Jones EG (1998). Viewpoint: The core and matrix of thalamic organization. *Neuroscience*, 85(2), 331–45. [PubMed: 9622234]
- Jones EG (2001). The thalamic matrix and thalamocortical synchrony. *Trends in Neuroscience*, 24(10), 595–601.
- Kharazia VN, & Weinberg RJ (1994). Glutamate in thalamic fibers terminating in layer IV of primary sensory cortex. *Journal of Neuroscience*, 14(10), 6021–6032. [PubMed: 7931559]
- Kim J, Pignatelli M, Xu S, Itohara S, & Tonegawa S (2016). Antagonistic negative and positive neurons of the basolateral amygdala. *Nature Neuroscience*, 19(12), 1636–1646. [PubMed: 27749826]
- Kim J, Zhang X, Muralidhar S, LeBlanc SA & Tonegawa S (2016). Basolateral to central amygdala neural circuits for appetitive behaviors. *Neuron* 93, 1464–1479 (2017). doi: 10.1038/nn.4414. Epub 2016 Oct 17.
- Kimura A, Donishi T, Sakoda T, Hazama M, & Tamai Y (2003). Auditory thalamic nuclei projections to the temporal cortex in the rat. *Neuroscience*, 117(4), 1003–1016. [PubMed: 12654352]

- Kimura A, Donishi T, Okamoto K, & Tamai Y (2004). Efferent connections of “posterodorsal” auditory area in the rat cortex: implications for auditory spatial processing. *Neuroscience*, 128(2), 399–419. [PubMed: 15350651]
- Kimura A, Donishi T, Okamoto K, Imbe H, & Tamai Y (2007). Efferent connections of the ventral auditory area in the cortex: Implications for auditory processing related to emotion. *European Journal of Neuroscience*, 25(9), 2819–2834. [PubMed: 17459110]
- Kimura A, Imbe H, & Donishi T (2010). Efferent connections of an auditory area in the caudal insular cortex of the rat: anatomical nodes for cortical streams of auditory processing and cross-modal sensory interactions. *Neuroscience*, 166(4), 1140–1157. doi: 10.1016/j.neuroscience.2010.01.032. Epub 2010 Jan 25. [PubMed: 20105453]
- Kim J, Pignatelli M, Xu S, Itohara S & Tonegawa S (2016). Antagonistic negative and positive neurons of the basolateral amygdala. *Nature Neuroscience*, 19(12), 1636–1646. doi: 10.1038/nn.4414. Epub 2016 Oct 17 [PubMed: 27749826]
- Kim J, Zhang X, Muralidhar S, LeBlanc SA, & Tonegawa S (2017). Basolateral to central amygdala neural circuits for appetitive behaviors. *Neuron*, 93(6), 1464–1479. doi: 10.1016/j.neuron.2017.02.034. [PubMed: 28334609]
- Konig JFR, & Klippel A (1963). *The Rat Brain. A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem*. Baltimore: The Williams and Wilkins Company.
- Krieg WJ (1946). Connections of the cerebral cortex. I. The albino rat. A. Topography of the cortical areas. *Journal of Comparative Neurology*, 84, 221–275. [PubMed: 20982805]
- Lacey CJ, Bolam JP, & Magill PJ (2007). Novel and distinct operational principles of intralaminar thalamic neurons and their striatal projections. *Journal of Neuroscience*, 27(16), 4374–4384. [PubMed: 17442822]
- Lanuza E, Moncho-Bogani J, & Ledoux JE (2008). Unconditioned stimulus pathways to the amygdala: effects of lesions of the posterior intralaminar thalamus on foot-shock-induced c-Fos expression in the subdivisions of the lateral amygdala. *Neuroscience*, 155(3), :959–968. doi: 10.1016/j.neuroscience.2008.06.028. Epub 2008 Jun 19. [PubMed: 18620025]
- Lapper SR, Bolam JP (1992). Input from frontal cortex and the parafascicular nucleus to cholinergic interneurons in the dorsal striatum of the rat. *Neuroscience*, 51(3), 533–545. [PubMed: 1488113]
- Ledoux JE (2000) Emotion circuits in the brain. *Annual Review of Neuroscience*, 23, 155–184.
- Ledoux JE, Farb CR, & Romanski LM (1991). Overlapping projections to the amygdala and striatum form auditory processing areas of the thalamus and cortex. *Neuroscience Letters*, 134(1), 139–144. [PubMed: 1815147]
- Ledoux JE, Farb C, & Ruggiero DA (1990). Topographic organization of neurons in the acoustic thalamus that project to the amygdala. *Journal of Neuroscience*, 10(4), 1043–1054. [PubMed: 2158523]
- Ledoux JE, Ruggiero DA, Forest R, Stornetta R, & Reis DJ (1987). Topographic organization of convergent projections to the thalamus from the inferior colliculus and spinal cord in the rat. *Journal of Comparative Neurology*, 264(1), 123–146. [PubMed: 2445791]
- Ledoux JE, Ruggiero DA, & Reis DJ (1985). Projections to the subcortical forebrain from anatomically defined regions of the medial geniculate body in the rat. *Journal of Comparative Neurology*, 242(2), 182–213. [PubMed: 4086664]
- Ledoux JE, Sakaguchi A, & Reis DJ (1984) Subcortical efferent projections of the medial geniculate nucleus mediate emotional responses conditioned to acoustic stimuli. *Journal of Neuroscience*, 4(3), 683–698. [PubMed: 6707732]
- Lee CC & Sherman SM (2008). Synaptic properties of thalamic and intracortical inputs to layer 4 of the first- and higher-order cortical areas in the auditory and somatosensory systems. *Journal of Neurophysiology*, 100(1), 317–326. [PubMed: 18436628]
- Lei W, Deng Y, Liu B, Mu S, Guley NM, Wong T, & Reiner A (2013). Confocal laser scanning microscopy and ultrastructural study of VGLUT2 thalamic input to striatal projection neurons in rats. *Journal of Comparative Neurology*, 521(6), 1354–1377. doi: 10.1002/cne.23235. [PubMed: 23047588]

- Linke R (1999). Differential projection patterns of superior and inferior collicular neurons onto posterior paralamina nuclei of the thalamus surrounding the medial geniculate body in the rat. *European Journal of Neuroscience*, 11(1), 187–203. [PubMed: 9987023]
- Linke R, & Schwegler H (2000). Convergent and complimentary projections of the caudal paralamina nuclei to the rat temporal and insular cortex. *Cerebral Cortex*, 10(8), 753–771. [PubMed: 10920048]
- Linke R, Braune G, & Schwegler H (2000). Differential projection of the posterior paralamina nuclei to the amygdaloid complex in the rat. *Experimental Brain Research*, 134(4), 520–532. [PubMed: 11081834]
- Malmierca MS, Blackstad TW & Osen KK (2011) Computer-assisted 3-D reconstruction of Golgi-impregnated neurons in the cortical regions of the inferior colliculus of rat. *Hearing Research*, 274(1–2), 13–26. doi: 10.1016/j.heares.2010.06.011. [PubMed: 20600744]
- Maren S (2001) Neurobiology of Pavlovian fear conditioning. *Annual Review of Neuroscience*, 24, 897–931.
- Mascagni F, McDonald AJ, & Coleman JR (1993) Corticoamygdaloid and corticocortical projections of the rat temporal cortex: a Phaseolus vulgaris leucoagglutinin study. *Neuroscience*, 57(3), 697–715. [PubMed: 8309532]
- McDonald AJ (1998). Cortical pathways to the mammalian amygdala. *Progress in Neurobiology*, 55(3), 257–332. [PubMed: 9643556]
- McHaffie JG, Stanford TR, Stein BE, Coizet V, & Redgrave P (2005). Subcortical loops through the basal ganglia. *Trends in Neuroscience*, 28(8), 401–407.
- Mellott JG, Foster NL, Ohl AP & Schofield BR (2014) Excitatory and inhibitory projections in parallel pathways from the inferior colliculus to the auditory thalamus. *Frontiers in Neuroanatomy*, 8(124), 1–11. doi: 10.3389/fnana.2014.00124. eCollection 2014. [PubMed: 24523676]
- Namburi P, Beyeler A, Yorozu S, Calhoon GG, Halbert SA, Wichmann R, Holden SS, Mertens KL, Anahtar M, Felix-Ortiz AC, Wickersham IR, Gray JM, & Tye KM (2015). A circuit mechanism for differentiating positive and negative associations. *Nature*, 520(7549), 675–678. doi: 10.1038/nature14366. [PubMed: 25925480]
- Namura S, Takada M, Kikuchi H, & Mizuno N (1997). Collateral projections of single neurons on the posterior thalamic region to both the temporal cortex and the amygdala: A fluorescent retrograde double-labeling study in rat. *Journal of Comparative Neurology*, 384(1), 59–70. [PubMed: 9214540]
- Niimi K, & Naito F (1974). Cortical projections of the medial geniculate body in the cat. *Experimental Brain Research*, 19(3), 326–342. [PubMed: 4594777]
- Niimi K, Ono K, & Kusunose M (1984). Projections of the medial geniculate nucleus to layer 1 of the auditory cortex in the cat traced with horseradish peroxidase. *Neuroscience Letters*, 45(2), 223–228. [PubMed: 6728316]
- Paxinos G, & Watson C (2007). *The Rat Brain in Stereotaxic Coordinates*. 6th Edition, New York: Academic Press.
- Pellegrino LJ, Pellegrino AS, & Cushman AJ (1979). *A Stereotaxic Atlas of the Rat Brain*. 2nd edition: Plenum, New York.
- Peruzzi D, Bartlett E, Smith PH, & Oliver DI (1997). A monosynaptic GABAergic input from the inferior colliculus to the medial geniculate body in rat. *Journal of Neuroscience*, 17(10), 3766–3777. [PubMed: 9133396]
- Polley DB, Read HL, Storace DA, & Merzenich MM (2007). Multiparametric auditory receptive field organization across five cortical fields in albino rat. *Journal of Neurophysiology*, 97(5), 3621–3638. [PubMed: 17376842]
- Radley JJ, Farb CR, He Y, Janssen WGM, Rodrigues SM, Johnson LR, Hof PR, LeDoux JE, & Morrison JH (2007). Distribution of NMDA and AMPA receptor subunits at thalamo-amygdaloid dendritic spines. *Brain Research*, 1134(1), 87–94. [PubMed: 17207780]
- Richardson RJ, Blundon JA, Bayazitov IT, & Zakharenko SS (2009). Connectivity patterns revealed by mapping of active inputs on dendrites of thalamorecipient neurons in the auditory cortex. *Journal of Neuroscience*, 29(20), 6406–6417. doi: 10.1523/JNEUROSCI.0258-09.2009. [PubMed: 19458212]

- Rodriguez-Sabate C, Llanos C, Morales I, Garcia-Alvarez R, Sabate M, & Rodriguez M (2015). The functional connectivity of intralaminar thalamic nuclei in the human basal ganglia. *Human Brain Mapping*, 36(4), 1335–1347. doi: 10.1002/hbm.22705. Epub 2014 Nov 27. [PubMed: 25429921]
- Roger M, & Arnault P (1989). Anatomical study of the connections of the primary auditory area in the rat. *Journal of Comparative Neurology*, 287(3), 339–356. [PubMed: 2778109]
- Romanski LM, & LeDoux JE (1993a). Organization of rodent auditory cortex: anterograde transport of PHA-L from MGv to temporal cortex. *Cerebral Cortex*, 3(6), 499–514. [PubMed: 7511011]
- Romanski LM, & LeDoux JE (1993b). Information cascade from primary auditory cortex to the amygdala: corticocortical and corticoamygdaloid projections of temporal cortex in the rat. *Cerebral Cortex*, 3(6), 515–532. [PubMed: 7511012]
- Rose HJ, & Metherate R (2005). Auditory thalamocortical transmission is reliable and temporally precise. *Journal of Neurophysiology*, 94(3), 2019–2030. [PubMed: 15928054]
- Rouiller EM, & Welker E (2000). A comparative analysis of the morphology of corticothalamic projections in mammals. *Brain Research Bulletin*, 53(6), 727–741. [PubMed: 11179837]
- Rubio-Garrido P, Perez-de-Manzo F, & Clasca F (2007). Calcium-binding proteins as markers of layer-I projecting vs. deep projecting thalamocortical neurons: A double-labeling analysis in rat. *Neuroscience* 149(1), 242–250. [PubMed: 17850982]
- Ryugo DK, & Killackey HP (1974). Differential telencephalic projections of the medial and ventral divisions of the medial geniculate body of the rat. *Brain Research*, 82(1), 173–177. [PubMed: 4611594]
- Ryugo DK, & Weinberger NM (1976). Corticofugal modulation of the medial geniculate body. *Experimental Neurology*, 51(2), 377–391. [PubMed: 1269567]
- Saint Marie RL, Stanforth DA, & Jubelier EM (1997). Substrate for rapid feedforward inhibition of the auditory forebrain. *Brain Research*, 765(1), 173–176. [PubMed: 9310410]
- Sherman SM (2001). A wake-up call from the thalamus. *Nature Neuroscience*, 4(4), 344–346. [PubMed: 11276218]
- Shi CJ, & Cassell MD (1997). Cortical, thalamic, and amygdaloid projections of rat temporal cortex. *Journal of Comparative Neurology*, 382(2), 153–175. [PubMed: 9183686]
- Smith PH, Bartlett EL, & Kowalkowski A (2006). Unique combination of anatomy and physiology in cells of the rat paralamina thalamic nuclei adjacent to the medial geniculate body. *Journal of Comparative Neurology*, 496(3), 314–334. [PubMed: 16566009]
- Smith PH, Bartlett EL, & Kowalkowski A (2007). Cortical and collicular inputs to cells in the rat paralamina thalamic nuclei adjacent to the medial geniculate body. *Journal of Neurophysiology*, 98(2), 681–695. [PubMed: 17537907]
- Smith PH, Manning KA, & Uhlrich DJ (2010). Evaluation of inputs to rat primary auditory cortex from the supragenulate nucleus and extrastriate visual cortex. *Journal of Comparative Neurology*, 518(18), 3679–3700. doi: 10.1002/cne.22411 [PubMed: 20653029]
- Smith PH, Uhlrich DJ, Manning KA, Banks MI (2012). Thalamocortical projections to rat auditory cortex from the ventral and dorsal divisions of the medial geniculate nucleus. *Journal of Comparative Neurology*, 520(1), 34–51. doi: 10.1002/cne.22682. [PubMed: 21618239]
- Storace DA, Higgins NC and Read HL (2010a). Thalamic label patterns suggest primary and ventral auditory fields are distinct core regions. *Journal of Comparative Neurology*, 518(10), 1630–1646. doi: 10.1002/cne.22345. [PubMed: 20232478]
- Storace DA, Higgins NC, & Read HL (2010b). Thalamocortical pathway specialization for sound frequency resolution. *Journal of Comparative Neurology*, 519(2), 177–193. doi: 10.1002/cne.22501.
- Swadlow HA, & Gusev AG (2001). The impact of “bursting” thalamic impulses at a neocortical synapse. *Nature Neuroscience*, 4(4), 402–408. [PubMed: 11276231]
- Takada M, Itoh K, Yasui Y, Sugimoto T, & Mizuno N (1985). Topographical projections from the posterior thalamic regions to the striatum in the cat, with reference to possible tecto-thalamo-striatal connections. *Experimental Brain Research*, 60(2), 385–396. [PubMed: 4054280]
- Turner BH, & Herkenham M (1991). Thalamoamygdaloid projections in the rat: A test of the amygdala’s role in sensory processing. *Journal of Comparative Neurology*, 313(2), 295–325. [PubMed: 1765584]

- Vaughan DW (1983). Thalamic and callosal connections of the rat auditory cortex. *Brain Research*, 260(2), 181–189. [PubMed: 6831202]
- Veening JG, Cornelissen FM, & Lieven PA. (1980). The topical organization of the afferents to the caudatoputamen of the rat. A horseradish peroxidase study. *Neuroscience*, 5(7), 1253–1268. [PubMed: 7402468]
- Weinberger NM (2011). The medial geniculate, not the amygdala, as the root of auditory fear conditioning. *Hearing Research*, 274(1–2), 61–74. doi: 10.1016/j.heares.2010.03.093. Epub 2010 May 11. [PubMed: 20466051]
- Winer JA, & Larue DT (1987). Patterns of reciprocity in auditory thalamocortical and corticothalamic connections: study with horseradish peroxidase and autoradiographic methods in the rat medial geniculate body. *Journal of Comparative Neurology*, 257(2), 282–315. [PubMed: 3571530]
- Winer JA, & Morest DK (1983). The medial division of the medial geniculate body of the cat: implications for thalamic organization. *Journal of Neuroscience*, 3(12),:2629–2651. [PubMed: 6655503]
- Winer JA, Morest DK, Diamond IT (1988). A cytoarchitectonic atlas of the medial geniculate body of the opossum, *Didelphys virginiana*, with a comment on the posterior intralaminar nuclei of the thalamus. *Journal of Comparative Neurology*, 274(3), 422–448. [PubMed: 2464620]
- Wolff SB, Gründemann J, Tovote P, Krabbe S, Jacobson GA, Müller C, Herry C, Ehrlich I, Friedrich RW, Letzkus JJ, & Lüthi A (2014). Amygdala interneuron subtypes control fear learning through disinhibition. *Nature*, 509(7501), 453–458. doi: 10.1038/nature13258. Epub 2014 May 11. [PubMed: 24814341]
- Woodson W, Farb CR, & Ledoux JE (2000). Afferents from the auditory thalamus synapse on inhibitory interneurons in the lateral nucleus of the amygdala. *Synapse*, 38(2), 124–137. [PubMed: 11018786]
- Zilles K, & Wree A (1985). Cortex: Areal and laminar structure In: Paxino G, editor. *The Rat Nervous System*, Volume 1 (pp 375–415). Sydney: Academic Press.
- Zilles K, Wree A, & Dausch N (1990). Anatomy of the neocortex; neurochemical organization In: Kolb B and Tees RC editors. *The Cerebral Cortex of the Rat*. (pp 113–150). Cambridge, MA MIT Press.

Figure 1.

a. Left. Redrawing of Fig. 1b from Linke (1999). Middle. Redrawing of a portion of Fig. 1 from Bordini and Ledoux (1994). Right. Redrawing of Fig. 1b from Marquez-Legorreta et al., (2016). All 3 show the agreement on the locations of most subnuclei of the auditory thalamus and illustrate the variable interpretation of the location of MGM and PIN. **b.**

Border locations of cortical layers in and around auditory cortex taken from a cresyl violet stained section adjacent to the unstained section illustrated in Fig. 3d. Abbreviations: Au1 = primary auditory cortex, AuD = auditory cortex, dorsal area, AuV = auditory cortex, ventral area, CP = cerebral cortex, D = dorsal, Ect = ectocortical cortex, L = lateral, LP = posterolateral thalamus, MGD = dorsal medial geniculate, MG = ventral medial geniculate, PP = peripeduncular nucleus, PRh = perirhinal cortex, PtPR = posterior parietal cortex, SG = supragenicular nucleus, SN = substantia nigra, S1 = primary somatosensory cortex, TeA = temporal association cortex, V2L = secondary visual cortex, lateral area.

Figure 2.

a. Camera lucida drawings of representative sections through the left MGB at various rostrocaudal locations. Dots illustrate cells labeled by a gross injection whose center is represented by the large dot in the 4th section. The gray vertical line indicates the dorsoventral extent of MG located to the left of the line so the top of the line would represent the dividing line between MG and MGD located above MG. The top of this line should also then indicate the dividing line between MGM/PIN below and to the right of the line and the suprageniculate nucleus just dorsal to MGM/PIN. Numbers indicate the location of the section relative to Bregma. Thus, in each section it is apparent that most of the labeled cells (88%) are below and to the right of the top edge of the line and in either MGM or PIN.

b. Camera lucida drawings of 3 representative sections through the inferior colliculus. Dots represent cells labeled by the gross injection shown in a. Cells in each section represent all labeled cells in the illustrated section as well as the next 3 sections rostral and 3 sections caudal to the illustrated section. Most of the cells (88.5%) were located in external or dorsal cortices and 11.5% of the cells were located in ICC. **c.** Camera lucida drawings of 2 representative sections through the superior colliculus. Dots represent cells labeled by the gross injection shown in a. Cells in each section represent all labeled cells in the illustrated section as well as the next 3 sections rostral and 3 sections caudal to the illustrated section. Most cells were located in intermediate (48%) or deep (49.5%) SC layers. Abbreviations:

Aq = Aqueduct, CIC = Central nucleus, D = dorsal, DCIC = Dorsal cortex, L = lateral, LCIC = Lateral external cortex, RCIC = Rostral external cortex, DpG = Deep gray layer, DpW = Deep white layer, InG = Intermediate gray layer, InW = Intermediate white layer, Op = Optic nerve layer, Zo = Zonal layer.

Figure 3.

Camera lucida drawings of the axonal projection of MGM/PIN to auditory cortical areas at 5 rostrocaudal locations (numbers indicate location in mm re. Bregma). Scale bar applies to all reconstructions. Abbreviations: Au1 = primary auditory cortex, AuD = auditory cortex, dorsal area, AuV = auditory cortex, ventral area, D = dorsal, Ect = ectothalamic cortex, L = lateral, PRh = perirhinal cortex, PtPar = posterior parietal cortex, S1 = primary somatosensory cortex, TeA = temporal association cortex, V2L = secondary visual cortex, lateral area.

Figure 4.

Light micrographs of labeled thalamocortical axons/boutons in layers I and II of auditory cortex in unstained sections. Left side is the cortical surface. **a.** Short pieces of thin axons (thin arrows) with en passant and en terminaux swellings in layer 1 are interspersed with an occasional large diameter axon (thicker arrow). **b.** Thin axons running primarily roughly parallel to the cortical surface in the upper half of layer 1 (white arrows) as well as ascending thin fibers (lower 4 black arrows). Scale bar applies to both micrographs.

Figure 5.

Distribution of MGM/PIN boutons in auditory cortex. **a-e.** Distribution of boutons on labeled axons in 5 strips of various regions in and around auditory cortex from a single section running from the cortical surface to the white matter. Each dot represents the location of a bouton. The larger dark sphere in layer 5 represents the location of a retrogradely labeled cell body. Dotted lines divide the strip into 50 µm parcels. Locations of the layer boundaries are indicated on the left. Plot of the number of boutons in each 50 µm parcel is indicated by the bars on the right. **f.** Camera lucida drawing of a section approximately 5.04 mm posterior to Bregma. Gray bars labeled A-E represent the location of the 5 strips illustrated in panels a-e. **g.** Percentage of terminals in Layer 1, layer 2 and the top half of layer 3 (2/t3, supragranular), layer 4 and the bottom half of layer 3 (b³/4, middle layers), and layers 5 and 6 (5/6, infragranular) for different cortical regions. AuD, Au1 and AuV are all parts of Te1 and are combined. The percentage of terminals in Layer 1 is consistent but as one moves from Te1 regions ventrally to TeA and Ect the number of terminals in supragranular and middle layers increases while those in infragranular regions decreases. Abbreviations: Au1 = primary auditory cortex, AuD = auditory cortex, dorsal area, AuV = auditory cortex, ventral area, D = dorsal, dcw = deep cerebral white matter, DLG = dorsal lateral geniculate nucleus, Ect = ectorhinal cortex, Igr = infragranular layers, L = lateral, LP = lateral posterior thalamic nucleus, MGB = medial geniculate body, Mid = middle layers, PRh = perirhinal cortex, PP = parietal cortex, Sgr = supragranular layers, TeA = temporal association cortex, V2L = secondary visual cortex, lateral area, VG = ventral geniculate nucleus.

Figure 6.

Distribution of MGM/PIN boutons at different rostrocaudal locations. **a-c.** Distribution of boutons on BDA-labeled axons in 3 strips of various regions at 3 rostrocaudal locations running from the cortical surface to the white matter. Each dot represents the location of a bouton. The larger dark sphere in layer 5 represents the location of a retrogradely labeled cell body. Dotted lines divide the strip into 50 μ m parcels. Location of the layer boundaries is indicated on the left. Plot of the number of boutons in each 50 μ m parcel is plotted on the right. **d-f.** Camera lucida drawing of three sections at 3 different rostrocaudal locations. Gray bars labeled A-C represent the strips illustrated in a-c. Similar to the strips illustrated in Fig. 4 taken from a single sections, the distribution of terminals in layer 1 is consistent but as one moves from a Te1 region ventrally to TeA and Ect the number of terminals in supragranular and middle layers increases while those in infragranular regions decreases. Abbreviations: 3V = third ventricle, Aq = aqueduct, Au1 = primary auditory cortex, AuD = auditory cortex, dorsal area, AuV = auditory cortex, ventral area, CA3 = hippocampus, cp = cerebral peduncle, CPu = striatum, D = dorsal, dcw = deep cerebral white matter, ECIC = external cortex of IC, Ect = ectorhinal cortex, Fi = fimbria, ic = internal capsule, L = lateral, LP = lateral posterior thalamic nucleus, MGB = medial geniculate body, Or = oriens layer, SC = superior colliculus, TeA = temporal association cortex, PRh = perirhinal cortex, PP = parietal cortex, V1B = primary visual cortex, V2L = secondary visual cortex, lateral area, VG = ventral geniculate nucleus.

Figure 7.

Innervation density. **a.** Camera lucida drawing (top left) of a section (at -3.24 mm re. Bregma) containing labeled terminals in amygdala (La), striatum (CPu) and auditory cortex. Gray area indicates extent of label in amygdala and striatum. Distribution of axonal swelling in the small dark gray squares labeled 1, 2 and 3 are illustrated in the larger squares with the same labels. N's indicate the number of boutons in each square. The terminal concentration is highest in layer 1 of cortex, slightly lower in striatum and still lower in amygdala. **b.** Light micrograph showing the typical configuration of labeled axons and their boutons in striatum. **c.** Light micrograph showing the typical configuration of labeled axons and their boutons in amygdala. **d.** Electron micrograph of a labeled myelinated MGM/PIN axon (asterisk) as it approaches amygdala. Scale bar in a1 applies to panels a1-3. Scale bar in c applies to b and c. Abbreviations: Au1 = primary auditory cortex, AuV = auditory cortex, ventral area, CPu = striatum (Caudate/putamen), D = dorsal, Ect = Ectorhinal cortex, Fi = Fimbria, ic = internal capsule, L = lateral, La = Lateral amygdaloid nucleus, PRh = Perirhinal cortex, rf = Rhinal fissure.

190x254mm (300 x 300 DPI)

Fig. 8.

Areas of MGM/PIN synaptic terminals and the post-synaptic structures they synapse on. **a.** Percentages of each size category of terminals in 3 regions of auditory cortex, Ect (N = 110) Aud1/D/V (N = 104) and TeA (N = 119). **b.** Percentages of each size category of terminals in amygdala (N = 121), striatum (N = 164) and all cortical areas combined (N = 323). **c.** Percentages of each size category of structures post-synaptic to MGM/PIN terminals in 3 regions of auditory cortex, Ect (N = 111) Aud1/D/V (N = 106) and TeA (N = 112). **d.** Percentages of each size category of structures post-synaptic to MGM/PIN terminals in amygdala (N = 124), striatum (N = 171) and all cortical areas combined (N = 329).

Figure 9.

MGv/PIN input to ECT cortex. **a.** In the infragranular layers labeled axons (asterisk) were typically myelinated. **b,e.** As they ascended into the supragranular layers it was not uncommon to see long stretches of unmyelinated axons forming swellings (circles in **e**, middle circle enlarged in panel **b**) that would generate synaptic specializations (Arrow in **b**). **c-d, f-g.** Examples of synaptic terminals ending on small dendrites/spines (asterisks). It was not uncommon to see more than one specialization (**f-g**, arrows). Scale bar in **a** applies to **a-b, c-d, f-g**.

254x190mm (300 x 300 DPI)

Figure 10.

MGM/PIN terminals in striatum. Electron micrographs of MGM terminals in the striatum synapsing on small (**b-f**) and larger (**a**) profiles (asterisks). A terminal on a spine (asterisk) that contains what might be a multivesicular body (arrow) is seen in **e**. An MGM/PIN terminal on a spine (asterisk) that contains what might be a spine apparatus (arrow) is seen in **f**. Scale bar in **a** applies to **a-d**. Scale bar in **e** applies to **e-f**.

Figure 11. MGM/PIN terminals in amygdala. Electron micrographs of MGM terminals in the amygdala synapsing on small (**a,b,d,e**) and large (**f**) dendrites (asterisks) and a cell body (**c**). Scale bar in **a** applies to **a-b, d-f**.