Convergent and Complementary Projections of the Caudal Paralaminar Thalamic Nuclei to Rat Temporal and Insular Cortex

Thalamic nuclei adjacent to the medial geniculate body play a pivotal role in processing of sensory stimuli during emotional situations. These nuclei, which include the suprageniculate nucleus (SG), the posterior intralaminar nucleus (PIN), the peripeduncular nucleus (PP) and the medial division of the medial geniculate body (MGm), project to both cortex and amygdala, but target areas and the extent of the projection of individual nuclei are not known yet. The aim of the present study was to analyze the contribution of individual nuclei to the cortical projection with modern sensitive tracing techniques. Small injections of MiniRuby or PHA-L were made into single thalamic nuclei. All thalamic nuclei have in common a projection into the upper portion of layer I of the temporal aspect of the cortical mantle. Furthermore, SG, PIN, MGm and PP each demonstrated a convergent projection to lower layer III and to layer IV of the ectorhinal and visceral cortex. Only MGm projects to layer VI of primary auditory and temporal association cortices. Within the perirhinal cortex zones of convergence and divergence exist. The present results demonstrate a differential thalamocortical projection of single thalamic nuclei to those cortical areas which are involved in the transmission of sensory signals to the amygdala via the thalamo-cortico-cortical pathway and to the hippocampus via the entorhinal cortex. The thalamic nuclei are thus in a position to activate the amygdala and to modulate the information flow of the thalamo-cortico-cortical pathway to both amygdala and hippocampus.

Introduction
A pivotal role for the fast acquisition of a fear-potentiated and conditioned response using acoustic stimuli has been ascribed to thalamic nuclei that border the medial geniculate body (MG) medially and ventromedially (LeDoux et al., 1986; Romanski and LeDoux, 1992; LeDoux, 1993). In particular, these nuclei are the suprageniculate nucleus (SG), the medial division of the MG (MGm), the posterior intralaminar nucleus (PIN), and the peripeduncular nucleus (PP). These nuclei have been called associated sensory nuclei (Winer and Morest, 1983) or caudal paralaminar nuclei due to their location adjacent to the internal medullary lamina and their pattern of projection to the cerebral cortex (Herkenham, 1980, 1986) (cf. Materials and Methods). In a number of studies using anatomical and physiological approaches it has been demonstrated in both cat and rat that these nuclei are tied into extrageniculate sensory pathways and receive convergent afferents from several sensory relay nuclei of the midbrain and brainstem such as the superior and inferior colliculi, the dorsal column nuclei and the spinal trigeminal colliculi, the dorsal column nuclei and the spinal trigeminal nucleus. All thalamic nuclei have in common a projection into the upper portion of layer I of the temporal aspect of the cortical mantle. Furthermore, SG, PIN, MGm and PP each demonstrated a convergent projection to lower layer III and to layer IV of the ectorhinal and visceral cortex. Only MGm projects to layer VI of primary auditory and temporal association cortices. These studies showed that the thalamic nuclei, which border the MG proper, project widely onto cortical regions in temporal and parietal cortex, and that the projection patterns of these nuclei significantly differed from that of, for example, the ventral division of the MG. The common feature of the projection of these nuclei is a projection to cortical layer I (Herkenham, 1980, 1986) but additional target layers have been reported. Unfortunately, neither of the aforementioned methods are able to unambiguously differentiate terminal labeling from fibers of passage and — more importantly — lesions or injections using amino acids are often very large, thus confounding the exact location of the injection site (cf. Fig. 5 in Vaughn, 1983). This issue is particularly important in small targets as it is the case with the nuclei adjacent to the MG. Recently, two reports in cat (Kurokawa et al., 1990) and rat (Shinonaga et al., 1994), respectively, using PHA-L re-examined this projection but concentrated onto single thalamic nuclei like the SG or the MGm. Kurokawa and co-workers reported a projection to the medial agranular area of the frontal cortex besides a projection into temporal areas (Kurokawa et al., 1990). Although the injections are small the exact locations within thalamic nuclei are not clear. Shinonaga et al. demonstrated in the cat a projection of MGm to the polar temporal cortex in the posterior sylvian gyrus but no detailed distribution within cortical areas and layers is given (Shinonaga et al., 1994).

In contrast to the relatively detailed knowledge of the thalamocortical projections of the MG proper (Winer, 1992; Romanski and LeDoux, 1993a and citations herein), the thalamocortical projections of the associated (Winer and Morest, 1983) or caudal paralaminar nuclei (Herkenham, 1980, 1986), i.e. SG, MGm, PIN, and PP, are at best insufficiently characterized, i.e. there are mainly studies using retrograde transport methods (Jones and Leavitt, 1974; Deacon et al., 1985; Guldin and Markowitsch, 1985; Arnault and Roger, 1990; Vaudano et al., 1991). In the rat, few reports exist that have used either radioactive amino acids (Herkenham, 1980, 1986) or anterograde degeneration techniques (Ryugo and Killackey, 1974; Patterson, 1977; Vaughn, 1983) to demonstrate terminal labeling within cortical areas. These studies showed that the thalamic nuclei, which border the MG proper, project widely onto cortical regions in temporal and parietal cortex, and that the projection patterns of these nuclei significantly differed from that of, for example, the ventral division of the MG. The common feature of the projection of these nuclei is a projection to cortical layer I (Herkenham, 1980, 1986) but additional target layers have been reported. Unfortunately, neither of the aforementioned methods are able to unambiguously differentiate terminal labeling from fibers of passage and — more importantly — lesions or injections using amino acids are often very large, thus confounding the exact location of the injection site (cf. Fig. 5 in Vaughn, 1983). This issue is particularly important in small targets as it is the case with the nuclei adjacent to the MG. Recently, two reports in cat (Kurokawa et al., 1990) and rat (Shinonaga et al., 1994), respectively, using PHA-L re-examined this projection but concentrated onto single thalamic nuclei like the SG or the MGm. Kurokawa and co-workers reported a projection to the medial agranular area of the frontal cortex besides a projection into temporal areas (Kurokawa et al., 1990). Although the injections are small the exact locations within thalamic nuclei are not clear. Shinonaga et al. demonstrated in the cat a projection of MGm to the polar temporal cortex in the posterior sylvian gyrus but no detailed distribution within cortical areas and layers is given (Shinonaga et al., 1994).

Since these studies have been conducted, the anatomical parcellation of the region around the MG has been refined enormously (LeDoux et al., 1985, 1987, 1990; Winer et al., 1988), in particular in the rat, and electrophysiological as well as hodological studies indicate that individual thalamic paralaminar nuclei might have different functions in the ascending extrageniculate sensory pathway (Hicks et al., 1984; Benedek et al., 1997; Linke, 1999a,b; Linke et al., 1999).

In a preliminary report we demonstrated that the projection of the PIN to temporal neocortex has a distinct pattern within several cortical regions (Linke, 1999a). The aims of the present study were therefore to analyze the contributions of the other thalamic paralaminar nuclei to the cortical projection, to
determine zones of convergence and non-convergence, and to obtain hints how these projections might be involved into thalamo-amygdaloid and thalamo-cortico-amygdaloid circuits.

Materials and Methods

Adult male albino rats of both Wistar (n = 10) and Sprague-Dawley (n = 17) strains (body wt 230–420 g) were analyzed for this study. All experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC) and the German Law on the Protection of Animals. All protocols were approved by the local council of animal care.

Surgery

Animals were deeply anesthetized with a mixture (3ml/kg body wt i.m.) of ketamine (100 mg/ml), xylazine (20 mg/ml) and acepromazine (20 mg/ml) and injected stereotactically with either Phaseolus vulgaris leucoagglutinin (PHA-L, 2.5% in 10 mM phosphate buffer (PB), pH 8.0, Vector Laboratories, Burlingame, CA; n = 8) or Miniruby (biotinylated dextran amine which was additionally labeled with rhodamine (D-3512) Molecular Probes, Eugene, OR; 10% in 10 mM PB, pH 7.5; n = 19) into the posterior thalamic nuclei adjacent to the medial geniculate body (SG, PIN, MGl, PP). Both tracers were iontophoretically injected using a custom-made precision current source. Details for each injection are listed in Table 1. In a brief, a glass micropipette filled with either Miniruby or PHA-L was inserted into the brain at coordinates for SG, PIN, MGl or PP respectively. After placement of the micropipette, a positive current of 5 µA was applied for 6−15 min with a 5 s on/off cycle. Tip diameters of micropipettes ranged from 12 to 30 µm. Coordinates were determined with the help of the stereotaxic atlas of Paxinos and Watson (Paxinos and Watson, 1986). At the end of the injection the needle was left in place for additional 5 min to prevent leakage of the tracer into the needle track. Survival times ranged from 7 to 12 days for the PHA-L-injected animals and from 12 to 24 days for the Miniruby-injected animals.

After the appropriate survival time (cf. Table 1) the animals received a lethal dose of sodium barbital (100 mg/kg, i.p.) and were transcardially perfused with 0.9% saline followed by a fixative containing 4% paraformaldehyde, 15% saturated picric acid and 0.1% glutaraldehyde in 0.1 M PB pH 7.4. In a few cases, the content of glutaraldehyde was raised to 1%. In two other cases a pH-shift fixation protocol was employed. Here, animals were perfused with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M sodium acetate buffer, pH 6.5, and 4% paraformaldehyde in 0.1 M sodium borate buffer, pH 9.3. Immediately before the onset of perfusion, all animals received a bolus injection of 1000 IU heparin–sodium (Liquemin®, Roche) into the left ventricle. Brains were removed from the skull and postfixed in the above fixative but without glutaraldehyde for additional 2 h.

Immunohistochemistry

Coronal vibratome sections (50 µm) or frozen sections (40 µm) of the brains were collected in four parallel series. Prior to cryosectioning, the brains were immersed in 20% sucrose overnight. One series was always stained with cresyl violet for evaluation of cytoarchitecture. Visualization of Miniruby was revealed by incubating one series of sections in ABC-elite solution (1:120, Vector Laboratories) for 2 h at room temperature or overnight at 4°C. Peroxidase histochemistry was carried out with heavy metal intensified DAB (Adams, 1981). Phaseolus vulgaris leucoagglutinin was demonstrated by incubating the sections in biotinylated goat anti-PHA-L (1:800 in a solution of 1% normal horse serum, 0.3% Triton X-100 in 0.1 M PB, pH 7.4, Vector Laboratories) either overnight at room temperature or at 4°C for 2 days. Then, sections were incubated in ABC-elite solution (1:120) for 2h and peroxidase histochemistry was carried out as described above. All sections were mounted on gelatin-coated slides, air dried, cleared in xylene and coverslipped.

Data Analysis

Each case was checked for the size and location of the injection and the labeling pattern in the amygdala and cortex. Each injection site was verified on an adjacent cresyl violet-stained section using blood vessels as landmarks. In all cases, the exact location of the needle tip could be located and assigned to a single thalamic nucleus.

Beginning with the caudal pole of the brain, the distribution of anterogradely labeled fibers and retrogradely labeled neurons in the cortex after injections into the thalamus was mapped with darkfield and brightfield illumination by means of a camera lucida. The labeling pattern of every third section in a series was drawn (this corresponds to a distance of 480 µm between two drawn sections) and sections were aligned to give the distribution of labeling in the temporal cortex (cf. Figs 4, 6, 8 & 11).

Photographs were taken with an Olympus PM20 photographic attachment coupled to a Zeiss Jenalumar microscope and photomontages were prepared. The photomontages were scanned into Adobe Photoshop at 300 d.p.i. and the most prominent seams between single photographs were eliminated digitally. Photographic plates were prepared and labeled digitally with Adobe Photoshop.

Parcellation and Nomenclature of the Medial Geniculate Body

The present delineation of the MG followed LeDoux et al. (LeDoux et al., 1985) and Winer et al. (Winer et al., 1988). As they do, we recognized a ventral and dorsal division of the MG (MGl, MGd, respectively). Medial to the MGl and ventral to the lateral posterior nucleus (LP) the SG is located. Ventral to the SG lies the medial division of the MG (MGl). Ventromedially to the MGl is the PIN, which further ventrally blends into the posterior subparafascicular nucleus. Ventral and lateral to the MGd lies the PP (cf. Fig. 1). However, due to its neuronal morphology and its connections to the auditory cortex, the MGl is recognized as an integral part of the MG (Winer and Morest, 1983; Clerici et al., 1990). The SG has not been systematically studied. Due to its connections and the response characteristics of its neurons it has been regarded as either visual (Winer and Morest, 1983; Hicks et al., 1984) or as part of the dorsal division of the MG (Clerici and Coleman, 1990; Clerici et al., 1990; Winer, 1992). In fact, it appears to be composed of two subnuclei (at least in the cat): a lateral one associated closely with the MGd, and a medial one associated with the visual thalamus (J.A. Winer, personal communication). The neurons of the PP and the PIN have long, sparsely branched dendrites (Winer et al., 1988; Clerici et al., 1990). This morphology differs significantly from that of the neurons in the MG proper, i.e. MGl, MGd and MGl (Morest and Winer, 1986; Clerici et al., 1990; Winer, 1992). The SG, MGl, PIN and PP were named caudal paralaminar nuclei by Herkenham due to their location and their cortical projection pattern (Herkenham, 1980, 1986). In the present paper, we adopted this name for these nuclei due to their additional common projection to the amygdaloid complex (LeDoux et al., 1985, 1990; Romanski and LeDoux, 1993a; Herkenham, 1980, 1986; Doron and LeDoux, 1999; Linke et al., 2000).

Parcellation of Cortical Areas

Parcellation of cortical areas were made using the atlas of Swanson (Swanson, 1992) and a series of cresyl violet-stained sections for each case. Likewise, Swanson's cortical area nomenclature was used. The temporal cortex of the rat, especially the perirhinal and adjacent areas, is an ill-defined region regarding parcellation into discrete areas and, hence, different names were used to define the same areas (Krieg, 1946; Paxinos and Watson, 1986; Zilles, 1990; Swanson, 1992; Burwell et al., 1995; Burwell and Amaral, 1993; Shu and Cassell, 1998). There is general agreement that the perirhinal cortex (P) is located dorsally and ventrally to the rhinal sulcus, and that further dorsally lies the primary auditory (AUD) cortex readily distinguished by its prominent granular layer and the well-described afferent projection of the ventral division of the MG (Winer and Larue, 1987; Romanski and LeDoux, 1993a). However, the parcellation of the region between these well-defined cortical areas is a matter of debate [see Fig. 1 in Burwell et al. (Burwell et al., 1995) and discussions by Burwell and Amaral (Burwell and Amaral, 1998a,b)]. In agreement with the latter authors we identified an area TEv ventral to AUD. This area is characterized by a distinct layer IV, which, however, is considerably thinner than that in AUD. The ECT below TEv has only scattered layer IV neurons. This layer IV has been disappeared completely in P which occupied mainly the bottom and the ventral bank of the rhinal sulcus. In the parcellation scheme of Zilles (1985, 1990) and LeDoux (Romanski and LeDoux, 1993a) TEv and ECT were together referred to as TEv. In Burwell and Amaral's scheme the present ECT corresponds to
their area 36 and the perirhinal cortex corresponds to their area 35 (Burwell and Amaral, 1998b).

Likewise, the rostral border between insular cortex and perirhinal cortex is still a matter of debate and also here different names were used (Shi and Cassel, 1998; McDonald, 1998). This comprises mainly the cortex located 2–3.8 mm behind the bregma which is included into ECT and TEv by Swanson (Swanson, 1992), termed parietal insular cortex by Shi and Cassel (Shi and Cassel, 1998) and parietal rhinal and parietal ventral cortex by McDonald (McDonald, 1998).

As well as with the rostral border between insular and perirhinal cortex a precautionary remark must be made regarding the region where AUD abuts SSs. The parcellation of Zilles (Zilles and Wree, 1995), Romanski and LeDoux (Romanski and LeDoux, 1993a,b) and others proposed an area TE3r between TE1 and Par2. TE1 corresponds to AUD and Par2 to SSs of Swanson (Swanson, 1992), respectively. The parcellation of Swanson did not recognize a rostral belt area although dorsal and ventral subdivisions of AUD are described. Also, Zilles (Zilles and Wree, 1995) states that an area TE3r is not readily distinguishable in Nissl preparations. Thus, it is possible that the projections that we located at the rostral border of AUD and that we assigned to SSs may belong to the rostral belt area of auditory cortex. Because the parcellation of the temporal cortex proposed by Swanson (Swanson, 1992) and Burwell and Amaral (Burwell and Amaral, 1998a,b) fits best to the observed pattern of thalamocortical projections, it was used throughout the present work.

Results

Injections of Miniruby or PHAL into thalamic nuclei resulted in anterograde labeling of cortical (present study) and subcortical structures (LeDoux et al., 1985, 1990; Linke et al., 2000). In addition, Miniruby labeled cells retrogradely. Anterogradely labeled terminals were found in the ventrolateral aspect of the cortical mantle mainly within and dorsal to the rhinal sulcus. In particular, fiber terminals occurred in P, TEv, AUD, Visc and AIp cortices with both tracers. Labeled fibers were regarded as terminals if they were fine and showed small swellings and bouton-like structures along their course. Coarser smooth fibers

---

**Figure 1.** Synopsis of injection sites into caudal paralaminar thalamic nuclei at three different rostrocaudal levels as indicated by numbers (distance from bregma) below each section. Injections into different nuclei were indicated by different symbols. Each symbol demarcates the core of the injection as revealed by inspection of cresyl violet-stained sections adjacent to those which demonstrates the largest extent of the injection site. Beneath each symbol the case number is indicated for comparison with Table 1. For abbreviations see list. Drawings are taken from ‘Brain Maps’ version 1.0 based on the atlas of Swanson (Swanson, 1992).
were regarded as fibers of passage. Retrogradely labeled cortical neurons were found in the above-mentioned regions only after Miniruby injections. Due to the smallness of injection sites (see below), each thalamic nucleus under study, i.e. SG, PIN, MGm and PP, demonstrated a unique pattern of anterogradely labeled terminals and retrogradely labeled neurons. Strain differences between Wistar and Sprague-Dawley rats were not observed.

**Injection Sites**

Figure 1 schematically depicts all injection sites in the posterior thalamus analyzed for this study. Most injections were very small, and hence labeled only a small portion of a given thalamic nucleus. In none of the cases was a spread of tracer into MGv found.

Figure 2 shows an example of an injection site into PIN as it appears in the tissue (case sd27). The photograph was taken at the largest extent of the tracer deposit. A faint outlining the triangular region between the APN, SG and MG but did not result in retrograde labeling of neurons which might contribute to the corticotopetal projection. Abbreviations see list. Calibration bar = 500 µm.

**Morphology of Axon Terminals and Cortical Neurons**

Miniruby as well as PHA-L injections resulted in anterograde labeling of axonal terminals and, in the case of Miniruby, retrograde labeling of cortical neurons. Labeling was often sparse and, thus, single axons could be differentiated and followed over some distance. Retrogradely labeled pyramidal neurons were predominantly found in layers V and VI. Occasionally, retrogradely labeled neurons were completely filled and demonstrated a Golgi-like appearance. However, the majority of labeled neurons displayed only a granular reaction product or a homogenously filled cell body with a pale primary dendrite (cf. Fig. 3A). In contrast, thalamocortical axons were stained completely. Along the course of fine axons swellings occurred. Side branches ended in terminal boutons (Fig. 3B). Figure 3B–D depict the appearance of single axons in layer IV of ECT (B), in layer I of ECT (C), and in layer I of of TIV (D). In all terminal fields single axons were extremely thin. Occasionally, a thick axon with side branches occurred in layer I (Fig. 3D).

**Projections of PIN**

From the 27 cases analyzed for this study, 10 injections were confined to PIN (cf. Fig.1; Table 1) at different rostrocaudal levels. In five of these injections, the tracer may have spread into MGm (sd3, wis60) or into the ventrally located subparafascicular nucleus (wis11, sd9, sd45), respectively. However, as judged by the lack of substantial terminal labeling in layer VI of primary and secondary auditory cortex (see below), diffusion of tracer was minimal. In all other cases, no tracer was found in other structures than PIN. As an example, the

### Table 1

<table>
<thead>
<tr>
<th>Case</th>
<th>Weight (g)</th>
<th>Tracer</th>
<th>Injection site (site of needle tip)</th>
<th>Duration (min)</th>
<th>Survival time (days)</th>
<th>Fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td>sd 3</td>
<td>200</td>
<td>PHA-L</td>
<td>PIN/MGm</td>
<td>13</td>
<td>10</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>wis 30</td>
<td>270</td>
<td>PHA-L</td>
<td>PIN</td>
<td>20</td>
<td>10</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>wis 11</td>
<td>250</td>
<td>PHA-L</td>
<td>PIN</td>
<td>15</td>
<td>7</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>wis 60</td>
<td>300</td>
<td>Miniruby</td>
<td>PIN</td>
<td>10</td>
<td>12</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>wis 61</td>
<td>300</td>
<td>Miniruby</td>
<td>PIN</td>
<td>10</td>
<td>12</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>sd 29</td>
<td>330</td>
<td>Miniruby</td>
<td>PIN</td>
<td>15</td>
<td>12</td>
<td>1% Glut</td>
</tr>
<tr>
<td>sd 27</td>
<td>370</td>
<td>Miniruby</td>
<td>PIN</td>
<td>10</td>
<td>24</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>sd 21</td>
<td>310</td>
<td>Miniruby</td>
<td>PIN</td>
<td>9</td>
<td>19</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>sd 57</td>
<td>300</td>
<td>Miniruby</td>
<td>PIN</td>
<td>10</td>
<td>16</td>
<td>pH-shift</td>
</tr>
<tr>
<td>sd 9</td>
<td>280</td>
<td>PHA-L</td>
<td>PIN</td>
<td>15</td>
<td>8</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>wis 70</td>
<td>300</td>
<td>Miniruby</td>
<td>PP</td>
<td>5</td>
<td>13</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>sd 35</td>
<td>330</td>
<td>Miniruby</td>
<td>FP/caud PIN</td>
<td>8</td>
<td>12</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>sd 28</td>
<td>260</td>
<td>Miniruby</td>
<td>PP/PIN</td>
<td>15</td>
<td>12</td>
<td>1% Glut</td>
</tr>
<tr>
<td>wis 18</td>
<td>340</td>
<td>PHA-L</td>
<td>PP</td>
<td>20</td>
<td>10</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>sd 34</td>
<td>360</td>
<td>Miniruby</td>
<td>MGm</td>
<td>8</td>
<td>12</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>sd 30</td>
<td>330</td>
<td>Miniruby</td>
<td>MGm</td>
<td>5</td>
<td>12</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>sd 62</td>
<td>300</td>
<td>Miniruby</td>
<td>MGm</td>
<td>10</td>
<td>18</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>wis 62</td>
<td>250</td>
<td>Miniruby</td>
<td>MGm</td>
<td>7</td>
<td>12</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>sd 26</td>
<td>340</td>
<td>Miniruby</td>
<td>MGm</td>
<td>10</td>
<td>24</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>wis 56</td>
<td>400</td>
<td>Miniruby</td>
<td>SG</td>
<td>10</td>
<td>24</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>wis 36</td>
<td>310</td>
<td>PHA-L</td>
<td>SG</td>
<td>20</td>
<td>10</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>sd 19</td>
<td>350</td>
<td>PHA-L</td>
<td>SG</td>
<td>20</td>
<td>12</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>sd 38</td>
<td>320</td>
<td>Miniruby</td>
<td>SG</td>
<td>15</td>
<td>22</td>
<td>1% Glut</td>
</tr>
<tr>
<td>sd 58</td>
<td>300</td>
<td>Miniruby</td>
<td>SE-caudal</td>
<td>10</td>
<td>12</td>
<td>pH-shift</td>
</tr>
<tr>
<td>sd 21</td>
<td>270</td>
<td>PHA-L</td>
<td>SG</td>
<td>20</td>
<td>11</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>wis 59</td>
<td>250</td>
<td>Miniruby</td>
<td>POL</td>
<td>10</td>
<td>12</td>
<td>0.1% Glut</td>
</tr>
<tr>
<td>sd 33</td>
<td>340</td>
<td>Miniruby</td>
<td>POL</td>
<td>7</td>
<td>12</td>
<td>0.1% Glut</td>
</tr>
</tbody>
</table>
distribution of anterogradely labeled terminals within the temporal cortex resulting from a small injection into PIN at an intermediate level (case sd27, Figs 4, 5) was described in greater detail. Terminal labeling after circumscribed injections into PIN was found in a wide area of the temporal cortical aspect from the entorhinal cortex ventrally to auditory cortex dorsally. Labeling in the entorhinal cortex was sparse and confined to the deep layers. Labeled fibers were found throughout the temporal cortex in the upper third of layer I. In the caudal part of the perirhinal cortex (6.0 mm behind bregma) the anterogradely labeled fibers were more or less confined to the dorsal bank of the rhinal sulcus including the ECT and ventral part of TEv, whereas more rostrally (∼3.5–3 mm behind bregma) terminals were found in the ventral bank of the perirhinal cortex. Dorsal to the perirhinal cortex, in ECT, TEv and AUD, sparse terminal labeling was also found but in these regions mainly in layers I and VI. In addition, anterogradely labeled fibers were found in lower layer III and layer IV of ECT (cf. Fig. 4) which was particularly prominent in the rostral portion (∼3.5–2.5 mm behind bregma).

Furthermore, scattered terminal labeling was observed in Visc, Alp and SsS. Labeling in Visc, although sparse, demonstrated a similar pattern like that in ECT with terminals in upper layer I, a plexus of terminals in layers III and IV and scattered labeling in layer VI.

Figure 5A shows a photomontage of the terminal labeling in the temporal cortex at ∼3.5 mm behind bregma and Figure 5B shows an adjacent cresyl violet-stained section. Terminal labeling is seen in the ventral bank of the perirhinal cortex in layers I–III and in a small patch dorsal to the rhinal sulcus in layers I and layers III and IV corresponding to ECT. Likewise, scattered terminal fibers are seen in layer I and VI of TEv. Anterograde labeling of layer I in the perirhinal cortex differed from that of dorsally located isocortical regions. In isocortical areas terminals were found almost exclusively in the upper layer I just below the pia mater. In the perirhinal cortex the terminals are located close to cellular layer II.

After injections of Miniruby into PIN a small number of retrogradely labeled neurons of layer V were detected in all cortical areas mentioned above except the entorhinal cortex. In the P, TEv, Alp and SsS, retrogradely labeled neurons in layers V and VI were observed.

**Projections of the SG**

Six injections were centered predominantly into SG (cf. Fig. 1; Table 1). Due to the smallness of injections, labeling in the cortex was generally sparse. The pattern of cortical labeling is illustrated for case sd58 (Figs 6, 7), which showed the least diffusion of tracer into neighboring structures like MGm and PIN below. Anterogradely labeled terminals were found in the P, ECT, TEv and AUD and Visc along a wide extension in the rostro-caudal direction (Fig. 6). In all cortical areas labeling in layer I was observed in equal low density. In AUD, terminals were
found predominantly in the rostral part. Labeling in the perirhinal region was very sparse and confined to a small region in the dorsal bank of the rhinal sulcus close to the ventral border of the ECT. Labeling in the P disappeared gradually when labeling in the rostral ECT became more distinct (3–4 mm behind bregma) especially in lower layer III and layer IV. This region was depicted in Figure 7. Here, sparse terminal labeling was seen in layers III and IV of the ECT and in upper layer I of the entire temporal cortex. The P was not labeled except for axons in layer I. Labeled fibers in layers VI were fibers of passage. Furthermore, a few fibers were seen in the ventrolateral part of the amygdala.

Further rostrally, sparse terminal labeling was found in Visc in lower layer III and layers IV and VI in addition to that in layer I (Fig. 6).

A few retrogradely labeled neurons occurred in the ECT and the AUD.

**Projections of PP**

Four injections were centered in PP at the caudal pole of MG (cf. Fig. 1; Table 1) with minimal spread of tracer into adjacent MGm and PIN. Figure 8 (case wis70, cf. Figs 1, 9C) illustrates the pattern of anterogradely labeled fibers in the cortex. As with the projections of the other thalamic nuclei, the projection expanded widely over the ventrotemporal aspect of the cortical mantle. Heavy anterograde labeling can be seen in in the ECT in layers I and lower layer III and layer IV at caudal levels (6.1–4.2 mm behind bregma). Likewise, in TEv this pattern can be observed; however, it is considerably weaker than in the ECT. The AUD receives mainly axons into layer I and occasionally into layer VI, which may be indicative of a minor involvement of MGm due to leakage of tracer into MGm. Most fibers in layers II–V of AUD were fibers of passage on their way from layer VI to layer I as judged by the absence of swellings along the axonal course. Further anteriorly, Visc and SSs received a faint but
Figure 5. (A) Projection pattern in the temporal cortex (~4 mm behind bregma) after an injection of Miniruby into PIN (sd27, cf. Figs 1, 2; darkfield illumination). White arrowheads indicate the parcellation of the temporal cortex. Areas are named in (B). (B) Adjacent Nissl-stained section. Cortical areas and layers (roman numbers) are indicated. Abbreviations see list. Calibration bars = 500 µm.
distinct projection into layer I. Occasionally, some terminals in layers III and IV were present, particularly in Visc. A weak but distinct projection was found to a rostral area corresponding to MOp. Retrogradely labeled cells were found mainly in layer V of ECT, AUD and Visc. In TEv neurons in layer VI were also labeled.

Figure 9 illustrates the projection of PP to temporal cortex at a caudal level (~6.0 mm behind bregma). Anterogradely labeled terminals are seen dorsal to the rhinal sulcus in a small patch (ECT) concentrated in lower layer III and layer IV with a sharp border to the perirhinal cortex. More dorsally, terminal labeling was seen in layer I and scattered fibers of passage were seen in layer VI and V particularly in AUD. In the latter, retrogradely labeled neurons in layer V are seen. Figure 10 illustrates this projection at a level closer to bregma (~3.5 mm behind bregma) comparable to the levels in Figures 5 and 7. A patch of terminals is seen at the bottom and the dorsal bank of the rhinal sulcus in lower layer III and layer IV. Dorsal to this region, the labeling in these layers becomes thinner and finally ends where TEv begins. In this region only few axons are found. Scattered terminal axons are also seen in layer I. Retrogradely labeled neurons are found in layer V of P and ECT.

Projections of MGm

Five injections were centered predominantly into MGm (cf. Fig. 1; Table 1) with only a few dendrites and an occasional retrogradely labeled neuron found within PIN. In no case were the SG, MGv, or PP involved. The pattern of labeling was demonstrated for case sd34 (cf. Fig. 1; Fig. 12). Terminal labeling (Fig. 11) was observed in P, TEv, ECT, AUD, Visc and SsS. All cortical areas received afferent fibers into layer I although layer I of the central core of AUD is devoid of terminals. In ECT, Visc, TEv and AUD additional input into layers III and IV could be seen. Whereas the labeling in lower layer III and layer IV of AUD was very sparse, labeling of these layers in ECT and TEv was heavy and distinct especially in the rostral part of those areas (3–4 mm behind bregma) (Fig. 11C). In addition to a projection into layer I, TEv and AUD received a rather dense projection into layer VI which was not found after injections into either SG, PIN...
Figure 7. (A) Photomontage of the temporal cortex demonstrating the cortical labeling after an injection of Miniruby into the SG (case sd58, cf. Figs 1, 2A; darkfield illumination). The picture is taken ∼4 mm behind bregma. White arrowheads mark the borders of cortical areas which are likewise marked and named in (B). (B) Adjacent Nissl-stained section. Cortical layers are indicated by roman numbers and cortical areas are demarcated. (C) Injection site of case sd58 at its largest extent as it appears in the tissue. The tracer deposit was in the ventral SG and labeling is seen to be transported ventrally but did not reach PIN. No labeling was found to enter APN, MGv or MGm. Abbreviations see list. Calibration bars = 500 µm.
or PP. Figure 12 illustrates the distribution of fibers at a comparable level as in the cases before (~3.5 mm behind bregma). Discrete terminal fields were seen in P and ECT in lower layer III and layer IV and in TEv and AUD in layer VI. A few fibers may be detected in layers III and IV in the primary auditory cortex. Retrogradely labeled neurons were found in layers V and VI of all areas mentioned above.

Discussion

The results of the present study can be summarized as follows: Thalamic nuclei that are located medially to the medial geniculate body, i.e. the caudal paralaminar nuclei SG, PIN, MGm and PP (Herkenham, 1986) project to distinct layers within several cortical areas in temporal and insular cortex. Although the anterograde projection patterns widely overlap, each nucleus has its individual projection pattern. A convergent projection of all nuclei was demonstrated into lower layer III and layer IV of the rostral ECT and Visc, and upper layer 1 of the entire temporal cortex. The projection of paralaminar thalamic nuclei to other areas of temporal and insular cortex varies. The pattern of terminal labeling and the location of these patterns in cortical areas (e.g. the terminal field in ECT) suggest a rostro-caudal topography of the projections of these thalamic nuclei. Due to the overlap of thalamocortical afferents with corticoamygdaloid projection neurons, the thalamic nuclei not only provide the amygdala with sensory information via a direct pathway (LeDoux, 1993) but are also in the position to modulate the information flow to the amygdala and hippocampus coming from an indirect cortico-cortical pathway.

Methodological Considerations

Due to the smallness of the thalamic region around the MG a major concern was that the injections sites were small enough to be attributed to single nuclei. Two tracers, PHA-L and Miniruby (a biotinylated dextran amine), were used in the present study to examine the thalamocortical pathways of caudal paralaminar nuclei. Both tracers have distinctive advantages and disadvantages. A major advantage of Miniruby over PHA-L is that injections sites are often very small with minor diffusion of tracer into neighboring regions. In most cases, the densely labeled central core had a diameter of not more than 250 µm, which corresponds to the width of SG or MGm in the region of the posterior thalamus. Furthermore, Miniruby injection sites demonstrated an elongated appearance as in the case of injections into narrow regions like SG (cf. Fig. 7C) or MGm (cf. Fig. 12C). In contrast, PHA-L injection sites are more circular.
irrespective of their location and had often a much larger halo. This indicates a reduced diffusion of Miniruby (Schmued et al., 1990). As a result of the small injection sites, terminal labeling in the cortex is sparse.

However, the sparseness of cortical labeling may result from diffusion within the tissue or decomposition of tracer substance during long survival times. Diffusion within the tissue over time and therefore dilution of tracer is unlikely for two reasons. First, we used survival times ranging from 12 to 24 days. The sizes of injection sites using comparable injection parameters (tip diameters of pipettes, current strength, duration of injection, cf. Table 1) do not vary in diameter in cases at either end of the

Figure 9. (A) Pattern of labeling in the caudal part of the temporal cortex of case wis70 (∼ 6 mm behind bregma). White arrowheads indicate borders of cortical areas which are named in (B). (B) Adjacent Nissl-stained section. Cortical areas and layers (roman numbers) are indicated. (C) Injection site of case wis70. The core of the injection was located below the MGv in PP and the subparafascicular nucleus. No spillage of tracer was seen into the other thalamic paralaminar nuclei, although a few neurons in SG appeared to be retrogradely labeled. Abbreviations see list. Calibration bars = 500 μm.
Furthermore, if diffusion had occurred, labeling in the cortex and subcortical regions should also be more diffuse in animals with longer survival times. This was not observed. The pattern of thalamic efferents (present paper) and thalamic afferents (Linke, 1999b) is solely dependent on the location of the injection site. Likewise, decomposition of tracer is unlikely to occur. Even after much longer survival times conspicuous labeling was reported after injections of dextran amines (Schmued et al., 1990). In contrast, longer survival times appear to increase the intensity of terminal labeling (Brandt and

Figure 10. (A) Pattern of labeling in the temporal cortex of case wis70 about 4 mm behind bregma. White arrowheads indicate areal borders of the cortex as indicated in (B). (B) Nissl-stained section slightly more rostral than that in (A). Cortical areas and layers (roman numbers) are indicated. Abbreviations see list. Calibration bars = 500 µm.
Apkarian, 1992). In our cases, no difference was detected in the appearance and density of labeling of neurons or axonal terminals after the survival times used in the present report. Thus, neither diffusion nor decomposition of tracer at the injection site or at terminal sites appear responsible for the sparseness of cortical labeling. A third possible reason for the sparse cortical labeling might result from structural and morphological differences of neurons within MGm, SG, PIN and PP, respectively. Several studies report that neurons within these nuclei differ in their morphology. Whereas in MGv, MGd and MGm neurons possess a tufted or stellate dendritic morphology, neurons in SG, PIN and PP have long, sparsely branched dendrites (Clerici et al., 1990; Morest and Winer, 1986; Winer, 1992). Injections of PHAL or BDA of comparable sizes to the present ones (∼300 µm in diameter) into the MGd (R. Linke and G. Braune, unpublished) produced a dense and circumscribed labeling into lower layer III and layer IV of TEv just dorsal to ECT and a dense labeling in layer I of the entire temporal cortex. A comparable pattern within layer IV of AUD has been obtained after small injections of PHAL into the MGv (Romanski and LeDoux, 1993b). It may well be that the sizes of injections were a little bit too small. However, larger injections, which resulted in heavier labeling, would conceal at the same time the exact locations within the thalamic nuclei. Furthermore, the higher probability of leakage of tracer into neighboring areas would confound the contribution of single thalamic nuclei to the cortical projection. At present, the use of Miniruby offers the best compromise between a maximum of labeled terminals and concentrated small injection sites. Despite its advantages, Miniruby has several disadvantages. First, it is also transported retrogradely, which may result in collateral axonal labeling (Chen and Aston-Jones, 1998), i.e. the labeling of the local axonal plexus of a retrogradely labeled cortical pyramidal neuron. This axonal plexus may be mistaken as an afferent projection from the thalamus. Axonal collateral labeling has happened occasionally in our material but was readily recognized. This rare type of labeling only occurred if a pyramidal neuron was completely filled and demonstrated a

Figure 11. Projection pattern after an injection into MGm (case sd34, cf. Figs 1, 12C). (A) Location of injection site. (B) Surface map of a rat brain with positions of sections indicated (cf. Fig. 4). (C) Terminals were found predominantly in the deep layers of AUD and TEv. Labeling in upper layers is generally weak but especially the central core of primary auditory cortex appeared to be spared by terminals. Numbers below sections give the distance from bregma in mm.
Golgi-like appearance. In general, retrogradely labeled neurons contained a granular reaction product in their somata or displayed a pale homogeneous filling which only labeled the primary dendrite. These neurons do not demonstrate a labeled local axonal plexus. A second disadvantage, which had been attributed to dextran amines, was labeling of undamaged fibers...
of passage. This is especially important, because the paralaminar thalamic nuclei are traversed by prominent fiber bundles. However, iontophoretic injections with small pipette tip diameters (cf. Table 1) are reported to minimize the risk of unspecific uptake by undamaged fibers of passage (Schmued et al., 1990). If considerable unspecific uptake by the fiber bundles that traverse the MGm, for example, had occurred, a massive labeling of terminals in the MGv and MGd should be seen. This was not the case.

As in every tracing study, the uptake of tracer by axons that were damaged along the needle track presents an inherent problem that cannot be avoided with absolute certainty. However, to minimize tissue damage we used micropipettes with small outer tip diameter (<30 µm) and long thin shanks. Furthermore, the usage of iontophoresis and the additional waiting period at the end of each injection should prevent the leakage of tracer into the needle track and, therefore, the uptake of tracer by axons that might be eventually damaged. Because in most cases no needle track could be reconstructed, we concluded that the uptake of tracer by damaged fibers did not contribute significantly to the observed projection patterns.

**Functional Anatomy of the Caudal Paralaminar Nuclei**

Electrophysiological (Wepsic, 1966; Benedek et al., 1997) and connectional studies (LeDoux et al., 1987; Linke, 1999b; Shiroyama et al., 1999) have demonstrated that the paralaminar thalamic nuclei, which border the MG at its medial side, are a site of convergent sensory input. The afferent projections originate in the superior colliculi (SC), the inferior colliculi (IC), the dorsal column nuclei, the spinal trigeminal nucleus and the dorsal horn of the spinal cord. Thus, auditory, visual, somatosensory, vestibular and nociceptive responses can be elicited in caudal paralaminar thalamic nuclei either alone or in various combinations (Wepsic, 1966). Despite their overall polymodal character, a rather high number of of neurons within these nuclei respond to only a single modality (Wepsic, 1966; Hicks et al., 1984; Bordi and LeDoux, 1994a,b). For example, neurons in the SG of the cat have large receptive fields and respond vividly to fast-moving visual stimuli (Hicks et al., 1984). Tracing studies in cat (Hicks et al., 1986), opossum (Morest and Winer, 1986) and rat (Linke, 1999b; Linke et al., 1999) revealed that the SG receives a prominent input from the superficial layers of the superior colliculus that are dominated by retinal input. After an injection of a retrograde tracer into the SG between 20 and 50% of retrogradely labeled neurons in the SC are located in the superficial layers. Injections into other thalamic paralaminar nuclei resulted in <10% of all labeled neurons within superficial layers (Linke, 1999b). In contrast to SG, the PIN, MGm and PP receive their SC projection mainly from polymodal deep layers (LeDoux et al., 1985; Arnault and Roger, 1987; Linke, 1999b).

A much more elaborate differential input to SG, PIN, MGm and PP originates from the inferior colliculus (IC). The major source of the colliculothalamic projections are the external and the dorsal nucleus of the IC (Arnault and Roger, 1987; LeDoux et al., 1990; Linke, 1999b). The dorsal nucleus has mainly commissural and cortical connections, whereas the external nucleus receives a variety of subcortical afferents (Aitkin, 1986). These nuclei are not well studied and available evidence suggests that they are polymodal regions (Aitkin, 1986), particularly the external nucleus. Nevertheless, the external nucleus has the most differentiated projection to the paralaminar thalamic nuclei. The SG receives its main input from IC from layer 2 neurons of the external nucleus. In contrast, PP receives its main input from layer 1 neurons of the external nucleus. The MGm
receives its input from layer 2 and magnocellular neurons of layer 3 (Linke, 1999b). Electrophysiological studies in the mouse revealed that neurons in the external nucleus are especially sensitive to specific intervals between acoustic stimuli and to short rise-times of acoustic stimuli (Willott et al., 1979).

Although detailed information of the exact terminations of afferent projections to thalamic paralaminar nuclei are not available, findings in bats (Wenstrup et al., 1994) and rats (LeDoux et al., 1987; Shiroyama et al., 1999) suggest two aspects of functional organization of this region. First, as can be inferred from the morphology of axonal endings in each nucleus, different types of projection neurons are involved. For example, after injections of HRP into the central nucleus of the IC axons in the SG are thin and en-passant boutons prevail. In the MGN, axons are coarser, boutons are larger and more clustered (Wenstrup et al., 1994). Second, convergent projections from different sources are only partially overlapping within paralaminar thalamic nuclei as demonstrated by LeDoux for spinal cord and IC projections (LeDoux et al., 1987). Both terminal fields overlap only in rostral portions of paralaminar thalamic nuclei but not in caudal portions. Accordingly, neurons responding to auditory and somatosensory stimuli where found in rostral regions and neurons that respond only to a single modality are found in caudal regions of these nuclei (Bordi and LeDoux, 1994b). A detailed tracing study of vestibular afferents to the thalamus demonstrated a selective afferent projection. Shiroyama and co-workers showed that only the spinal vestibular nucleus provides an afferent projection into mainly the SG (Shiroyama et al., 1999).

A further aspect related to the functional organization of these nuclei addressed the different types of neurons that are found in these nuclei. Studies in cat (Winer and Morest, 1983; Winer, 1985), opossum (Morest and Winer. 1986; Winer et al., 1988) and rat (Clerici et al., 1990) revealed several different neuronal cell types which may differ in their projection targets and their peptide content. For example, Namura et al. in a double-labeling study provide evidence that the majority of neurons project to either the amygdala or the cortex (Namura et al., 1997). Only a small percentage is double-labeled. Furthermore, within PIN a subset of projection neurons to the perirhinal cortex contain the calcitonin gene-related peptide (CGRP) which is thought to be involved in visceral sensory pathways (Yasui et al., 1989).

To summarize, the overall organization of the thalamic paralaminar nuclei suggests a convergence of several sensory modalities within these nuclei. However, a more detailed consideration of single nuclei revealed that the sensory afferents to each of the thalamic paralaminar nuclei are to a certain degree selective, giving rise to the suggestion that each nucleus has a specific role within the ascending sensory pathway. Therefore, it may be conceivable that the SG is involved in the processing of visual information (combined with auditory and vestibular components), whereas the MGm is mainly involved in the processing of auditory stimuli (combined with somatosensory components). PIN and PP may process somatosensory, visceral and nociceptive stimuli (combined with auditory components). Although combined physiological and morphological data are lacking, available data from electrophysiological studies at the single neuron level lead to the impression that the caudal paralaminar nuclei consist of an intricate web of parallel unimodal and polymodal channels that convey specific aspects of sensory stimuli to both the amygdala and to those cortical areas that are heavily connected to both the amygdala and the hippocampal region.

### Organization of the Thalamocortical Projection

A number of studies have documented that thalamic nuclei surrounding the MG project to cortical secondary sensory and association areas as well as to the amygdala (Jones and Leavitt, 1974; Ottersen and Ben-Ari, 1979; Guldin and Markowitsch, 1983; Deacon et al., 1983; LeDoux et al., 1985, 1990; Vaudano et al., 1991). A recent double-labeling study even demonstrates that the amygdala and the temporal cortex is innervated by the same neurons via axon collaterals (Namura et al., 1997). However, the retrograde strategies are not able to reveal the areal distribution of axons in the temporal cortex and – what appears to be more important – the laminar pattern of afferent axons within a given area (specific versus unspecific projection systems) (Jones, 1985; Groenewegen and Berendse, 1994). Information about the areal and laminar distribution of single thalamic nuclei is critical for an understanding of the coordinated activity of thalamus, amygdala and cortex (Romanski and LeDoux, 1992; Armony et al., 1997; Quirk et al., 1997) during sensory processing. This is all the more important because it has been shown that the thalamic nuclei around the MG receive a differential sensory input (see above) and project differentially to the amygdala (Linke et al., 2000).

A summary of the afferent and efferent cortical projections of thalamic paralaminar nuclei is given in Fig. 13. Three major features arise from these results. First, there is a convergent input from the paralaminar thalamic nuclei into layers III and IV of the ECT, particularly into its rostral part, and there is a convergent input into layer I of the temporal cortical mantle. As mentioned above, there is little agreement about the nomenclature and affiliation of this region to parietal, insular or perirhinal cortex (McDonald, 1998). Connectional studies demonstrate that rostral ECT has a robust projection mainly to the dorsolateral and ventrolateral divisions of the lateral nucleus of the amygdala, whereas the P has its main projection to the ventromedial portion of the lateral nucleus (Romanski and LeDoux, 1993b; McDonald, 1998; Shi and Cassell, 1998). Furthermore, ECT projects heavily onto the P and lightly onto the lateral entorhinal cortex (Burwell and Amaral, 1998b). The perirhinal cortex (P), which receives a differential input from the paralaminar thalamic nuclei, projects massively to the lateral entorhinal cortex, which is the gateway to the hippocampus proper (Burwell and Amaral, 1998a). Although less clear cut, a second cortical area of thalamic convergence is Visc. It is not known whether separate subsets of thalamic neurons project to ECT and Visc. The involvement of Visc into the projection is probably related to visceral and nociceptive sensations, which are also relayed via the thalamic nuclei (Yasui et al., 1989).

Due to their differential sensory input (LeDoux et al., 1987; Linke, 1999b) each thalamic nucleus may provide the ECT with a unique aspect of sensory information. For example, the afferent corticothalamic projection patterns of SG and PIN appear very similar (cf. Figs 5, 7) although differences exist in the projection to the perirhinal cortex. Tracer studies provided evidence that the dorsal perirhinal cortex receives mainly auditory and visual information whereas the ventral perirhinal cortex receives mainly auditory and somatosensory information (McDonald, 1998). The afferent projection to SG is dominated by visual information (Hicks et al., 1984; Benedek et al., 1997; Linke, 1999b), whereas the afferent projection to PIN can at best be described as dominated by auditory and somatosensory input (Aitkin et al., 1978; 1981; Aitkin, 1986; Linke, 1999b). Retrograde tracing studies demonstrate that the afferent projections to SG and PIN, at least from the superior and inferior colliculi,
originated to a large extent from distinct subdivisions of both superior and inferior colliculi, respectively (Linke, 1999b). Hence, although there is an overlap of the afferent projection of SG and PIN in the perirhinal, ectorhinal and also visceral cortex, the information each thalamic nucleus relays might be different.

The second convergent projection system originating from the thalamic paralaminar nuclei is the projection into upper layer I. This projection is found in the entire temporal aspect of the cortical mantle. Little is known about the organization and functions of layer I. Within the projection zone of thalamic afferents the most distal dendrites of pyramidal neurons, bipolar non-pyramidal neurons and non-pyramidal amygdalofugal projection neurons of layer II (unpublished observations) are found. It has been demonstrated by Cauller and Connors that afferent synapses on most distal dendrites of pyramidal neurons exert a powerful influence on their firing probability (Cauller and Connors, 1992). In layer I also other unspacific projection systems (cholinergic and catecholaminergic ones) terminate. Together, these projections are thought to play a role in attentional processing and short-term memory formation.

A third major feature of the thalamocortical projection of the paralaminar thalamic nuclei is the complementary organization within cortical regions. For example, a comparison of the projection patterns of PIN and MGm revealed a different projection pattern within cortical areas. As can be seen in Figures 7 and 9, PIN projects heavier to layer I of AUD than MGm but the latter has a strong projection to layer VI, which is virtually absent in PIN. As in the case of SG and PIN, MGm and PIN likewise differ in their afferent input from the superior and inferior colliculi (Linke, 1999b). The different projection patterns suggest that PIN and MGm may activate different sets of cortical neurons and that the effectiveness of activating cortical neurons might be different for PIN and MGm. A second example is the projection of PIN and MGm to P. Whereas MGm projects mainly into the rostral dorsal bank, the projection of PIN is found more caudally. Projections of PIN to the rostral P are found within the ventral bank of the rhinal sulcus.

Taken together, there are zones of convergence and zones of non-convergence. Thalamic nuclei have convergent projections to several cortical areas (e.g. ECT and Visc) and layers (layer I) but also non-convergent zones within layers exist. Thus, the observed afferent projections to cortical regions appear to be highly organized in the temporal neocortex so that each nucleus may play its unique role in the activation process of cortical neurons.

**Functional Considerations**

The present analysis demonstrates that the paralaminar nuclei, which project to the amygdala, also project to those cortical regions which give rise to amygdalopetal projections (Ottersen, 1982; Arnault and Roger, 1990; LeDoux et al., 1991; McIntyre et al., 1996; Shi and Cassell, 1997). For example, extensive overlap of thalamocortical axons with retrogradely labeled cortico-amygdaloid layer II neurons have been found in ECT and Visc (R. Linke, unpublished data). However, a direct synaptic contact has yet to be established. Thus, cortical regions receive sensory information from the thalamic nuclei as fast as the amygdala. The thalamic nuclei are thus in the position to modulate the information flow of the modality-specific cortico-cortical pathways to the amygdala. The existence of such a connection has been suggested by Quirk et al. (Quirk et al., 1997) who measured response latencies in the lateral amygdala and in cortical areas Te3/Te1v (ECT and TEv in the present nomenclature) after tone onset. The response latencies in both the lateral amygdala and auditory cortical areas after tone onset were identical (12 ms) (Quirk et al., 1997). The physiological evidence and the present anatomical data indicate that those cortical regions which were integrated into the ‘slow’ thalamo-cortico-amygdaloid pathway – i.e. secondary auditory cortical areas (ectorhinal cortex/TEv or Te3/Te1v respectively) and perirhinal cortex – will also receive information relayed via those thalamic nuclei tied into the ‘fast’ pathway. The location of axonal terminals of the corticothalamic projection of the paralaminar nuclei predominantly in layer I together with unsppecific ascending systems (cholinergic, serotoninergic, noradrenergic ones) have a powerful influence on layer V pyramidal neurons (Cauller and Connors, 1992) and appear to play a key role in attentional processing and memory formation, especially short-term memory or ‘event holding’ (Vogt, 1991). Recent experiments in the laboratory of LeDoux (Quirk et al., 1997; Armony et al., 1998) demonstrate a late response (1500 ms after tone onset) of cortical neurons after fear-conditioning.

**Conclusions**

In summary, the corticopetal projections from the thalamic nuclei, which surround the medial geniculate body, demonstrate a diversity of laminar projections patterns despite a strong axonal convergence onto selective temporal cortical areas like the ECT. These thalamic nuclei receive a convergent but selective input from midbrain and brainstem centers (LeDoux et al., 1990; Linke, 1999b) and thus participate in establishing an extrageniculate sensory system composed of multiple unimodal and polymodal channels arranged in parallel. These extrageniculate projection systems appear to have far-reaching and powerful influences on cortical processing of sensory signals (Barth and McDonald, 1996; Brett and Barth, 1997).

**Notes**

We would like to thank G. Braune for kindly providing some of her experimental material, and B. Ketzler and S. Röhl for technical assistance. The present work was supported by Deutsche Forschungsgemeinschaft SFB 426 (TP B5).

Address correspondence to Dr Rüdiger Linke, Institut für Anatomie, Otto-von-Guericke-Universität Magdeburg, Leipziger Strasse 44, D-39120 Magdeburg. Email: ruediger.linke@medizin.uni-magdeburg.de.

**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIp</td>
<td>agranular insular cortex</td>
</tr>
<tr>
<td>APN</td>
<td>anterior prectual nucleus</td>
</tr>
<tr>
<td>AUD</td>
<td>auditory cortex</td>
</tr>
<tr>
<td>bic</td>
<td>brachium of the inferior colliculus</td>
</tr>
<tr>
<td>bsc</td>
<td>brachium of the superior colliculus</td>
</tr>
<tr>
<td>cpd</td>
<td>cortical peduncle</td>
</tr>
<tr>
<td>EC</td>
<td>entorhinal cortex</td>
</tr>
<tr>
<td>ECT, Ect</td>
<td>entorhinal cortex</td>
</tr>
<tr>
<td>fr</td>
<td>fasciculus retroflexus</td>
</tr>
<tr>
<td>INC</td>
<td>interstitial nucleus of Cajal</td>
</tr>
<tr>
<td>LGd</td>
<td>lateral geniculate body dorsal division</td>
</tr>
<tr>
<td>LP</td>
<td>lateral posterior nucleus</td>
</tr>
<tr>
<td>LT</td>
<td>lateral terminal nucleus accessory optic tract</td>
</tr>
<tr>
<td>MGd, v, m</td>
<td>medial geniculate body, dorsal, ventral, medial division</td>
</tr>
<tr>
<td>ml</td>
<td>medial lemniscus</td>
</tr>
<tr>
<td>MPT</td>
<td>medial prectual nucleus</td>
</tr>
<tr>
<td>MRN</td>
<td>magnocellular reticular nucleus</td>
</tr>
<tr>
<td>ND</td>
<td>nucleus Darkschewitsch</td>
</tr>
<tr>
<td>NOT</td>
<td>nucleus of the optic tract</td>
</tr>
<tr>
<td>OP</td>
<td>olivary prectual nucleus</td>
</tr>
<tr>
<td>opt</td>
<td>optic tract</td>
</tr>
<tr>
<td>P</td>
<td>perirhinal cortex</td>
</tr>
<tr>
<td>PAG</td>
<td>periaqueductal gray</td>
</tr>
</tbody>
</table>
References


posterior paralaminar thalamic nuclei to the amygdaloid complex of the rat. Exp Brain Res (in press).