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ORIGINAL ARTICLE

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Evidence for an auditory subsector within the zona incerta of rats

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Abstract This study explores the patterns of connections between the zona incerta (ZI) of the thalamus and the major auditory centres of the rat brain. Two series of experiments were performed using neuroanatomical tract tracing methods (biotinylated dextran or cholera toxin subunit b). First, tracers were injected into the ZI and resultant patterns of labelling in the auditory centres were examined. Labelling was seen in distinct subdivisions of the temporal cortex (Te3), medial geniculate complex (medial division; MGm), inferior colliculus (external cortex; ICe) and cochlear nucleus (dorsal and ventral divisions). For the most part, these subdivisions are involved in more associative or integrative aspects of audition. In a second series of experiments, three of these centres (Te3, ICe, cochlear nucleus) were injected with tracers and the labelling patterns formed in the ZI were explored. These results show that each of these connections makes a very distinct territory or subsector within the most lateral region of the ZI. This subsector of connectivity with the auditory centres does not respect the well defined cytoarchitectonic sectors of ZI, being made up of small slabs in both the dorsal and ventral sectors. Overall, the results suggest that ZI may integrate auditory information together with the other exteroceptive and interoceptive information that it receives and then influences subsequently global states of arousal and/or attention.

Keywords Temporal cortex · Inferior colliculus · Medial geniculate complex · Cochlear nucleus · Thalamus

Abbreviations *ABC* avidin biotin peroxidase complex · *BD* biotinylated dextran · *Cd* dorsal division of the cochlear nucleus · *cp* cerebral peduncle · *CTb* cholera toxin subunit B · *Cv* ventral division of the cochlear nucleus · *DAB* 3,3-diaminobenzidine tetrahydrochloride · *DR* dorsal raphe · *Hb* habenula · *ICc* central nucleus inferior colliculus · *ICd* dorsal cortex inferior colliculus · *ICe* external cortex inferior colliculus · *III* oculomotor nucleus · *lfp* longitudinal pontine fasciculus · *LG* lateral geniculate complex · *LP* lateral posterior nucleus · *MGd* dorsal division of medial geniculate complex · *MGm* medial division of medial geniculate complex · *MGv* ventral division of medial geniculate complex · *ml* medial lemniscus · *NTBS* nickel tris base saline · *Oc2* occipital cortex 2 · *PAG* periaqueductal grey matter · *Par1* parietal cortex 1 · *PB* parabrachial nucleus · *PBS* phosphate buffered saline · *Pc* posterior commissure · *Pf* parafascicular nucleus · *Pn* pontine nuclei · *Po* posterior thalamic nucleus · *PPT* pedunculopontine nucleus · *PR* pontine reticular nucleus · *PRh* perirhinal cortex · *Pt* pretectum · *pyr* pyramidal tract · *RSA/G* retrosplenal cortex · *Rt* thalamic reticular nucleus · *SC* deep layers of the superior colliculus · *SCd* deep layers of the superior colliculus · *scp* superior cerebellar peduncle · *SCs* superficial layers of the superior colliculus · *SN* substantia nigra · *SO* superior olivary nucleus · *Te1* temporal cortex 1 · *Te2* temporal cortex 2 · *Te3* temporal cortex 3 · *Tz* trapezoid body · *V* trigeminal nucleus · *Ves* or *Vest* vestibular nucleus · *VII* facial nucleus · *VP* ventral posterior nucleus · *Vs* spinal trigeminal nucleus · *ZI* zona incerta · *ZIc* zona incerta caudal sector · *ZId* zona incerta dorsal sector · *ZIr* zona incerta rostral sector · *ZIv* zona incerta ventral sector

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Introduction

The zona incerta (ZI) is a distinctive feature of the thalamus of all mammals. Together with the thalamic reticular nucleus and the ventral lateral geniculate nucleus, the ZI has developmental origins in the ventral thalamus and hence does not have a heavy projection to the neocortex (Jones 1985; Mitrofanis and Mikuletic 1999; Power and Mitrofanis 1999b; cf. Lin et al. 1990; Nicolelis et al. 1992, 1995). There are four distinct ZI zones or sectors, rostral, dorsal, ventral and caudal, each distinguished

mainly on the basis of cytoarchitecture, immunocytochemical staining and patterns of connectivity (Kim et al. 1992; Ma et al. 1992; Nicolelis et al. 1992, 1995; Kolmac and Mitrofanis 1999a). With regard to cytoarchitecture, the different sectors are usually separated by a thin cell free border and have cells of distinct shapes, sizes and densities; for example, the rostral sector contains small spindle shaped and densely packed cells, while the dorsal sector is made up of larger, generally oval shaped and more loosely packed cells (Kawana and Watanabe 1981; Watanabe and Kawana 1982; Romanowski et al. 1985; Ma et al. 1992; Nicolelis et al. 1992, 1995; Kolmac and Mitrofanis 1999a). With regard to immunocytochemical character, several studies have shown that GABA and parvalbumin immunoreactive cells are found mainly in the ventral sector; NADPH diaphorase and glutamate immunoreactive cells are located principally within the dorsal sector and tyrosine hydroxylase and somatostatin immunoreactive cells are found within the rostral sector (see Nicolelis et al. 1992, 1995; Kolmac and Mitrofanis 1999a). Finally, with regard to connections, the different sectors have been shown, for the most part, to have distinct inputs and outputs (Ricardo 1981; Roger and Cadusseau 1985; Romanowski et al. 1985; Shammah-Lagnado et al. 1985; Nicolelis et al. 1992). For instance, many nuclei of the brainstem and the intralaminar thalamic nuclei have connections principally with the dorsal sector of ZI (Kim et al. 1992; May et al. 1997; Kolmac et al. 1998; Power et al. 1999), the higher order thalamic nuclei and the deep layers of the superior colliculus with the ventral sector (Power et al. 1999) and the hypothalamus with the rostral sector (Wagner et al. 1995). The precise function of ZI is not known, although it has been suggested recently to form a thalamic centre for the integration of various types of brainstem information, for example somato- and/or viscerosensory (Nicolelis et al. 1992, 1995; Kolmac et al. 1998; Power et al. 1999; Power and Mitrofanis 2001). In addition, ZI may also process and influence particular movements (e.g. locomotive and sociosexual) (see Mogenson et al. 1985; Edwards and Isaacs 1991), since it has numerous connections with various motor centres (see Mogenson et al. 1985; Mitrofanis and deFonseka 2001; Mitrofanis 2002).

More recently, detailed analyses of ZI connections with modality specific centres have revealed distinct subsectors that largely ignore the cytoarchitectonic boundaries described above. For example, motor centres (cerebellum, red nucleus) interconnect with the medial regions of the rostral, dorsal, ventral and caudal sectors (Mitrofanis and deFonseka 2001; Mitrofanis 2002), somatosensory centres [parietal (area 1) cortex, trigeminal nuclei, spinal cord] project to the mid regions of the dorsal and ventral sectors (Nicolelis et al. 1992; Shaw and Mitrofanis 2002), "limbic" centres (brainstem reticular nuclei, cingulate cortex, amygdala, hypothalamus, basal forebrain) interconnect with large areas of the dorsal and rostral sector (Wagner et al. 1995; Kolmac et al. 1998; Mitrofanis and Mikuletic 1999; Kolmac and Mitrofanis 1999b; Reardon and Mitrofanis 2000), while the major visual centres (retina, dorsal lateral geniculate nucleus, superficial layers of the superior colliculus and occipital cortex) have afferents to a small area within the lateral edge of the dorsal, ventral and caudal sectors of the ZI (Power et al. 2001).

In this study, the patterns of connections between the major auditory centres and the ZI are explored, in particular whether there is a distinct auditory receptive territory within the ZI. To this end, the ZI connections with the major auditory centres, including the temporal cortex, medial geniculate complex, inferior colliculus, superior olivary nucleus, trapezoid body and cochlear nucleus, were examined using neuroanatomical tract tracing. Rats were used in this study, a species that has been the focus of many previous examinations of auditory pathway organisation and ZI connectivity (see Nicolelis et al. 1992, 1995; Webster 1995; Kolmac et al. 1998; Power et al. 1999). The results should furnish insights into whether the auditory centres of the brain contribute information to ZI, and whether they may play a part in overall ZI function.

Materials and methods

Subjects

This series of experiments involved 22 adult male (~8 weeks old) Sprague Dawley rats (250–300 g). These animals were housed in a 12-h light cycle with food and water available at all times. All experiments were approved by the Animal Ethics Committee of the University of Sydney.

Tracers

Cholera toxin subunit B (CTb; low salt, List Biological Labs; 1% in dH_2O) and biotinylated dextran (BD; 10 k, 10% in dH_2O; Molecular Probes) were used as tracers in this study. Previous studies have reported that these tracers travel anterogradely and retrogradely, they each make small injection sites and they are not taken up readily by intact or damaged fibres of passage (Angelucci et al. 1996; Kolmac et al. 1998; Power et al. 1999; Power and Mitrofanis 1999a, 2001). Two tracers were used so that the results would not be limited to the transport capabilities of one tracer alone: some tracers have been described to travel anterogradely or retrogradely better than others along certain pathways (see Coleman and Mitrofanis 1996).

Tracer injections and staining

Rats were anaesthetised with ketamine (100 mg/kg; tranquilliser) and Rompun (10 mg/kg; muscle relaxant) and placed into a stereotaxic apparatus. Tracers were injected, either by iontophoresis or by pressure into the ZI (CTb, *n*=5), cochlear nucleus (BD, *n*=1; CTb, *n*=2) inferior colliculus [external cortex (ICe); BD, *n*=2; CTb, *n*=2] and temporal cortex (Te3; BD, *n*=2; CTb, *n*=2) by using the stereotaxic coordinates of Paxinos and Watson (1986). For pressure injections, tracer (either CTb or BD) was drawn into a glass micropipette and ~0.1 μl was injected into the targeted area or nucleus. For iontophoresis injections, CTb or BD was drawn into the pipette and tracer was injected into the targeted structure after passing 5–10 μA, 7 s on/7 s off, for ~40 min. After 7 days survival, rats were anaesthetised by intraperitoneal injection of sodium pentobarbital (Nembutal; 60 mg/ml) and then perfused transcardially, initially with phosphate buffered saline (PBS) and

then with 4% buffered formaldehyde. Thereafter, the brain was blocked, postfixed overnight in the same fixative and then immersed in PBS with the addition of 20% sucrose until the blocks sank. All brains were sectioned coronally on a freezing microtome at 50 µm thickness and every second section was collected in PBS. For CTb immunocytochemistry, sections were immersed in a solution containing 70% ethanol and 3% hydrogen peroxide for 10 min and then washed thoroughly in PBS. Sections were then placed in 4% normal rabbit serum/0.1% Triton (Sigma) in PBS for 1 h, and then into goat anti choleranoid (1:10,000; List Biological Labs) for 2 days at room temperature. Sections were then incubated in biotinylated anti goat (1:300; IgG, Sigma) for 4 h at room temperature (both antibodies with diluted in 2% normal rabbit serum/0.1% Triton/PBS). Next, they were immersed in avidin biotin peroxidase complex (ABC; 1:100 in PBS; Vector) for 1 h and subsequently in nickel TRIS base saline (NTBS) for 30 min. In between each step outlined above, sections were washed thoroughly in PBS. The sections were then reacted with 3,3-diaminobenzidine tetrahydrochloride (DAB; 25 mg; Sigma) dissolved in NTBS (~75 ml) and H_2O_2 (5 µl of 30% solution; Sigma). Finally, sections were mounted on chrom alum gelatin subbed slides and dried overnight. They were then dehydrated in a series of alcohols, cleared in Histoclear and coverslipped with DPX. For BD histochemistry, sections were incubated for 4 h at room temperature in ABC and then as described above following the CTb procedure.

Analysis

Coronal sections of brain were drawn with reference to the atlas of Paxinos and Watson (1986) and the anterogradely labelled terminals and retrogradely labelled cells in the ZI or auditory centres after tracer injections were plotted with use of a camera lucida. For the ZI (Figs 4, 5 and 6), three sections spanning the rostrocaudal axis were mapped. The different sectors were identified after neutral red counterstaining (as were the different nuclei of the thalamus and brainstem) and after referring to immunostained (glutamic acid decarboxylase, parvalbumin, nitric oxide synthase) sections prepared for a previous study (Kolmac and Mitrofanis 1999a). For the auditory centres considered, namely temporal cortex, medial geniculate complex, inferior colliculus, superior olivary nucleus, trapezoid body and cochlear nucleus, cytoarchitectonic subdivisions were distinguished after Nissl counterstaining and with reference to the atlases of Paxinos and Watson (1986). Three sections from rostral, middle and caudal regions of each nucleus/area were mapped (Fig. 3). In an effort to compare patterns of labelling from different cases, the ZI, brainstem, thalamus and cortex was mapped from closely matched sections of the different injection sites. Drawings and plots of thalamus, brainstem and cortex were then scanned onto a computer graphics programme (Microsoft PowerPoint) and the schematic diagrams were constructed.

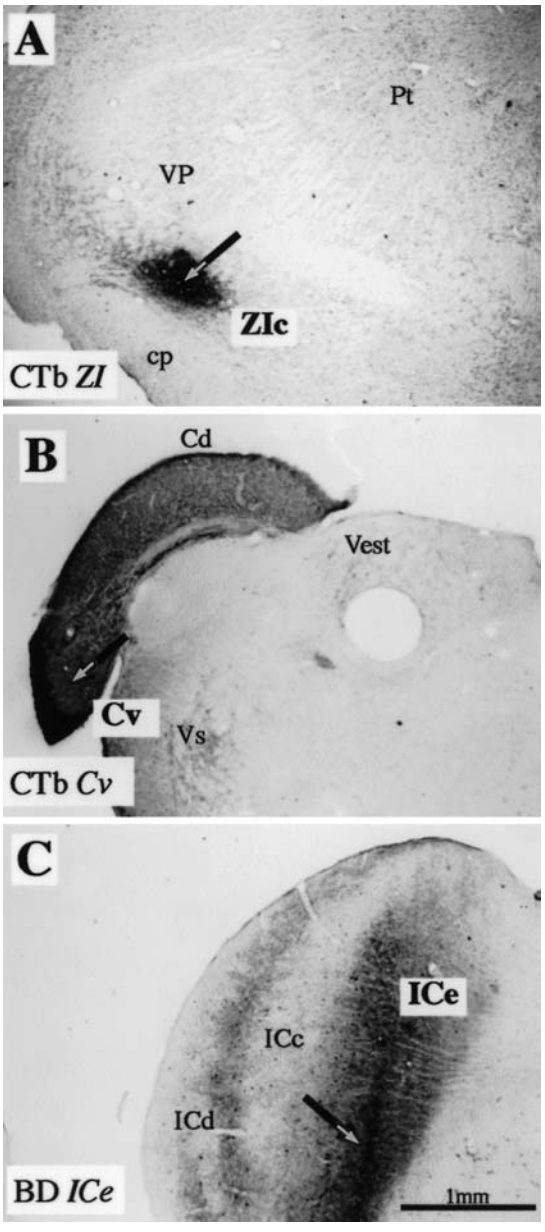


Fig. 1 Examples of CTb and BD injection sites into different neural centres of the rat. **A** CTb injection site into the caudal sector of ZI (*ZIc*). **B** CTb injection site into the ventral division of the cochlear nucleus (*Cv*). **C** BD injection site into the external cortex of the inferior colliculus (*ICe*). All sections were counterstained lightly with neutral red. *Arrows* indicate focal point of injection sites

Results

The results will focus on the distribution of labelled profiles in the auditory centres after tracer injections into the ZI and in the ZI itself after injections into the major auditory centres. First, the tracers used and injection sites will be considered.

Tracers and injection sites

Each CTb or BD injection site showed little spread from the focal point (arrows Fig. 1A, B, C). The extent of each of the injection sites was checked after Nissl (neutral red) counterstaining: only the cases that had injection sites confined to the target were used in this study (n=16).

Tracer pick-up by intact and/or damaged fibres of passage was limited, since injection of these tracers directly into the white matter (e.g. cortical white matter, superior cerebellar peduncle or medial lemniscus) resulted in few anterogradely labelled terminals or retrogradely labelled cells being seen (n=3; these cases were not considered for further analysis in this study). Hence, the bulk of labelling described in this study is likely to be

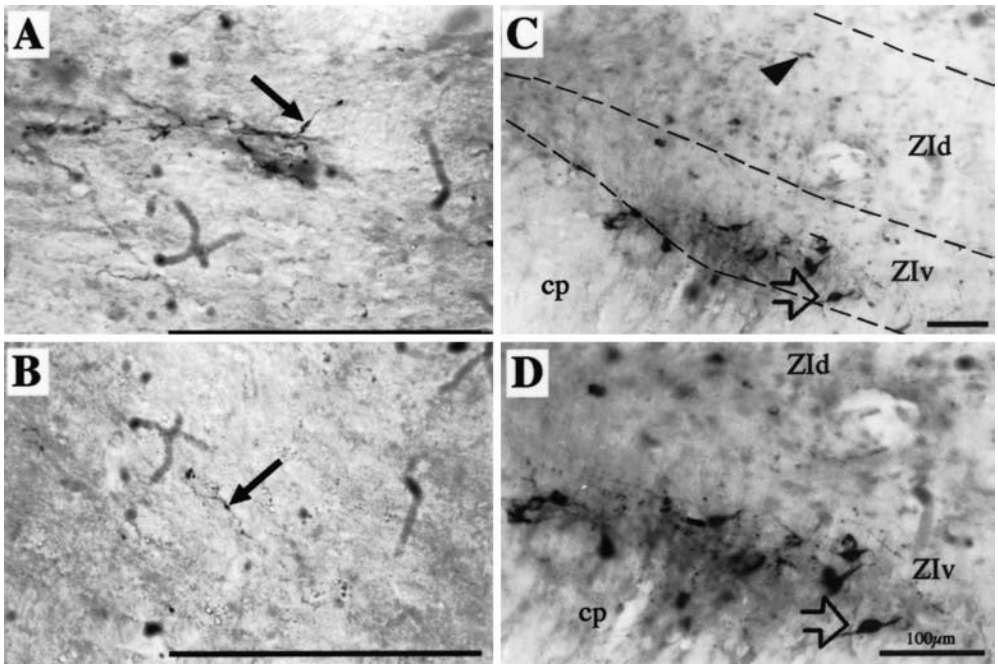


Fig. 2 Examples of the labelling seen in the ZI after injections of BD (**A**, **B**), and CTb (**C**, **D**) into major auditory centres of rats – Te3 (**A**) Cv (**B**) ICe (**C**, **D**). The *black arrows* in **A** and **B** indicate a terminal bouton; the *arrowhead* in **C** indicates labelled cell in dorsal sector of ZI (*ZId*); *unfilled arrows* indicate labelled cells in ventral sector of ZI (*ZIv*). Sections **C** and **D** were counterstained lightly with neutral red. **D** is an enlarged part of **C**. Injection site in **B** is centred on Cv, with some spread into Cd

from tracer uptake by terminals, somata or dendrites, and not by fibres of passage.

Anterograde and retrograde labelling was seen after both BD and CTb injections. Anterograde labelling was in terminals with distinct swellings or boutons (arrows, Fig. 2A, B), linked by thin fibres, while retrograde labelling was in cells with distinct somata and proximal regions of primary dendrites (arrowhead, unfilled arrows Fig. 2C, D). In general, one could not readily discern the difference in labelling between the BD and CTb, although there was a tendency for the BD cases to result in better defined labelled terminals and the CTb cases to yield more robustly labelled cells. Notwithstanding, the resulting patterns of labelling into each centre were very similar after injection of either tracer. These findings are largely consistent with those that have reported previously on the transport capabilities of both tracers (Angelucci et al. 1996; Coleman and Mitrofanis 1996; Power and Mitrofanis 1999a, 2001; Power et al. 1999).

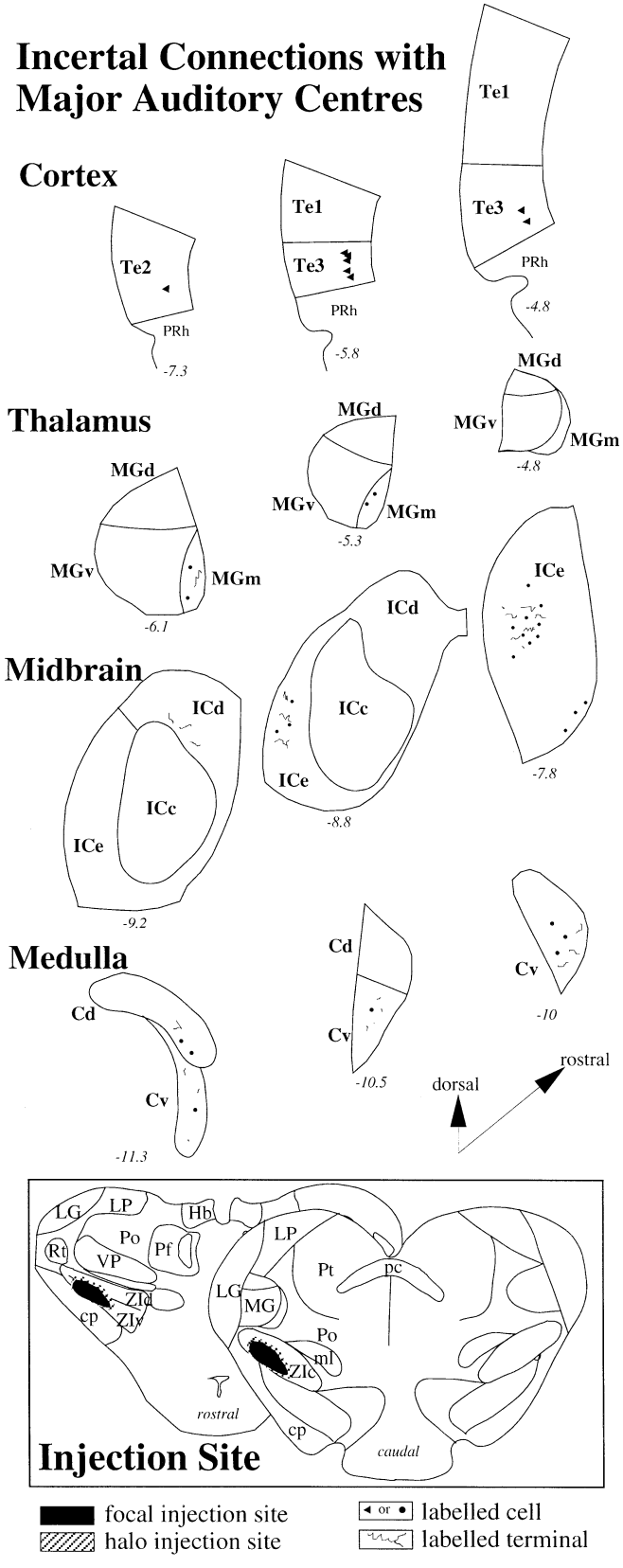
Each neural centre was injected with the use of stereotaxic coordinates (Paxinos and Watson 1986) and the accuracy and location of the resultant injections were determined first, by defining the cytoarchitecture of the area or nucleus injected (after counterstaining with neutral red) (Paxinos and Watson 1986), and second, by examining the resulting labelling patterns in the dorsal thalamus (medial geniculate complex) or brainstem (inferior colliculus), since each of these injections yields different labelling patterns in these brain regions (see Webster 1995; Zilles and Wree 1995). These two methods proved a most effective means by which the location of the injection sites could be pinpointed (see also Coleman and Mitrofanis 1996; Coleman et al. 1997; Mitrofanis and Mikuletic 1999).

Incertal injections

As a first step in the analysis of the auditory connections of the ZI, the ZI itself was injected and the labelling in the major auditory centres, temporal cortex, medial geniculate complex, inferior colliculus, superior olivary complex, trapezoid body and the cochlear nucleus, was examined.

The distribution of labelled terminals and cells in the major auditory centres after an injection of CTb into ZI is shown schematically in Fig. 3. This particular injection site was limited to the lateral region of the ZI; other injections into this same region of the nucleus yielded similar results (n=5). Injection sites located in the medial half of the nucleus, however, yielded no labelling in the major auditory centres, although rich labelling was seen in other regions of the brain, for example in the thalamus and brainstem. These, more medially located injections sites, were not analysed further in this study (n=3).

After either BD or CTb injections into the lateral region of the ZI, labelled profiles were seen in all the abovementioned auditory centres except for the superior olivary and trapezoid body of the pons (no labelling was seen in these pontine nuclei after injections in medial ZI either). Overall, labelling from the lateral region of the ZI did not "blanket" all regions of each auditory centre; rather labelling was apparent in particular parts of each centre. In the temporal cortex, labelling was seen mainly in Te3 and occasionally in Te2. No labelling was ever seen in Te1, the primary auditory cortex. Labelling was limited to cells, with no labelled terminals ever being seen in the cortex after ZI injections. Labelled cells were seen in layer V of Te2/3, and not elsewhere (Fig. 3). In the medial geniculate complex of the thalamus, sparse



cellular and terminal labelling was seen limited to the medial division (MGm; Fig. 3). No labelled profiles were ever seen in the ventral (MGv) and dorsal (MGd) divisions of the complex. Within the inferior colliculus, labelling was seen mainly in the external cortex of the nucleus (ICe), and to a lesser extent, its dorsal cortex (ICd); the large central nucleus (ICc) remained free of label (see Fig. 3). In the cochlear nucleus, labelled terminals and cells were seen within both the ventral (Cv) and dorsal (Cd) divisions (Fig. 3).

The labelling seen after each ZI injection was seen on the ipsilateral side, except for the cochlear labelling which was contralateral. There was no evidence for bilateral connectivity for any of the auditory centres with the ZI.

Auditory injections

In the foregoing section, it was shown that distinct parts of the various auditory centres have connections with the ZI. In the next series of experiments, three of these centres were injected with tracer (CTb or BD) and the resultant labelling patterns were examined in the ZI. The centres injected were Te3, ICe and Cv, each of which contained labelled profiles after ZI injections. From these experiments, the existence of an "auditory" subsector in the ZI could be determined.

The distribution of ZI labelling after an injection of CTb into Te3 is shown in Fig. 4. The injection site is shown in Fig. 4A, and was confined largely to Te3, but with some spread into Te1. The general labelling in the midbrain and thalamus after such an injection is shown in Fig. 4B. Here, heavy labelling was evident in MGm and MGd, and light labelling was seen in the MGv, as well as the ICe of the midbrain (see Webster 1995; Zilles and Wree 1995). The labelling within the ZI is shown in more detail in Fig. 4C and it was made up exclusively of terminals. No labelled cells were ever seen in ZI after Te3 injections. Labelled terminals appeared in the lateral region of the dorsal and ventral sectors of the ZI, and were organised into two distinct slabs or bands (Fig. 4C). One slab was positioned in the lateral half of ventral sector, close to the cerebral peduncle while the other was located more dorsally, on the boundary between dorsal and ventral sectors (Fig. 4C). This distinctive pattern was seen after all cortical injections of tracer (n=4), regardless of their location in either more rostral or caudal re-

Fig. 3 Schematic diagrams showing the distribution of labelled terminals and cells in the major auditory centres after injection of CTb into the ZI of the thalamus. Three sections, from rostral to caudal, have been drawn for the temporal cortex, medial geniculate complex, inferior colliculus and the cochlear nucleus. The numbers at the bottom of each figure represent distance in mm from bregma. Note that the cochlear labelling is contralateral, while the others are ipsilateral. The injection site is shown in the *inset* at the bottom. The striking feature of this labelling is that it was found in distinct parts of each of the major auditory centres

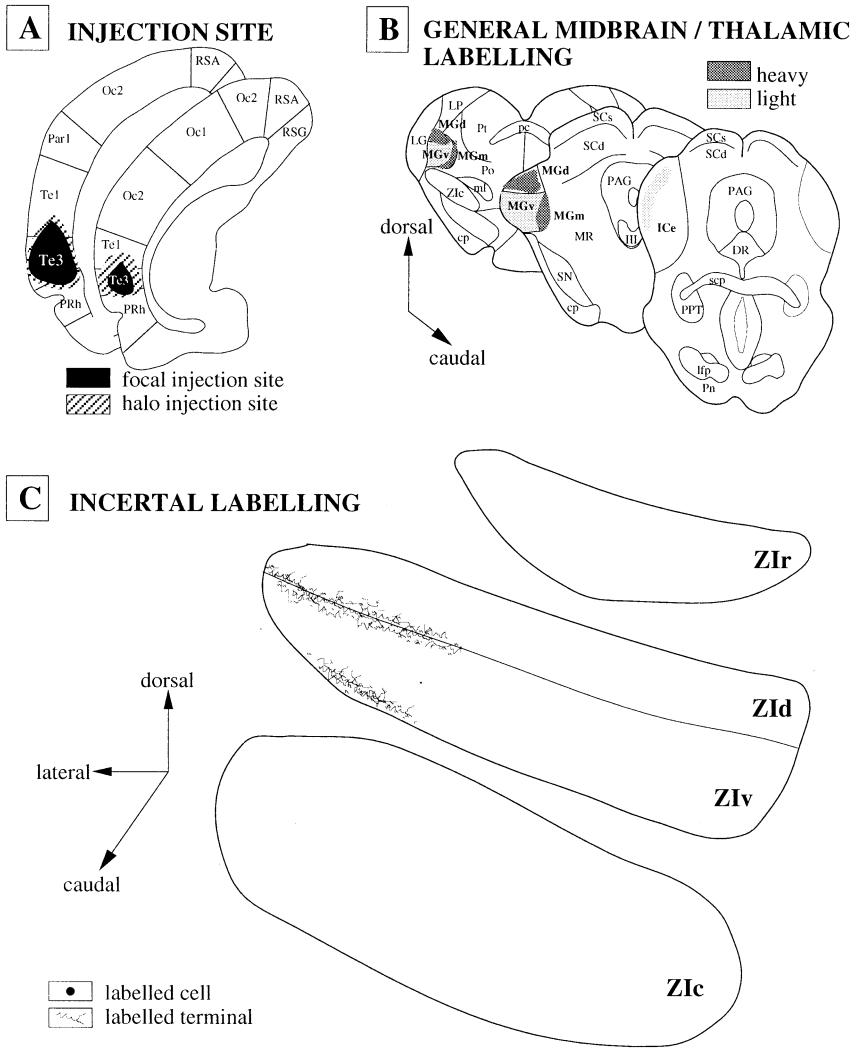


Fig. 4 Schematic diagrams of a CTb injection site in the Te3 cortex (**A**); the resultant general labelling in the auditory midbrain and thalamus (represented as shading) (**B**); and the resultant detailed labelling seen in the ZI (represented as individual labelled axons and cells). Note the distinct slabs of label in the lateral region of the ZI

gions of the Te3. This would indicate little topography of projection from Te3 to the ZI.

A very similar distribution of labelling was seen in the ZI after tracer injections into the ICe (Fig. 5). In the case shown in Fig. 5, the labelling in the ZI was seen in two spatially distinct slabs, in similar locations to those described for the Te3 injections. The major slab of label was in the ventral sector, adjacent to the cerebral peduncle (Fig. 5C; unfilled arrows Fig. 2), while the other slab was found more dorsally, within the lateral third of the nucleus (Fig. 5C; arrowhead Fig. 2). Unlike the Te3 labelling, however, the labelling from the ICe was made up both of cells and terminals, rather than just terminals as from the Te3 injections (Fig. 5C; see Fig. 2). Further, the more dorsally located labelled slab was found over a larger area of the dorsal sector, not being limited to the boundary with the ventral sector (Fig. 5C). In the other cases examined (total of four), the injections sites of which were located in more rostral regions of the ICe, labelling was located in the same region of the ZI, indicating little evidence for topography. From each injection site within the ICe, heavy labelling was localised largely to the MGm while lighter labelling was seen in the MGv and MGd (Fig. 5B).

Figure 6 shows the distribution of labelling following an injection of CTb into Cv, with some spread into the Cd. In this case, all in the others (n=3), labelling in the ZI was restricted to the lateral edge of the nucleus, as seen in the ICe and Te3 injections. Labelled cells and terminals were seen in two slabs, one in the ventral sector, and the other at the border of the ventral and dorsal sectors. Within the midbrain for each cochlear injection, heavy labelling was localised to the ICe while lighter labelling was seen in the ICd and ICe afterwards (Fig. 6B) (Webster 1995).

Discussion

There are two major findings of this study; first, that there are preferential connections between the ZI and particular subdivisions of the major centres of the auditory system, and second, that these connections relate to a distinct territory or subsector within the ZI. The signif-

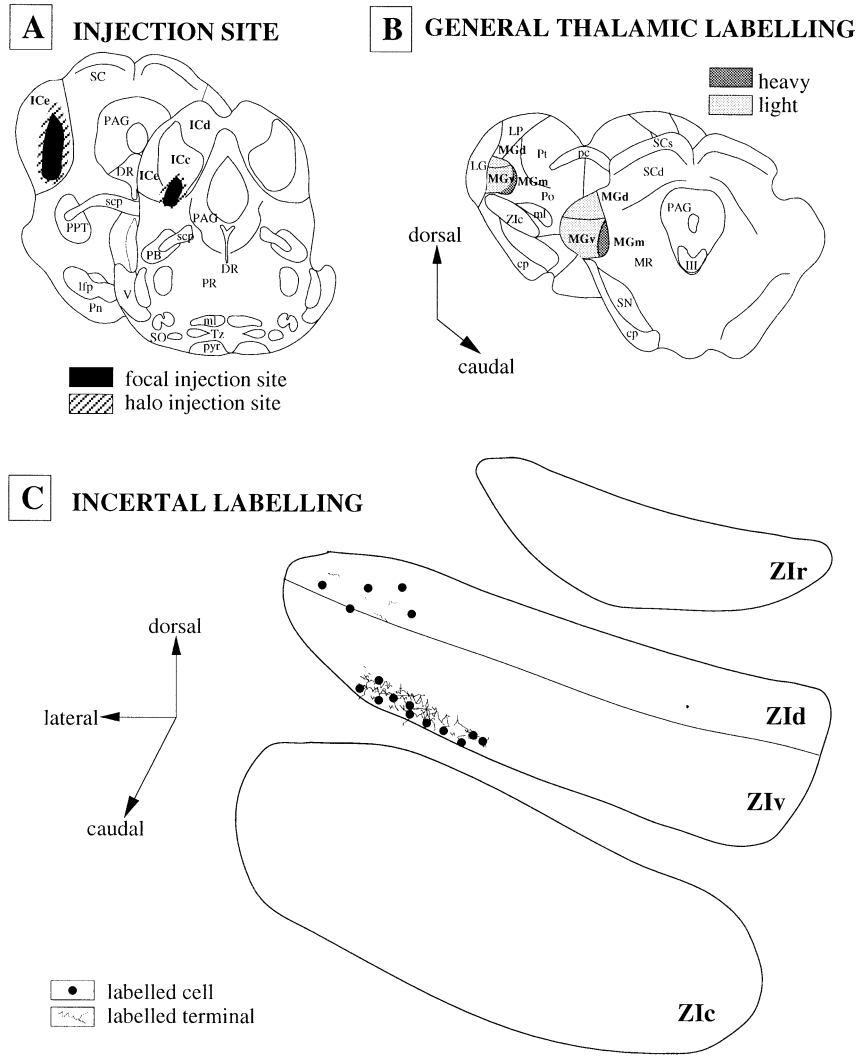


Fig. 5 Schematic diagrams of a BD injection site in the ICe (**A**); the resultant general labelling in the auditory thalamus (represented as shading) (**B**); and the resultant detailed labelling seen in the ZI (represented as individual labelled axons and cells). Note the distinct slabs of label in the lateral region of the ZI

icance of these findings will be considered below. First, a comparison with previous studies will be made.

Several previous studies have made mention of auditory connections with the ZI, but these have been part of more generalised reports and the precise details were not considered (Ricardo 1981; Shiosaka et al. 1985; LeDoux et al. 1987; Sakanaka et al. 1987). Hence, the paucity and patterns of termination within ZI for most auditory centres was generally unclear from previous reports. Perhaps the only centre that has had its connections with the ZI considered in a little more detail than others is the inferior colliculus (Shiosaka et al. 1985; LeDoux et al. 1987; Sakanaka et al. 1987). After tracer injections into the inferior colliculus, a thin slab of label was reported in the ZI, adjacent to the cerebral peduncle. Such a slab was also apparent after collicular injections in this study. The more detailed analysis carried out in the present study reveals an additional group of labelled terminals and cells located more dorsally in the ZI.

This study shows that auditory afferents (from Te3, ICe, cochlear nucleus) converge in the ZI in two slabs in the lateral region of the nucleus. One slab is located in the ventral sector, adjacent to the cerebral peduncle; the other is located at the boundary between the dorsal and ventral sectors (Fig. 7). Hence, the results suggest that there may be a distinct subsector of ZI associated with audition. Other modality specific subsectors have also been identified in the ZI, and include a motor related subsector in the medial regions of the rostral, dorsal, ventral and caudal sectors (Mitrofanis and deFonseka 2001), a somatosensory subsector spanning across the midregions of the dorsal and ventral sectors (Nicolelis et al. 1992), a limbic subsector in the dorsal and rostral sector (Kolmac et al. 1998; Mitrofanis and Mikuletic 1999) and a visual subsector converging onto a small area within the lateral edge of the ZI (Power et al. 2001) (Fig. 7). These subsectors, together with the auditory one described in this study, are largely independent of the well defined cytoarchitectonic subsectors of the ZI, since they include regions of either the rostral, dorsal, ventral and caudal sectors. Thus, ZI organisation may be based on a set of modality specific subsectors, superimposed on the main cytoarchitectonic sectors (Fig. 7). The significance of this organisation is not clear, but the follow-

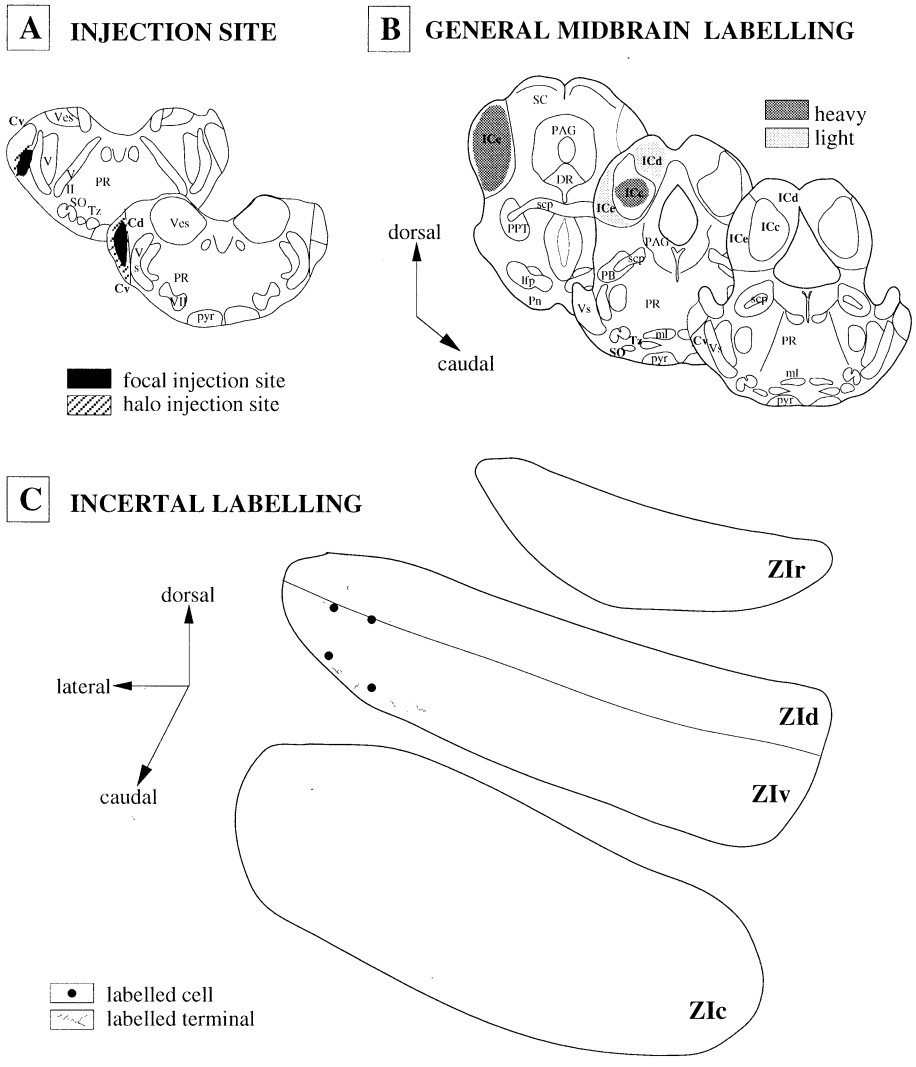


Fig. 6 Schematic diagrams of a BD injection site in the Cv (**A**); the resultant general labelling in the auditory midbrain (represented as shading) (**B**); and the resultant detailed labelling seen in the ZI (represented as individual labelled axons and cells). Note the sparsely labelled profiles in the lateral region of the ZI

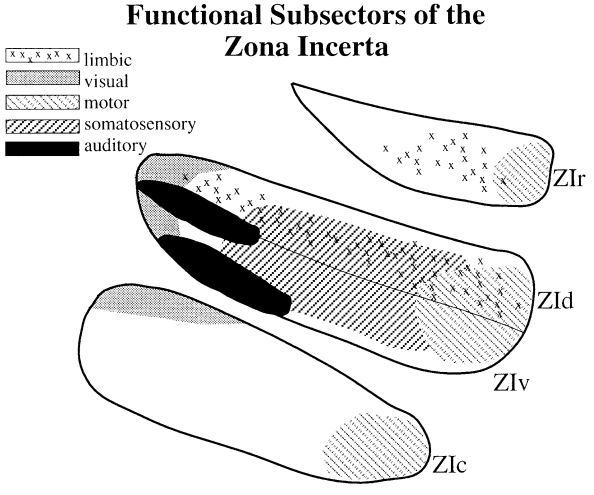


Fig. 7 Summary schematic diagram showing the formation of modality specific subsectors, "superimposed" over the cytoarchitectonic sectors of the ZI. There may be some overlap between adjacent subsectors (e.g. visual and auditory). Limbic: Kolmac et al. (1998), Mitrofanis and Mikuletic (1999); visual: Power et al. (2001); motor: Mitrofanis and deFonseka (2001); somatosensory: Nicolelis et al. (1992), Shaw and Mitrofanis (2002); auditory: present study

ing has been suggested (Mitrofanis 2002). Modality specific brain centres may each receive neurochemically diverse inputs from ZI cells of distinct sectors. For instance, the inferior colliculus may receive a glutamatergic input from cells of the dorsal sector, as well as a GABAergic input from cells of the ventral sector (see Kolmac and Mitrofanis 1999a). Hence, the ZI may furnish both excitatory and inhibitory projections to the same target. A future double labelling study and/or electrophysiological should clarify these issues.

The precise role for ZI in audition is not known, but the novel finding that ZI interconnects with particular subdivisions of the major auditory centres, each involved in different aspects of audition, provides a possible clue. For several reasons, the ZI does not appear to have a role in transmitting the key characteristics of a sound, such as the tone, pitch and frequency (so-called "core" pathway) (Tokunaga et al. 1984; LeDoux et al. 1987; Kaas et al. 1999). First, the ZI has no connections with the major regions dealing with this aspect of audition, namely Te1, MGv and ICc. Second, there appears no clear topography of projection, since projections from the different auditory centres (from Te3, ICe, cochlear nucleus) con-

verge onto the same ZI territory, rather than terminating in spatially distinct zones of the nucleus (e.g. forming tonotopic maps).

There is also little evidence implicating the ZI in the process of sound localisation, since the auditory projections to the ZI show no apparent topography (see above) and more importantly, they do not overlap the ZI region (main body of ventral sector) that projects to the deep layers of the superior colliculus, a structure deemed pivotal in localising a sound in relation to the visual field (Aitkin et al. 1981; Schweizer 1981; Tokunaga et al. 1984; Cadusseau and Roger 1985; Kim et al. 1992; Kolmac et al. 1998). In further support of this notion, the present results show that ZI has no connections with the superior olivary nucleus and trapezoid body, two nuclei involved in sound localisation (Webster 1995).

It is likely that the ZI is linked to a more integrative and associative function in audition (so-called "belt" pathway), since the ZI has diffuse, albeit preferential connections with the key "belt" pathway regions, namely, Te3, MGm, ICe (Tokunaga et al. 1984; LeDoux et al. 1987; Kaas et al. 1999). Indeed, a major function ascribed to the ZI recently has been as a thalamic centre for multimodal integration (Kolmac et al. 1998; Power and Mitrofanis 1999a, 2001). The ZI may sample inputs from the major "associative" auditory centres and then integrate them, through the considerable intersector connections of the ZI (Power and Mitrofanis 1999a, 2001), with the smorgasbord of other exteroceptive and interoceptive sensory afferents it receives (see Fig. 7; Introduction). Subsequently, ZI may influence general arousal and/or attentive states through its large projection to the dorsal thalamus, in particular to the intralaminar and higher order nuclei (Power et al. 1999; Power and Mitrofanis, 1999a, 2001).

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References

Aitkin LM, Irvine DRF, Webster WR (1984) Central neural mechanisms of hearing. In: Smith D (ed) Handbook of physiology. American Physiological Society, New York, pp 737–878

Angelucci A, Clascà F, Sur M (1996) Anterograde axonal tracing with the subunit B of cholera toxin: a highly sensitive immunohistochemical protocol for revealing fine axonal morphology in adult and neonatal brains. J Neurosci Meth 65:101–112

Cadusseau J, Roger M (1985) Afferent projections to the superior colliculus in the rat, with special attention to its deeper layers. J Hirnforsch 26:667–681

Coleman KA, Mitrofanis J (1996) Organisation of the visual reticular thalamic nucleus of the rat. Eur J Neurosci 8:388–404

Coleman KA, Baker GE, Mitrofanis J (1997) Topography of fibre organisation in the corticofugal pathways of rats. J Comp Neurol 381:143–157

Edwards DA, Isaacs S (1991) The zona incerta lesions: effects on copulation, partner preference and other sociosexual behaviours. Behav Brain Res 44:145–150

Jones EG (1985) The thalamus. Plenum Press, New York

Kaas JH, Hackett TA, Tramo MJ (1999) Auditory processing in primate cerebral cortex. Curr Opin Neurobiol 9:164–170

Kawana E, Watanabe K (1981) A cytoarchitectonic study of the zona incerta in the rat. J Hirnforsch 22:535–541

Kim U, Gregory E, Hall WC (1992) Pathway from the zona incerta to the superior colliculus in the rat. J Comp Neurol 321:555–575

Kolmac CI, Mitrofanis J (1999a) Distribution of various neurochemicals within zona incerta: an immunocytochemical and histochemical study. Anat Embryol 199:265–280

Kolmac CI, Mitrofanis J (1999b) Organisation of the basal forebrain projection to the thalamus in rats. Neurosci Lett 272:151–154

Kolmac CI, Power BD, Mitrofanis J (1998) Patterns of connections between the zona incerta and brainstem in rats. J Comp Neurol 396:544–555

LeDoux JE, Ruggiero DA, Forest R, Stornetta R, Reis DJ (1987) Topographic organisation of convergent projections to the thalamus from the inferior colliculus and spinal cord in the rat. J Comp Neurol 264:123–146

Lin CS, Nicolelis MA, Schneider JS, Chapin JK (1990) A major direct GABAergic pathway from the zona incerta to neocortex. Science 248:1553–1556

Ma TP, Hu XJ, Anavi Y, Rafols JA (1992) Organisation of the zona incerta in the macaque: a Nissl and Golgi study. J Comp Neurol 320:273–290

May PJ, Sun W, Hall WC (1997) Reciprocal connections between the zona incerta and the pretectum and superior colliculus of the cat. Neuroscience 77:1091–1114

Mitrofanis J (2002) Distinctive patterns of connectivity between the zona incerta and the red nucleus of rats. Anat Embryol (in press)

Mitrofanis J, deFonseka R (2001) Organisation of connections between the zona incerta and the interposed nucleus. Anat Embryol 204:153–159

Mitrofanis J, Mikuletic L (1999) Organisation of the cortical projection to the zona incerta of the thalamus. J Comp Neurol 412:173–185

Mogenson GJ, Swanson LW, Wu M (1985) Evidence that projections from substantia innominata to the zona incerta and mesencephalic locomotor region contribute to locomotor activity. Brain Res 334:65–76

Nicolelis MA, Chapin JK, Lin RC (1992) Somatotopic maps within the zona incerta relay parallel GABAergic somatosensory pathways to the neocortex, superior colliculus and brainstem. Brain Res 577:134–141

Nicolelis MA, Chapin JK, Lin RC (1995) Development of direct GABAergic projections from the zona incerta to the somatosensory cortex of the rat. Neuroscience 65:609–631

Paxinos G, Watson CJ (1986) The rat brain in stereotaxic coordinates, 2nd edn. Academic Press, New York

Power BD, Mitrofanis J (1999a) Evidence for extensive interconnections within the zona incerta. Neurosci Lett 267:9–12

Power BD, Mitrofanis J (1999b) Specificity of projection among cells of the zona incerta. J Neurocytol 28:481–493

Power BD, Mitrofanis J (2001) Zona incerta: substrate for contralateral interconnectivity in the thalamus of rats. J Comp Neurol 436:52–63

Power BD, Kolmac CI, Mitrofanis J (1999) Evidence for a large projection from the zona incerta to the dorsal thalamus. J Comp Neurol 404:554–565

Power BD, Leamey CA, Mitrofanis J (2001) Evidence for a visual subsector within the zona incerta. Vis Neurosci 18:179–186

Reardon FM, Mitrofanis J (2000) Organisation of the amygdalothalamic pathways in rats. Anat Embryol 201:75–84

Ricardo JA (1981) Efferent connections of the subthalamic region in the rat. II. The zona incerta. Brain Res 214:43–60

Roger M, Cadusseau J (1985) Afferents to the zona incerta in the rat: a combined retrograde and anterograde study. J Comp Neurol 241:480–492

Romanowski CAJ, Mitchell IJ, Crossman AR (1985) The organisation of the efferent projections of the zona incerta. J Anat 143:75–95

Sakanaka M, Shibasaki T, Lederis K (1987) Corticotrophin releasing factor containing afferents to the inferior colliculus of the rat brain. Brain Res 414:68–76

Schweizer H (1981) The connections of the inferior colliculus and the organisation of the brainstem auditory systems in the greater horseshoe bat. J Comp Neurol 201:25–29

Shammah-Lagnado SJ, Negrao N, Ricardo JA (1985) Afferent connections of the zona incerta: a horseradish peroxidase study in the rat. Neurosci 15:109–134

Shaw VE, Mitrofanis J (2002) Lamination of spinal cells projecting to the zona incerta in rats. J Neurocytol (in press)

Shiosaka S, Kawai Y, Shibasaki T, Tohyama M (1985) The descending αMSHergic projections from the zona incerta and lateral hypothalamic area to the inferior colliculus and spinal cord in the rat. Brain Res 338:371–375

Tokunaga A, Sugita S, Otani K (1984) Auditory and non auditory subcortical afferents to the inferior colliculus in the rat. J Hirnforsch 25:461–472

Wagner CK, Eaton MJ, Moore KE, Lookingland KJ (1995) Efferent projections from the region of the medial zona incerta containing A13 dopaminergic neurones: a PHA-L anterograde tract-tracing study in the rat. Brain Res 677:229–237

Watanabe K, Kawana E (1982) The cells of origin of the incertofugal projections to the tectum, thalamus, tegmentum and spinal cord in the rat: a study using autoradiographic and horseradish peroxidase methods. Neuroscience 10:2389–2406

Webster WR (1995) Auditory system. In: Paxinos G (ed) The rat nervous system, 2nd edn. Academic Press, New York

Zilles K, Wree A (1995) Cortex: areal and laminar structure. In: Paxinos G (ed) The rat nervous system, 2nd edn. Academic Press, New York