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TOPOGRAPHY IN THE PROJECTIONS OF LATERAL POSTERIOR THALAMUS WITH CINGULATE AND MEDIAL AGRANULAR CORTEX IN RELATION TO CIRCUITRY FOR DIRECTED ATTENTION AND NEGLECT

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Abstract

In the rat, the lateral posterior thalamic nucleus (LP) has reciprocal connections with areas of the cortex and the striatum involved in directed attention and its dysfunctional counterpart, contralateral neglect. It has also been shown that the medial portion of the mediorostral part of LP (mLPMR) is of special interest because it has connections with the dorsocentral striatum, a key node in this circuitry. In the present study we used neuroanatomical tracers to map the specific connections and topography of LP with the anterior cingulate cortex (ACC) and medial agranular cortex (AGm). We primarily used Alexa Fluor conjugates of the retrograde tracer cholera toxin subunit B, and injected two different colored conjugates into ACC and AGm in the same animal in order to directly compare the differential topography of the thalamocortical connections of mLPMR. The bidirectional tracer, dextran amine, was also used to examine anterograde corticothalamic projections of AGm and ACC. We found that mLPMR consists of two distinct groups of neurons, with the more dorsal group projecting to ACC and the more ventral group projecting to AGm. This is mirrored by a similar corticothalamic topography. These findings suggest that the ventral mLPMR is specifically associated with AGm and dorsocentral striatum, while dorsal mLPMR is associated with ACC. They also suggest that ACC may play a role in the circuitry for directed attention and contralateral neglect, as it is known to do in humans.

Keywords

thalamocortical; contralateral neglect; tract tracing; cholera toxin; retrograde; neuroanatomy

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Section: Structural Organization of the Brain

1. Introduction

The rat lateral posterior thalamic nucleus (LP) has connections with associative regions of cerebral cortex (Reep et al., 1990; Reep et al., 1994; Sefton et al., 2004; Sukekawa, 1988) and with superior colliculus (McHaffie et al., 2005; Sefton et al., 2004; Taylor et al., 1986), suggesting that it participates in multimodal integration and behavior. More specifically, the connections of LP indicate that it may play a key role in the circuitry for directed attention, and its dysfunctional counterpart, contralateral neglect. Recent studies from our group have indicated that the connections of LP are complex and topographic (Chandler et al., 1992; Kamishina et al., 2006; Kamishina et al., 2008).

LP has reciprocal connections with medial agranular cortex (AGm) and posterior parietal cortex (PPC). Unilateral lesions of either AGm or PPC produce contralateral neglect of visual, auditory and tactile stimuli, as do knife cuts that disconnect the corticocortical axons linking AGm and PPC (Reep et al., 2004). Additionally, LP projects to the dorsocentral striatum (DCS), which is the site of corticostriatal projections from AGm and PPC (Kamishina et al., 2008; Reep et al., 2003). DCS is a key node in the network subserving directed attention and it is necessary for recovery from neglect (Van Vleet et al., 2003a). Therefore, a detailed understanding of the connections of LP is of particular interest due to its central role in the network of brain regions implicated behaviorally in directed attention and neglect.

The central region of DCS (cDCS) receives LP input from only the far medial portion of mediorostral LP (mLPMR) (Kamishina et al., 2008). Furthermore, we have found that mLPMR has reciprocal connections with AGm (Kamishina et al., 2006), and AGm projects to cDCS (Kamishina et al., 2008; Reep et al., 2003). Thus, there is a complete thalamic-cortical-striatal loop linking mLPMR, AGm, and cDCS. Cortical area PPC has reciprocal connections with central LPMR, which projects to the dorsal periphery of DCS (Kamishina et al., 2006; Kamishina et al., 2008; Reep et al., 2003). The dorsal periphery of DCS is a target of input from PPC (Kamishina et al., 2008). Therefore, a second thalamic-cortical-striatal loop links central LPMR, PPC, and dorsal peripheral DCS.

Of particular interest is the specific topography within LPMR, since this area serves as an important relay node in the circuitry due to its connections to cortical areas AGm and PPC, and to DCS. Our group has discovered that there is very little overlap in the populations of neurons projecting from LPMR to AGm and PPC. Double injections of retrograde tracers into AGm and PPC revealed no double-labeled neurons in LPMR, and virtually complete segregation of these neighboring cell populations (Kamishina et al., 2006). The neurons projecting to AGm are located in the ventral portion of mLPMR (Kamishina et al., 2006), and retrograde transport after tracer injections in cDCS produces neuron labeling in the same region (Cheatwood et al., 2003). The dorsal portion of mLPMR was labeled in cases where the injection site in AGm encroached upon the medially adjacent anterior cingulate cortex (ACC). Figure 1 gives an overview of the location of ACC compared to other areas of the cortex.

Given the role of this circuitry in multimodal processing and neglect, the purpose of the present study was to use retrograde tracers to test the hypothesis that dorsal mLPMR projects to ACC, whereas ventral mLPMR projects to AGm, with no overlap between these neuronal populations. We used the newly available Alexa Fluor (AF) conjugates of cholera toxin subunit B (CTB). Thereby, we wished to ascertain a more complete picture of the connectivity of mLPMR, and its potential role in circuitry related to directed attention, neglect, and processing of multimodal information.

2. Results

Although we focused our analysis on the labeling only within LPMR and nearby nuclei, other labeling was also seen in many other nuclei as previously reported by other studies for this pathway. Figure 1 indicates the anterior-posterior (AP) range from bregma of cortical injection sites made in this study.

2.1. Retrograde Cases

2.1.1. Injections made into the caudal half of ACC—The injection site in AGm for case 417 was located at AP -1.3, and the ACC injection was located at AP -0.2 (Fig. 2A-B). The ACC injection for case 420 was also at AP -0.2, but the AGm injection was at AP -0.1. The injection site centers for case 420 were located on the same section. Additionally, the ACC injection in case 420 was the bidirectional tracer 3k dextran amine (3kDA), while the ACC injection in case 417 was the retrograde tracer AF 488 CTB. In case 420, the AGm injection site was actually on the border of AGm/ACC, with some of the tracer within ACC.

Both cases showed similar thalamic labeling patterns. Figure 2C-E shows representative sections from case 417 with a focus on mLPMR. Additionally, figure 2F-G shows high powered confocal micrographs showing the detailed morphology of CTB-labeled neurons in case 417, and confirms that CTB did indeed transport retrogradely to primarily label the cell bodies. In each case, the ACC injection site resulted in labeling in the more dorsal portion of mLPMR, while the AGm injection sites produced labeling in a more ventral portion of mLPMR. There was no overlap of labeling in either case, and confocal imaging confirmed no apparent double-labeled cells. In case 417, there was no labeling between the dorsal and ventral labeled portions of mLPMR, while the two different groups of labeling in case 420 were contiguous on their border. This is likely a reflection of the injection site locations, because case 420 had injections at the same AP level while case 417 had injections at different AP levels. In case 420, the 3kDA tracer revealed both anterograde (discussed below in section 2.2) and retrograde labeling in the same location in the more dorsal portion of mLPMR.

Case 431 had injections into ACC and AGm at AP -0.2, but had a clear separation of injection sites (fig. 2H). Unlike case 417, this case had both injections at the same AP level. The AF 488 CTB injection was focused near the border of dorsal/ventral ACC, and AF 594 CTB was injected into AGm but was very close to the AGm/ACC border so it most likely also affected ACC. The ACC injection site appears very diffuse, but is focused in ACC. However, there also appears to be a faint band of AF 488 CTB tracer uptake in layer 1 of both ACC and AGm. An apparent overlap of the AGm injection site into ACC was apparent based on some yellow double-labeled neurons in mLPMR (figure 2I-M). High-powered confocal images in figure 2L and M confirm the presence of double labeled cells (yellow in appearance) and show an overlap of red and green neurons at the border between dorsal and ventral mLPMR. Nevertheless, the AGm injection consistently labeled neurons in the more ventral portion of mLPMR and the ACC injection labeled neurons in the more dorsal portion of mLPMR.

Case 428 (not illustrated) exhibited a similar pattern of labeling as case 431. Injections of AF 488 CTB were made in ACC and AF 594 CTB was injected into AGm at the same AP level (AP -0.9). Imaging revealed that the two injection sites were directly adjacent to each other near the ACC/AGm border. The red injection, which was intended for AGm, encroached somewhat into ACC. Labeling in the thalamus in case 428 revealed overlapping and double-labeling in mLPMR, similar to that seen in case 431, and consistent with the finding that the injection site of AF 594 CTB exhibited overlap into area ACC. However, even though the labeling pattern was overlapping, the AF 488 CTB ACC labeling was focused in the more dorsal portion of mLPMR, and the AF 594 CTB AGm/ACC injection showed labeling that was more focused in ventral mLPMR.

Case 423 also had similar patterns of labeling to these cases. The ACC injection site was at AP -1.0 and produced labeling in the dorsal portion of mLPMPR. However, there was an apparent problem with the uptake of AF 594 CTB into AGm since there was no labeling present anywhere in the brain.

2.1.2. Injections made into the rostral half of ACC—In case 426, injections of AF 488 CTB into ACC and AF 594 CTB into AGm were made at the same AP level of +0.8 (figure 3A). There is an apparent injection track of AF 488 CTB that goes through AGm. There also appears to be significant uptake of AF 488 CTB tracer in layer 1 of both ACC and AGm. The ACC injection labeled neurons in the dorsal portion of mLPMPR near the border of the centrolateral nucleus (CL) (figure 3B-G). Likewise, the AGm injection labeled neurons in the more ventral portion of mLPMPR. However, there were a fair amount of double-labeled cells present between the two areas of labeling and within the dorsal portion (figure 3E, F-G). This suggests that there was either some overlap of the AGm injection into ACC, a result of the AF 488 CTB injection tract present through AGm, or even as a result of the uptake of tracer by layer 1 of AGm. The double-labeled cells were present on the more caudal sections of mLPMPR. There were also some neurons labeled from ACC in the more lateral part of LPMR.

In Case 429 (not illustrated), injections of AF 488 CTB into ACC and AF 594 CTB into AGm were made at the same AP level of +0.9. The AGm injection did not overlap into ACC. A small injection tract was noticeable from the ACC injection that may be within AGm. The ACC injection produced labeled neurons that were located less dorsally in mLPMPR than other cases with injections in ACC. However, the AGm injection produced labeled neurons ventral to the ACC labeling in mLPMPR, similar to the pattern seen in other cases.

The following cases, which had injections into rostral ACC, are not illustrated but revealed confirmatory results for the previously reported cases. Case 439, which had double injections at AP +1.0, had similar patterns of labeling to case 426 where the ACC injection labeled dorsal cells of mLPMPR and the AGm injection labeled ventral cells of mLPMPR. The ACC injection site (AP +1.6) for case 432 overlapped into the AGm injection site and produced a large amount of double labeled cells at the dorsal/ventral mLPMPR border. However, the AGm injection site revealed consistent labeling in the more ventral mLPMPR. Case 425 had an ACC injection site at AP +2.0, but the AGm injection site produced no labeling anywhere in the brain, most likely due to a surgical error. The ACC injection did however produce labeling in the more dorsal portion of mLPMPR, along similar lines to the other cases.

2.2. Dextran amine cases

Out of the 8 cases that used 3kDA in AGm, all cases showed labeling in LPMR from the AGm injection site. Due to the less-viscous nature of 3kDA as compared to CTB, we were only able to successfully inject 3kDA into ACC in one case. We discuss only two cases here, but these are representative of the other successful cases.

Injections of the bidirectional tracer tetramethylrhodamine 3kDA were made into AGm in case 372 (AP -0.6) and into ACC in case 420 (AP -0.1). The injection site of case 372 partially overlapped into the lateral agranular cortex (AGl) (figure 4A). The injection site of case 420 was confined to ACC (figure 4C). Note that case 420 also had a second injection of AF 488 CTB made into AGm near the boundary of the ACC injection site (see section 2.1.1 above), but was excluded from figure 3 since it produced only retrograde labeling.

Labeling in both cases produced mostly dense anterograde axonal labeling, but some retrogradely labeled neurons are also visible. In the context of LPMR labeling, the AGm injection in case 372 produced dense anterograde labeling only in the more ventral portion of mLPMPR and some light labeling in posterior thalamic nucleus (Po) and mediodorsal thalamic

nucleus (MD) (figure 4B). The ACC injection in case 420 produced dense anterograde labeling in the dorsal portion of mLPMR, and lightly in the laterorostral part of LP (LPLR), with some retrogradely labeled cells in lateral MD (MDl) (figure 4D). There was also noticeable anterograde labeling in the caudal portion of the lateral dorsal thalamic nucleus (LD).

3. Discussion

The present results suggest that the most medial portion of mLPMR has a more fine grained topography than previously realized. Our results show that mLPMR has a dorsal group of neurons that is connected with ACC, while a more ventral group projects to AGm. In addition, the reciprocal corticothalamic projections to mLPMR from AGm and ACC exhibit a similar topography.

Our results demonstrate a clear separation of the projections from mLPMR to ACC and AGm. However, this separation diminished when the injection sites were located near the boundary of ACC/AGm. Nevertheless, in every case the labeling related to ACC was always more dorsal to the labeling related to AGm. This pattern of labeling was consistent through various levels of ACC, from +2.0mm rostrally to -1.0mm caudally. The AGm injections that encompassed various levels also produced consistent patterns of labeling. Within our range of study, the same rostrocaudal extent of neuron labeling was seen in dorsal mLPMR, regardless of the rostrocaudal location of the injection site in ACC. This could be due to individual LP neurons having branching axons to ACC. Additionally, we often noticed double-labeled cells near the dorsal/ventral mLPMR border when the AGm injection site encompassed part of the ACC. This could suggest that dorsal mLPMR contains branching axons to ACC, since the double-labeled cells were absent when the AGm injection site was contained exclusively in AGm. We do not suspect that LP has neurons that branch to both AGm and ACC, since double labeled cells were only present in cases where the AGm injection appeared to overlap into ACC, but it is possible that dorsal mLPMR has branching axons to different areas of ACC, or the ventral AGm border, and a future study should be undertaken to examine this possibility.

These findings have several implications for the functional neuroanatomy of directed attention since they show that LPMR contains a subset of different cell groups that are involved in different components of the network for directed attention. Only the ventral portion of mLPMR projects to cDCS, which is important in spatial attention (Kamishina et al., 2008). Further studies need to be undertaken to clarify the striatal projections of dorsal mLPMR and the behavioral significance of these connections.

In humans, the cingulate cortex is one of the regions implicated in the attentional deficits of neglect and thus spatial attentional processes in general (Buklina, 2002; Heilman and Valenstein, 1972; Hopfinger et al., 2000; Leibovitch et al., 1998; Mesulam, 1981; Mesulam, 1999; Sturm et al., 2006; Swan, 2001; Watson et al., 1973). Heilman and Valenstein (1972) reported a patient who had neglect as a result of a lesion confined to the anterior cingulate cortex (and adjacent white matter). Similarly, Watson et al. (1973) found that lesions to the cingulate cortex in the macaque monkey produced neglect. More recent functional imaging studies in humans have suggested that the ACC plays an important role in attentional control (Crottaz-Herbette and Menon, 2006; Milham et al., 2001).

Although further studies need to be done, it is possible that in the rodent the cingulate cortex also participates in the network for directed attention. This conjecture is consistent with the theory presented by Mesulam (1981), who also suggests that an interruption in a cortical network for directed attention produces neglect in humans. Most recently in rats, Ng et al. (2007) have found that the anterior and posterior cingulate cortex are crucial for being able to shift attention between closely related meaningful cues and thus may be important for screening

out irrelevant information. In the rodent, disruption of any cortical component of the network produces neglect.

It is very difficult to selectively lesion ACC without damage to AGm, and vice versa. In rat neglect studies, typical lesions of AGm also include variable amounts of damage to ACC (Corwin et al., 1986; King and Corwin, 1992; King and Corwin, 1990; Van Vleet et al., 2003b). In most previous anatomical tracing studies that have injected a retrograde tracer into the cingulate cortex, the injection site has also inadvertently involved AGm. Additionally, injection of an anterograde tracer directly into only the dorsal portion of mLPMPR is even more difficult to isolate precisely.

This is the first study examining the connections of LP using the new fluorescent conjugates of CTB, which is a very sensitive retrograde tracer. While other work using non-fluorescent CTB has been undertaken, the fluorescent conjugates used in this present report allowed us to precisely compare the input to ACC and AGm in the same animal. Compared to other tracers such as dextran amine, CTB is generally very viscous and can often be injected into a very small and discrete region. Thus, we were able to successfully inject the tracer into ACC without any overlap into AGm. Some cases where one injection site was located near the border between ACC and AGm produced useful information through double-labeled cells in mLPMPR. However, we often found that CTB diffused through layer 1 of the cortex easily, and even on the contralateral side, which can be seen in cases 431 and 426 (figure 2H and figure 3A, respectively). We believe this was due to the location of the ACC injection being so close to the sagittal sinus, rather than a property of the tracer itself. Since pressure injections were used, it may be the case that some of the tracer leaked into the sagittal sinus and was then reabsorbed by terminals in layer 1 of the cortex near the injection site. In our experience, cases that involved a deeper cortical injection did not have this diffusion problem, but these confounds were unavoidable due to the location of ACC, and may be dependent on the actual volume injected. The optimized methodology and the availability of new technologies is probably the reason why previous reports have not identified the role of ACC in the neuroanatomy of neglect. Additionally, it is possible that other studies have either overlooked or downplayed connections to LP since most previous work has focused on the connectivity between ACC, anterior thalamic nuclei and MD.

In addition to defining the topography of mLPMPR, this study also noted labeling in other thalamic nuclei, consistent with previous studies. The thalamic projections to ACC previously reported are mainly from anteromedial (AM), ventromedial (VM), centrolateral (CL), paracentral (PC), and mediodorsal (MD) thalamic nuclei (Hoover and Vertes, 2007; Horikawa et al., 1988; Shibata, 1993; Shibata and Kato, 1993; van Groen et al., 1999; Vertes, 2002; Wang and Shyu, 2004), and we noted retrograde labeling in all these nuclei after injection into ACC (also see Hoover and Vertes (2007) for an overview). Previous studies have identified the thalamic projections of the ACC as primarily to AM (Beckstead, 1979; Shibata and Naito, 2005; van Groen et al., 1999), anterodorsal (AD) (Shibata and Naito, 2005), anteroventral (AV) (Beckstead, 1979; Shibata and Naito, 2005), and LD (Shibata and Naito, 2005). Note that the results from our 3kDA case shows that ACC has a projection to LD, as confirmed by Shibata et al. (2005), but our results did not show any input from LD and this confirms the findings of Hoover and Vertes (2007).

Very few studies have reported data about the connections between ACC and LP. However, some of the cases from previous reports that examined other connections also include findings related to LP. Vertes (2002) was able to inject the anterograde tracer *phaseolus vulgaris*-leucoagglutinin into ACC without any overlap in AGm and illustrated a projection to the dorsal portion of mLPMPR in his figure 11. Likewise, figure 4 from Beckstead (1979) shows a projection from ACC to dorsal mLPMPR using autoradiography. Figure 7 from Sesack et al.

(1989) also shows a projection from ACC to mLPMR using *phaseolus vulgaris*-leucoagglutinin, although it is difficult to judge if the labeling was present dorsally or ventrally within LPMR. In table 2 of Condé et al. (1995) there is a report of input to ACC from LP, although the specific topography is not reported. Additionally, we were able to inject the bidirectional tracer 3kDA into ACC in case 420, and were able to note anterograde and retrograde labeling within the dorsal portion of mLPMR. Although we were only able to do this for one case, our findings are in line with previous studies and we are confident of the results. The injection into AGm in case 372 showed anterograde and retrograde labeling in the ventral portion of mLPMR. Our findings, combined with these previous findings suggest that there are reciprocal connections of ACC with dorsal mLPMR, as there are with AGm and ventral mLPMR.

4. Experimental Procedure

All experiments were performed on adult male Long Evans Hooded rats (n=19; 300-350gms; obtained from Harlan Sprague Dawley, inc.). All procedures were conducted in accordance to the US Public Health Service Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, revised 1996) and was approved by the University of Florida IACUC. All efforts were made to minimize the amount of pain experienced and number of animals used.

4.1. Surgery

For anesthesia, animals were first placed in a small induction chamber and given a small dose of Isoflurane (Webster Labs) before being given an intraperitoneal injection of ketamine/xylazine cocktail (87 mg/kg ketamine + 13 mg/kg xylazine; 0.1ml / 100g body weight; Webster Labs). Supplemental dosages of this cocktail were given throughout the surgery to maintain adequate depth of anesthesia.

Once deep anesthesia was confirmed, animals were placed in a stereotaxic surgical device with blunt earbars. Incision was made through the scalp and bregma was measured and marked. A craniotomy was then performed using a Dremel drill. Glass pipettes (a tip diameter of 30-40 μ m) were filled with tracer and the tracer was injected into the region of interest. Coordinates for the cortex were defined using the Paxinos and Watson (1998) atlas. After injections, the surgical site was cleaned, packed with gelfoam, sutured, and the animals were given an injection of ringers lactate.

4.2. Tracer injections

Double fluorescent retrograde tracers injected into two different regions were utilized for this study. The primary tracer used was the retrograde tracer cholera toxin subunit B (CTB) conjugated to Alexa Fluor (AF) 488 and 594 (0.5% in neutral phosphate buffer; Molecular Probes, Invitrogen inc.). Nine of the cases also used the bidirectional tracer dextran amine (3kDA) conjugated to tetramethylrhodamine (3k molecular weight; 10% in phosphate buffer, pH 3; lysine fixable; Molecular Probes, Invitrogen inc.). The rest of the cases had two injections of the conjugates of CTB.

Injections of the two different conjugates of CTB tracers were made with a picospritzer into ACC and AGm, with the most rostral section being +2.0mm and the most caudal section being -1.0mm from bregma. The coordinates for the injections were varied within this range. For a given brain, most of the injections of the two tracers were within 0.5mm of each other, and often times were at the same AP level. In case 420, we replaced the AF 488 CTB injection in ACC with 3kDA.

4.3. Post-operative protocols

After survival periods of 7 days, the animals were transcardially perfused with PBS and paraformaldehyde and the brain was removed and placed into a postfix sucrose solution. Brains were cut coronally on a sliding microtome at thickness of 40 μ m. For each brain, we mounted at least two spaced series of fluorescent sections and one cresyl violet series.

Sections were visualized using a Zeiss Axioplan II microscope with the following filter sets: FITC (model 31001, Chroma Technology Corp.) and Texas Red (model 31004, Chroma Technology Corp.). Photos were taken with a Retiga 4000R monochrome camera in QCapture pro 6.0 (Qimaging). Some images were taken with an Olympus IX81 spinning disk confocal microscope. Images were optimized in Adobe Photoshop CS3 and traced in Adobe Illustrator CS3. Designations for the boundaries of the cortex were made using the Paxinos 4th edition atlas (Paxinos and Watson, 1998), while the thalamus boundaries and terminology were made using the Paxinos 5th edition atlas (Paxinos and Watson, 2005), and LP topography was based on the report of Takahashi (1985). Our cortical terminology used was modified from the terminology used by Paxinos and Watson (1998): AGm is synonymous to secondary motor cortex, AGl is synonymous to primary motor cortex, and ACC included both cingulate area 1 and 2. Figures constructed were limited in scope to the topography in LPMR. The figures constructed were representative sections of all the cases, and the non-illustrated cases had similar and consistent patterns of labeling.

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Abbreviations

3kDA, 3000 MW dextran amine
 ACC, anterior cingulate cortex
 AD, anterodorsal thalamic nucleus
 AF, Alexa Fluor
 AGl, lateral agranular cortex
 AGm, medial agranular cortex
 AM, anteromedial thalamic nucleus
 AP, anterior-posterior coordinate
 AV, anteroventral thalamic nucleus
 cDCS, dorsocentral striatum, central part
 CL, centrolateral thalamic nucleus
 CTB, cholera toxin subunit B
 DCS, dorsocentral striatum
 HL, hindlimb cortex
 LDDM, lateral dorsal thalamic nucleus, dorsomedial part
 LP, lateral posterior thalamic nucleus
 LPLR, lateral posterior thalamic nucleus, laterorostral part
 LPMR, lateral posterior thalamic nucleus, mediorostral part
 MD, mediodorsal thalamic nucleus
 MDl, mediodorsal thalamic nucleus, lateral part
 mLPMR, medial portion of the lateral posterior thalamic nucleus, mediorostral part
 Oc1, occipital cortex, area 1
 Oc2L, occipital cortex, area 2, lateral part
 Oc2M, occipital cortex, area 2, medial part

Po, posterior thalamic nucleus
 PC, paracentral thalamic nucleus
 PL, prelimbic cortex
 PPC, posterior parietal cortex
 RSa, retrosplenial agranular cortex
 RSG, retrosplenial granular cortex
 S1, primary somatosensory cortex
 VM, ventromedial thalamic nucleus
 VP, ventral posterior thalamic nucleus

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Fig. 1. Dorsal view of the left hemisphere of the rat cortex. The horizontal dashed line represents the dorsal midline. The unfolded medial wall appears above this line. The vertical dashed lines indicate the AP range of injection sites used in this study. For abbreviations, see list.

Fig. 2.

Representative sections of the thalamocortical connections of mLPMR to AGm and ACC using the retrograde tracers AF 488 CTB (green) and AF 594 CTB (red). Numbers in bottom right corners are AP distances from bregma. AF 594 CTB was injected into central AGm (A.) and AF 488 CTB was injected into ACC (B.), at different AP levels. C-E: The ACC injection produced dense retrograde labeling in dorsal mLPMR. The AGm injection produced dense labeling in ventral mLPMR. F: Confocal micrograph (40x) from case 417 showing the detailed morphology of neurons labeled with AF 488 CTB. G: High powered (60x) confocal micrograph of about three neurons labeled with AF 594 CTB in case 417. H: Injection site of AF 488 CTB into central ACC and AF 594 CTB into central AGm in case 431. I-M: Patterns of labeling for

case 431. Retrograde transport from ACC produced dense labeling in dorsal mLPMR. Retrograde transport from AGm produced dense labeling in ventral mLPMR. Note that the magnification of I-K are double the magnification as those in C-E. L: High powered confocal micrograph (20x) of the box shown in J that shows the pattern of cell labeling at the border between dorsal and ventral LPMR. M: High powered confocal micrograph (20x) of the box shown in K that shows the pattern of cell labeling at the border between dorsal and ventral LPMR. Note the presence of yellow double-labeled cells at the border between dorsal/ventral LPMR. Scale bars= 200 μ m, unless otherwise noted. For abbreviations, see list.

Fig. 3.

A: Injection site of AF 488 CTB into rostral ACC and AF 594 CTB into rostral AGm, at the same AP level, in case 426. B-D: Retrograde transport from ACC produced dense labeling in dorsal mLPMR. Retrograde transport from AGm produced dense labeling in ventral mLPMR. E: Higher power confocal micrograph (20x) of the box shown in C. F: Higher power confocal micrograph (20x) of the box shown in D. G: Even higher power confocal micrograph (40x) of the box shown in F showing the detailed morphology of double-labeled cells. Scale bars= 200μm, unless otherwise noted. For abbreviations, see list.

Fig. 4. Corticothalamic connections of AGm and ACC using the bidirectional tracer 3kDA. A: Case 372. Injection of 3kDA was made into AGm, with some overlap into AGl. B: Anterograde transport produced dense axonal labeling in ventral mLPMR with retrogradely labeled cells intermingled. There was also anterograde labeling in Po and MD. C: Case 420. Injection of 3kDA was made into ACC, with no overlap into AGm. D: Anterograde transport produced dense labeling in dorsal mLPMR, with some retrogradely labeled cells intermingled. There is also some anterograde labeling in LPLR and CL. Scale bars= 200μm. For abbreviations, see list.