Research Report

Cortical connections of the rat lateral posterior thalamic nucleus

Hiroaki Kamishina,⁎ William L. Conte, Sarika S. Patel, Rachel J. Tai, James V. Corwin, Roger L. Reep

⁎Corresponding author.
E-mail address: kamicna@iwate-u.ac.jp (H. Kamishina).

Abstract

Spatial processing related to directed attention is thought to be mediated by a specific cortical–basal ganglia–thalamic–cortical network in the rat. Key components of this network are associative cortical areas medial agranular cortex (AGm) and posterior parietal cortex (PPC), dorsocentral striatum (DCS), and lateral posterior (LP) thalamic nucleus, all of which are interconnected. Previously, we found that thalamostriatal projections reaching DCS arise from separate populations of neurons of the mediorostral part of LP (LPMR). The far medial LPMR (fmLPMR) terminates in central DCS, a projection area of AGm, whereas central LPMR terminates in dorsal DCS, a projection area of PPC. This represents segregated regional convergence in DCS from different sources of thalamic and cortical inputs. In the present study, thalamocortical and corticothalamic projections arising from and terminating in LPMR and neighboring thalamic nuclei were studied by anterograde and retrograde tracing techniques in order to further understand the anatomical basis of this neural circuitry. A significant finding was that within LPMR, separate neuronal populations provide thalamic inputs to AGm or PPC and that these cortical areas project to separate regions in LPMR, from which they receive thalamic inputs. Other cortical areas adjacent to AGm or PPC also

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Abbreviations: ACC, anterior cingulate cortex; AD, anterodorsal thalamic nucleus; AGl, lateral agranular cortex; AGm, medial agranular cortex; AM, anteromedial thalamic nucleus; AV, anteroventral thalamic nucleus; BDA, biotinylated dextran amine; Cg1, cingulate cortex, area1; Cg2, cingulate cortex, area2; CL, central lateral thalamic nucleus; CM, central medial thalamic nucleus; CV, cresyl violet; DCS, dorsocentral striatum; DLG, dorsal lateral geniculate nucleus; fmLPMR, far medial portion of lateral posterior thalamic nucleus, mediorostral part; Fr2, frontal cortex, area 2; FrA, frontal association cortex; HL, hindlimb cortex; ic, internal capsule; LD, laterodorsal thalamic nucleus; LDLM, laterodorsal thalamic nucleus, dorsomedial part; LDVL, laterodorsal thalamic nucleus, ventrolateral part; LP, lateral posterior thalamic nucleus; LPLR, lateral posterior thalamic nucleus, laterorostral part; LPMR, lateral posterior thalamic nucleus, mediorostral part; IPPC, posterior parietal cortex, lateral portion; MD, mediiodorsal thalamic nucleus; MDC, mediiodorsal thalamic nucleus, central part; MDL, mediiodorsal thalamic nucleus, lateral part; MDM, mediiodorsal thalamic nucleus, medial part; MO, medial orbital cortex; mPPC, posterior parietal cortex, medial portion; Oc1, occipital cortex, area 1; Oc2L, occipital cortex, area 2, lateral part; Oc2M, occipital cortex, area 2, medial part; Par1, parietal cortex, area 1; PC, paracentral thalamic nucleus; PF, parafascicular thalamic nucleus; Po, posterior thalamic nucleus; PPC, posterior parietal cortex; PrL, prelimbic cortex; PtpD, dorsal posterior parietal cortex; Re, reuniens thalamic nucleus; Rh, rhomboid thalamic nucleus; RSD, retrosplenial dysgranular cortex; RSGc, retrosplenial granular c cortex; Rt, reticular thalamic nucleus; S1BF, primary somatosensory cortex, barrel filed; S1FL, primary somatosensory cortex, forelimb area; S1HL, primary somatosensory cortex, hindlimb area; VA, ventroanterior thalamic nucleus; VL, ventrolateral thalamic nucleus; VLO, ventrolateral orbital cortex; VPL, ventral posterolateral thalamic nucleus

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demonstrated reciprocal connections with LP or surrounding nuclei in a topographic manner. Our findings suggest that the cortical–basal ganglia–thalamic network mediating directed attention in the rat is formed by multiple loops, each having reciprocal connections that are organized in a precise and segregated topographical manner.

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1. Introduction

The rat lateral posterior thalamic nucleus (LP) is a key component of the neural circuitry for directed attention and its dysfunctional counterpart, contralateral neglect. This circuitry includes cortical, thalamic and striatal components that appear to be organized in functionally compartmented loops, as are known to exist for sensory, motor and limbic functions (Reep and Corwin, 2008). In rats, unilateral lesions of either medial agranular cortex (AGm or Fr2) or posterior parietal cortex (PPC), or disconnection of the axons linking them, produce neglect (Burcham et al., 1997; Corwin and Reep, 1998). This neglect is multimodal, involving visual, auditory and somatic sensory stimuli. Rats with cortical lesions may recover spontaneously or as a result of specific treatments (Corwin and Reep, 1998; Brenneman et al., 2008).

Nucleus LP is reciprocally connected with AGm and PPC, as well as with visual areas occipital cortex, area 1 (Oc1), occipital cortex, area 2, lateral part (Oc2L) and occipital cortex, area 2, medial part (Oc2M) (Sukekawa, 1988; Reep et al., 1994; Reep and Corwin, 1999; Sefton et al., 2004), and these cortical areas are themselves interconnected (Reep et al., 1990, 1994, 1996; Vandevelde et al., 1996; Reep and Corwin, 1999).

Thalamic nucleus LP also projects to dorsocentral stratum (DCS), a site where corticostriatal projections from AGm and PPC converge (Erro et al., 2002; Cheatwood et al., 2003; Reep et al., 2003; Cheatwood et al., 2005). The DCS is an essential node in the circuitry for directed attention. Unilateral lesions of DCS produce neglect that does not recover spontaneously or after treatment with a dopamine agonist, in contrast to neglect resulting from cortical lesions (Van Vleet et al., 2000, 2002, 2003a, 2003b). The main input from LP to DCS has been shown in retrograde tracing studies to arise from the far medial portion of rostral LP, fmLPMR (Erro et al., 2002; Cheatwood et al., 2003). Recently, we found using anterograde tracing that fmLPMR projects to the matrix compartment of central DCS and to the dorsal periphery of DCS (Kamishina et al., 2008). In contrast, central LPMR projects only to the dorsal periphery of DCS. The laterorostral part of LP (LPLR) and other thalamic nuclei surrounding LP project sparsely to dorsolateral and dorsomedial regions of the striatum but do not project to DCS. Thus, fmLPMR, the portion of LP that projects to AGm, also projects to central DCS, a major target of AGm. Double anterograde fluorescent labeling confirmed that axons from fmLPMR intermingle with axons from AGm, thus raising the possibility that they synapse on common medium spiny striatal output neurons (Cheatwood et al., 2005). In a parallel manner, central LPMR projects to PPC and to the dorsal periphery of DCS, a major target of PPC, suggesting that terminals from central LPMR and PPC may also converge on single medium spiny striatal neurons. The dorsal periphery of DCS receives input from fmLPMR, central LPMR, and PPC. All three of these inputs terminate in segregated foci, but it is not known if there is overlap among foci from these different sources. These findings indicate that DCS is a region of convergence for thalamostriatal and corticostriatal projections from regions that are themselves interconnected, and that there is some topographic separation of inputs from different thalamic and cortical sources.

The significant differences within LP regarding thalamostriatal projections to DCS suggest that there may also be differences with regard to the topography of thalamocortical and corticostriatal projections involving LP, AGm, and PPC. Such differences would have functional implications for the operation of the circuitry for directed attention. Corticothalamic projections are known to promote synchronized oscillatory activity that is thought to underlie the coherent action of large groups of thalamic neurons (Contreras et al., 1996; Jones, 2001), and thalamocortical neurons exert a powerful effect on cortical neurons through convergent, synchronous activation (Bruno and Sakmann, 2006). Corticothalamic projections influence the occurrence of tonic versus burst firing of thalamocortical neurons (Sherman, 2001). In general, layer V neurons provide an excitatory driving function to thalamic neurons, whereas layer VI neurons exert a modulatory influence through their effect on the tonic versus bursting mode of thalamic neurons (Sherman, 2001; Cudeiro and Sillito, 2006; Sherman and Guillery, 2006; Sherman, 2006). However, corticothalamic relationships are unknown at the structural or functional level for inputs to the rat LP from AGm and mPPC (medial PPC), and the topography of thalamocortical relationships between LP and AGm and PPC is not well mapped. For example, although it has been shown that LP projects to AGm and PPC, do these projections arise from separate groups of neurons in LP? If so, are these populations of neurons segregated or intermingled? These two possibilities represent divergent ways in which information could be distributed from LP to the cortex. Similarly, because we know that fmLPMR is the source of input to DCS, particularly to its central part, it is of interest to determine which corticothalamic projections reach fmLPMR. In light of these questions, we sought to determine with greater precision the topography of projections from LP to AGm and PPC. In addition, we mapped the reciprocal projections from these cortical areas to LP, and observed how these related to projections from neighboring cortical areas.

2. Results

2.1. Thalamocortical projections

Terminal labeling patterns from thalamus to cortical areas were examined by injecting the anterograde axonal tracer 10k
biotin-dextran amine (10k BDA) in various portions of thalamic nuclei in 12 cases and by injecting retrograde axonal tracers (red or green fluorescent 3k DA) in cortical areas in 7 cases. Here we present representative cases; other cases are described only when they represent unique findings.

2.1.1. Injections focused in fmLPMR
Three cases received a 10k BDA injection into fmLPMR. The injection centers were located in the ventral portion of the fmLPMR in cases 297 and 238, and in its dorsal portion in case 239. Case 297 was selected for illustration (Fig. 1) because this

-- Fig. 1 -- Anterograde cortical labeling in cases 297 and 328 following 10k BDA injection in ventral fmLPMR and central LPMR, respectively. Photomicrographs of the injection site centers in case 297 (A) and case 328 (A'), with a–p coordinates indicated. Representative spaced cortical sections with anterograde labeling in case 297 (B–G) and in case 328 (B’–G’). Numbers shown in left top corners on each section represent the a–p location of the sections in reference to the standard atlas of Paxinos and Watson (2005). The right edge of the pictures corresponds to the midline. For abbreviations, see list. Conventions for this figure are repeated similarly in subsequent figures.
case had the most robust labeling in the cortical areas involved in neglect.

2.1.1.1. Cases 297 and 238. The injection site was centered in the ventral portion of fmLPMR (Fig. 1A). In rostral cortical sections (a–p 2.5 to −0.2), terminal labeling was observed in distinct laminar patterns located within AGm and cingulate cortex area 1 (Cg1), with strong density of axon terminals in layers I and III, and light, scattered labeling in layer V (Figs. 1B–D). Cg1 laminar patterns in case 238 were similar to case 297 since both cases had greatest axon terminal density in layers I and III. In case 297, labeling in AGm continued caudally (a–p −1.4), decreasing its intensity, at which level involvement of layers I–III of lateral agranular cortex (AGl) was found (Fig. 1E). In the most caudal sections (a–p −3.6 caudally) of both case 297 and case 238, dense labeling was found in layer IV of Oc2M,

Fig. 2 – Anterograde cortical labeling in cases 295 and 326 following 10k BDA injection in lateral LPMR and lateral LPLR, respectively. Photomicrographs of the injection site in case 295 (A) and case 326 (A’). Representative spaced cortical sections with anterograde labeling in case 295 (B–G) and in case 326 (B’–F’).
and Oc2L (Figs. 1F, G). However, while case 297 had the densest retrosplenial dysgranular cortex (RSD) labeling in layer II and very dense PPC labeling in layer IV, case 238 had very dense RSD labeling in layer IV and dense PPC labeling in layers V–VI. In addition, cortical density in case 297 was generally moderate in layers I–II of RSD and in layers II–VI of Oc2M and Oc2L, with the exception of high cortical density areas. In RSD layers II–VI of case 238, cortical density was present with scattered density found in layers III and V while layers II, IV, and VI had moderate density.

2.1.2. Case 239 (not illustrated). The injection center was located in the dorsal portion of the fmLPMR. Similar to cases 297 and 238, this case also had labeling in AGm and Cg1 in rostral sections between a–p 2.5 and 0.5 but the labeling intensity was lighter. Unlike cases 297 and 238, labeling in Cg1 became prominent in more caudal sections between a–p 0.0 and −0.7 and extended ventrally to involve cingulate cortex, area2 (Cg2). This labeling continued caudally and extended primarily into RSD and, to a lesser degree, retrosplenial granular cortex (RSGc) between a–p −1.4 and −4.8. Relatively light labeling was found in the lateral portion of PPC and also in Oc2L and Oc1.

2.1.3. Injections focused in lateral LPMR

In case 328, the injection site was located slightly more lateral than those of above cases and centered in the central portion of LPMR at a–p −3.2 (Fig. 1A). The labeling pattern in cortical areas of this case was similar to case 297 but labeling in AGm shifted slightly more caudally and had heavier labeling in PPC and visual cortices. In rostral sections, light labeling was found in layers II–V of AGm. In AGm, labeling became fainter as it traversed down the cortex, showing distinct density in layer II, moderate density in layer III, and light density in layers IV–V. Labeling in layers I, V, and VI of Cg1 and Cg2 appeared between a–p 1.9 and 0.0 (Figs. 1B–C). Labeling in layers II–V of AGm and layers I, II, and V of Cg1 became heavier caudal to a–p −0.2 and continued until a–p −1.7 (Figs. 1D–E). In caudal sections, strong labeling was found in layer IV of the PPC between −3.6 and −4.0 (Fig. 1F). Moderate density was present in layers II–III of Oc2M. Also, dense labeling in Oc1 layers II and IV and moderate density in Oc1 layer III was present between −4.3 and −4.6 (Fig. 1G). Very light labeling in layers I and II of RSD and RSGc was also found between −1.7 and −4.5 (Figs. 1F–G).

2.1.4. Injections focused in far lateral LPLR

The injection site of case 326 was located in the far lateral LPLR at a–p −4.0 (Fig. 2A). This injection produced light labeling in AGm and Cg1 rostrally and moderate labeling in PPC and dorsal posterior parietal cortex (PPcD) caudally. Light labeling was present in layer I. Scattered labeling was observed in layers II and III. PPC labeling was densest in layer IV. In rostral sections, light labeling was present in layers I–III in AGm and Cg1 as well as in Cg2 between a–p 2.5 and −1.0 (Figs. 2B–C). Between a–p −1.7 and −2.6 light labeling was seen in AGm and AG1, mainly in layer I and III (Figs. 2D–E). Denser labeling was found in more caudal sections which involved layer IV of PPC, layer IV of Oc2M, and PtPD (Figs. 2F–G). There was distinct labeling in RSD layers I–III, especially dense layer I and III labeling. Additionally, minimum labeling was observed in RSGc.

2.1.5. Injections focused in lateral LDDM/medial LDVL

In case 252 (not illustrated), the injection site was located in the dorsal boundary of LDDM and LDVL at a–p −2.5. There was no cortical labeling in rostral sections. In caudal sections, there was faint labeling in PPC layers I–II and light, slightly denser labeling in PPC layer III at a–p −3.6. There was also light labeling in layers I–II of Oc2M between a–p −4.4 and −4.8.

2.1.6. Injections focused in central lateral thalamic nucleus (CL)

In case 341, the injection site was confined to the dorsal portion of CL at a–p −3.6 (Fig. 3A). This case demonstrated labeling in rostral cortical sections, most heavily in Cg1 and Cg2, and almost no labeling in caudal cortical sections except in Oc2M and Oc2L. In most rostral sections between a–p 5.6 and 4.7, labeling was dense in medial cortical areas including frontal cortex, area 2 (FrA), medial orbital cortex (MO), AGm,
and prelimbic cortex (PrL). Labeling in this location continued caudally and involved layers I–III of Cg1 and PrL, with greatest density in layers I–III between a–p 3.7 and 2.5 (Fig. 3B). Labeling then became strong in layers I and III of Cg1 and Cg2 between 2.2 and 0.2 in caudal sections (Figs. 3C–E). Labeling in Cg1 and Cg2 became lighter caudal to a–p 0.0. In caudal sections, moderately dense labeling was found in layers I and III of Oc2M, Oc2L, and Oc1 and light, scattered labeling was found in layers II and IV of Oc2M, Oc2L, and Oc1 between −4.2 and −7.7 (Figs. 3F, G).

2.1.7. Injections focused in posterior thalamic nucleus (Po)
The injection site of case 296 primarily involved Po at a–p −3.3 (Fig. 3A'). There was relatively dense labeling in layers II–III of AGm and AGL, and light labeling in Cg1 in rostral sections between a–p 3.7 and −1.4 (Figs. 3B'–D'). Labeling in AGm and
AGl continued caudally between a−p − 1.7 and −2.6, becoming lighter than rostral sections (Fig. 3E). Labeling in RSD layers I–III, with greatest density on the border between layers II and III, and faint labeling in RSGc layer I was found between −1.7 and −2.6 which became heavier from a−p − 3.4 caudally (Figs. 3E′−G′). Moderate labeling was found in the layers II–III of lateral PPC as well as in PIPD (Fig. 3F′). Strong labeling was seen on the border between layers III and IV in Oc2L between −4.4 and −4.8, and Oc1 between −4.4 and −5.5 (Fig. 3G′).

2.2. Fluorescent retrograde tracer injections in cortical areas

Case 290 and 291 received tetramethylrhodamine-conjugated 3k DA in AGm and fluorescein-conjugated 3k DA in PPC. In case 290, one injection site was centered in AGm (a−p − 1.4) but appears to have also encroached on Cg1 (Fig. 4A). The other injection site was centered in PPC (a−p − 3.2) (Fig. 4B).

Retrogradely labeled neurons from AGm were found in fmLPMR (Fig. 4C). Rostromedial LD (LDDM) and, to a lesser degree, rostralateral LD (LDVL) also contained labeled neurons. There were also labeled neurons in anterodorsal thalamic nucleus (AD), anteromedial thalamic nucleus (AM), anteroventral thalamic nucleus (AV), CL, rhomboid thalamic nucleus (Rh), and reuniens thalamic nucleus (Re) in more rostral sections, most likely due to the overlap of the injection into Cg1. Retrogradely labeled neurons from PPC distributed more broadly in LPMR and LPLR (Fig. 4D). Neurons in LD projecting to PPC were located similar to those projecting to AGm. In addition, neurons projecting to PPC were found in AD, AV, CL, and paracentral thalamic nucleus (PC) in rostral sections. Distribution of labeled neurons in case 291 was similar to that of case 290, except that labeled neurons in LD after PPC injection were mainly located in LDVL. In both cases, no double labeled cells were seen, indicating that single neurons in LP do not project to both AGm and PPC.

A single injection of tetramethylrhodamine-conjugated 3k DA was made in AGm in 4 cases (cases 383, 387, 389, 393) and in AGl in 1 case (case 385). All cases with AGm injection produced a similar distribution of labeled neurons as seen in the double labeling cases 290 and 291. After AGl injection in case 385, labeled neurons were found in central LPMR, Po, LDDM, and medial LDVL. Rostrally, labeled neurons were also found in AD, AV, CL, and ventrolateral thalamic nucleus (VL).

Fig. 4 – Double-retrograde thalamic labeling in case 290 following tetramethylrhodamine-conjugated retrograde 3k DA injection in AGm and fluorescein-conjugated retrograde 3k DA injection in PPC. Photomicrographs of the injection site in AGm (A) and PPC (B). Labeling in LPMR exhibits topography whereby neurons projecting to AGm (red) are located more medially than those projecting to PPC (C and D). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Fig. 5 – Anterograde thalamic labeling in cases 404 and 109 following 10k BDA injection in rostral AGm and mid AGm, respectively. Photomicrographs of the injection site in case 404 (A) and case 109 (A'). Representative spaced thalamic sections with anterograde labeling in case 404 (B–D) and in case 109 (B'–D'). Numbers shown in right bottom corners on each section represent the a–p location of the sections in reference to a standard atlas of Paxinos and Watson (2005). The right edge of the pictures corresponds to the midline. For abbreviations, see list.
Fig. 6 – Anterograde thalamic labeling in cases 100 and 340 following 10k BDA injection in caudal AGm and AGl, respectively. Photomicrographs of the injection site in case 100 (A) and case 340 (A'). Representative spaced thalamic sections with anterograde labeling in case 100 (B–D) and in 340 (B'–D').
2.3. Corticothalamic projection

2.3.1. Injections focused in AGm

There were a total of 11 cases with 10k BDA injections in AGm. Based on the observed thalamic labeling pattern, our cases could be divided into three groups with different rostro-caudal injection levels in AGm.

2.3.1.1. Rostral AGm—cases 404 and 380. Case 404 had an injection in rostral AGm at a-p 2.5 (Fig. 5A). In this case, thalamic terminal field labeling was observed between a-p −2.1 and −4.0. In rostral sections, labeling with moderate intensity was found in mediodorsal thalamic nucleus, lateral part (MDL) and CL (Fig. 5B). Labeling in these nuclei became stronger in more caudal sections around a-p −3.1 (Fig. 5C) and again became lighter caudal to a-p −3.3 (Fig. 5D). Faint labeling was also observed in the ventral part of the far medial LPMR between a-p −3.3 and −4.0 (Fig. 5D).

Case 380 (not illustrated) had an injection in rostral AGm at the level of a-p 4.2. In this case, thalamic terminal field labeling was observed between a-p −1.7 and −4.8. Rostrally, labeling was found only in ventroanterior thalamic nucleus/ventrolateral thalamic nucleus (VA/VL) (−1.7 to −1.9). In more rostral sections, in addition to VA/VL labeling, faint labeling was found in more medially located thalamic nuclei including central medial thalamic nucleus (CM), CL, and Rh (a-p −2.2). Labeling in CL and the ventral portion of VL continued caudally until a-p −2.6. Densest labeling was observed in mediodorsal thalamic nucleus, medial part (MDM) and MDL between a-p −3.4 and −3.6. Relatively faint labeling was found at the border of LPMR and Po of their medial edge between a-p −3.6 and −3.8.

2.3.1.2. Mid AGm—cases 109, 377, 289, 350, 351, 397, and 381. A similar pattern of thalamic labeling was observed in cases with 10k BDA injections in AGm between a-p 0.8 and −1.3. The thalamic labeling pattern was clearly different from those of rostral AGm injections (cases 380 and 404) and those of more caudal AGm injections (Cases 378 and 100). In all cases, the densest labeling was located in the fnLPMR, the portion of LPMR that is known to project to central DCS (Kamishina et al., 2008). In case 109, the injection site in AGm was located at a-p 0.1 (Fig. 5A). In rostral sections, strong labeling was found in CM, AV, and light labeling in the dorsal portion of VL and reticular thalamic nucleus (Rt) (−1.9 to −2.4) (Fig. 5B). Labeling in LDDM (−2.6 to −2.9) was also observed (Fig. 5C). The labeling in VL continued caudally into Po at the level of a-p −2.6 and extended until a-p −4.1 (Figs. 5C’ and D’). The densest labeling was in the ventral portion of the far medial LPMR at a-p −3.4 (Fig. 5D’).

2.3.1.3. Caudal AGm—cases 100 and 378. Injections in more caudal AGm produced a different pattern of thalamic labeling from those of rostral and mid AGm injections. A significant difference was the location of the terminal field labeling in LPMR, which was located in a more lateral portion in cases with caudal AGm injections. In case 100, the injection site was located in AGm at a-p −2.7 (Fig. 6A). In rostral sections, there was strong labeling in Rt, VA/VL, AV, and AM (−1.9 to −2.2) (Fig. 6B). Labeling with moderate intensity was observed in LDDM and the dorsal portion of Po (Fig. 6C). Dense labeling was seen in the border of the lateral portion of LPMR, medial LDVL, and dorsal Po (−3.3 to −3.8) (Fig. 6D). In these sections, weak CL labeling was also present (Fig. 6D).

2.3.2. Injections focused in AGl

2.3.2.1. Case 340. The labeling pattern in the thalamus in this case was readily distinguishable from those of AGm injections at a similar rostrocaudal level. Case 340 had an injection site in AGl at a-p −1.3 (Fig. 6A). In rostral sections, faint labeling was observed in VA/VL, AV, and PC between −1.9 and −2.2 (Fig. 6B). Light labeling was found in the border of lateral LDDM, medial LDVL, and dorsal Po between −2.9 and −3.1 (Fig. 6C). In more caudal sections, labeling in LP was observed in the ventral portion of lateral LPMR between −3.6 and −5.2, which also included medial LPLR and dorsal Po (Fig. 6D’).

2.3.3. Injections in Cg1

2.3.3.1. Cases 286, 288, and 109 (AGm and Cg1). Cases 286 and 288 produced almost identical labeling in thalamus. In both cases, labeling was always heaviest in medially located nuclei CL, MDL, and mediodorsal thalamic nucleus, central part (MDC). Case 286 had an injection site focused in Cg1 at a-p 1.2, with overlap into AGm (Fig. 7A). In rostral sections between −1.4 and −2.4, labeling was observed in Rt, AV, AM, PC, CM, CL, VL, and VM (Fig. 7B). Labeling in VL continued caudally to include the dorsolateral portion of Po (Fig. 7C). Although the intensity was lighter, labeling patterns in LD and LP were similar to cases with mid AGm injection; labeling was found in LDDM (−2.6 to −2.9) (Fig. 7C) and in the far medial LPMR (−3.3 to −4.1) (Fig. 7D). Labeling in LP was concentrated in the dorsal part of LPMR. The injection site of case 109 was located at the border of Cg1 and AGm, and thus had a mixed labeling pattern, as was also noted with case 286.

2.3.4. Injections focused in PPC

2.3.4.1. Cases 77, 69, and 245. All of these cases produced a distinct patch of labeling in the boundary of the lateral LPMR and medial LDVL at −3.4. Caudally, this labeling extended in the dorsal Po at around −3.8. Case 77 had the injection center located in a more ventral area in PPC (with some white matter involvement) (Fig. 7A) than case 69 and produced labeling in the more ventral portion of LPMR/LDVL. In rostral sections of cases 69 and 245, light labeling was observed in the dorsal Rt and VL (−2.2 to −2.4), and in the border of lateral LDDM and medial LDVL (−2.9 to −3.1). In case 77, relatively strong labeling was seen in LDDM; however, this labeling might have been due to the encroachment of the injection site into the underlying white matter (Fig. 7C). A distinct patch of labeling was seen encompassing the lateral LPMR, medial LPLR, and dorsal Po in caudal sections (Fig. 7D’).

2.3.5. Injections focused in Oc2M

2.3.5.1. Cases 101, 353, and 95. Injections in Oc2M produced a thalamic labeling pattern very similar to that of PPC injections. Cases 101 and 95 had a similar injection center in
Fig. 7 - Anterograde thalamic labeling in cases 286 and 77 following 10k BDA injection in Cg1 and mPPC, respectively. Photomicrographs of the injection site in case 286 (A) and case 77 (A'). Representative spaced thalamic sections with anterograde labeling in case 286 (B–D) and in case 77 (B’–D’).
Fig. 8 – Anterograde thalamic labeling in cases 101 and 96 following 10k BDA injection in Oc2M and S1HL, respectively. Photomicrographs of the injection site in case 101 (A) and case 96 (A'). Representative spaced thalamic sections with anterograde labeling in case 101 (B-D) and in case 96 (B'-D').
Oc2M (Fig. 8A). In rostral sections of case 101, relatively heavy labeling was found in Rt, AM, AD, CM (-1.6 to -1.9) (Fig. 8B), LDDM, LDVL, and CL (-2.4) (Fig. 8C). Labeling in LP was laterally located (-3.6 to -3.8), with the densest center being in the medial LPLR (Fig. 8D). Labeling in Po was less significant in these cases compared to cases with PPC injections.

Case 353 (not illustrated) had a slightly more rostral Oc2M injection and produced a similar labeling pattern to cases 101 and 95 but with some unique features. Unlike cases with more caudal Oc2M injections (cases 101 and 95) which had heavy medial LPLR labeling, a patch of labeling was observed in the lateral LPMR at -3.6 that extended in the medial LPLR and dorsal Po caudally (-3.8 to -4.4). The labeling pattern in more rostral sections in this case was similar to those of PPC injections; there was light labeling in the dorsal Rt (-1.7) and the border of lateral LDDM and medial LDVL (-2.9 to -3.1).

### 2.3.6. Injections focused in Par 1 and primary somatosensory cortex, hindlimb area (SIHL)

**2.3.6.1. Cases 96, 90, 247, and 113.** Case 96 had a confined injection site in SIHL (Fig. 8A). Rostral sections had heavy labeling in the dorsal part of Rt and VA/VL between -2.1 and -2.4 (Figs. 8B–C). There was light labeling in the medial LDVL at -2.4 (Fig. 8C), but no labeling in LP was observed. In more caudal sections, heavy labeling was seen in the dorsal Po and VPL between -3.6 and -4.1 (Fig. 8D).

The most rostral Par 1 injection was represented by case 90 (not illustrated), which had some involvement of medially adjacent AGl. There was no LP or LD labeling throughout the rostrocaudal extent of the thalamus. Rostrally, similar to case 96, very heavy labeling was located in the dorsal part of Rt and VA/VL at -1.7 (VA/VL labeling might be due to involvement of AGl). These extended caudally into the lateral periphery of ventral posterolateral thalamic nucleus (VPL) at -2.2. The ventrolateral portion of VPL had heavy labeling caudally from which level heavy labeling was also observed in Po at -3.1. Heavy labeling in Po continued caudally until -4.1.

The injection sites of cases 247 and 113 (not illustrated) were more caudal than cases 90 and 96. The locations of these injections were similar but case 113 had a bigger deposit of tracer, which produced wider distributions of labeling in the thalamus. In rostral sections, distinct labeling was found in the dorsal Rt and the lateral part of VPL between -2.4 and -2.6 in both cases. Neither cases produced labeling in LP. Labeling in ventral to medial LDVL was found in case 113 at -3.1. Heavy Po labeling was seen in both cases between -3.8 and -4.1.

### 3. Discussion

We described in the present study the connectivity of LP and cortical areas AGm, PPC, and visual association cortices with which they are connected. Within LP, LPMR is our major interest because it projects to the DCS, an associative striatal area reciprocally connected with AGm, PPC, and with visual areas Oc1, Oc2L, and Oc2M (Reep et al., 2003). Furthermore, LPMR is directly connected with these cortical areas (Sukekawa, 1988; Reep et al., 1990, 1994). Our previous work identified a rather fine topographic organization of thalamostriatal projections arising from LPMR (Kamishina et al., 2008). Whereas the fMLPMR projects to the central DCS where cortical inputs from AGm terminate, the central LPMR projects to the dorsal periphery of DCS where cortical inputs from PPC terminate. Thus, it is probable that thalamostriatal and corticostriatal projections arising from these interconnected thalamic and cortical areas converge and interact respectively in the central portion and dorsal periphery of DCS. In the present study, we found that LPMR and these cortical areas are not only reciprocally connected as suggested previously (Reep et al., 1994; Reep and Corwin, 1999) but are also topographically organized. The results of this study extend our current knowledge about the anatomical basis of the cortical-basal ganglia-thalamic-cortical network mediating directed attention in the rat.

#### 3.1. Cortical connections of lateral posterior thalamic nucleus

Similar to thalamostriatal projections of LPMR, thalamocortical projections reaching different sets of cortical areas were found to originate from separate neuronal populations in LPMR. These neuronal populations in LPMR can be divided, based on their target cortical areas, into those located in the far medial part of LPMR (which can be further divided into ventral and dorsal parts), central part, and lateral part. The ventral part of fMLPMR projects heavily to AGm between a relatively narrow a–p extent (1.4 to -1.4) and lightly to more caudal cortical areas mPPC, Oc2M, and Oc2L (case 297). Earlier studies (Sukekawa, 1988; Hicks and Huerta, 1991) and our fluorescent double-retrograde AGm/PPC cases suggested that projections from LP to AGm originate predominantly from its medial portion. Our earlier study also showed a similar thalamocortical projection pattern where injection of retrograde tracer in AGm at a compatible a–p level resulted in retrograde labeling in the ventral part of fMLPMR (Reep and Corwin, 1999).

The general topography of corticothalamic projections from AGm is known to be organized in a continuous gradient whereby more caudal portions of AGm have connections with progressively more lateral and caudal regions of the thalamus (Reep and Corwin, 1999). The differences in the connectivity of the thalamus and various a–p levels of AGm are consistent with the differing functional roles played by the rostral and caudal portions of AGm (see Burcham et al., 1997; Corwin and Reep, 1998). In the present study, rostral AGm was shown to project to medially located thalamic nuclei (case 404) as found previously (Reep et al., 1984, 1987). More caudal portions of AGm have been shown to project to more lateral parts of thalamus including LD, LP, and Po, in addition to the lateral part of mediadorsal thalamic nucleus (MD) and Cl. (Takahashi, 1985; Sukekawa, 1988).

We showed in the present study that the portion of AGm receiving inputs from ventral fMLPMR (i.e. between a–p 0.8 and -1.3) projects most heavily to ventral fMLPMR (case 109). This location of AGm in the rostrocaudal axis is the site where unilateral lesions cause the most severe neglect in rats (Corwin and Vargo, 1993; Van Vleet et al., 2003b). Case 100 represented the most caudal 10k BDA injection in AGm. Its corticothalamic terminal fields in LPMR shifted laterally and involved the lateral LPMR, medial LDVL,
and dorsal Po similar to the findings of our previous study (Reep and Corwin, 1999). Far medial LPMR including its ventral part projects strongly to central DCS, the target area of corticostriatal projections from AGm (Reep and Corwin, 1999; Cheatwood et al., 2003; Reep et al., 2003; Kamishina et al., 2008). Thus, there is a loop reciprocally connecting the ventral fmLPMR, central DCS, and mid AGm.

We demonstrated that the dorsal part of fmLPMR also projects to central DCS and AGm, but its intensity in AGm is much lighter than the projection originating from ventral fmLPMR. Instead, the dorsal fmLPMR has much stronger connections with anterior cingulate cortex (ACC) (case 239) compared to the projection originating from ventral fmLPMR. Instead, the dorsal fmLPMR has much stronger connections with anterior cingulate cortex (ACC) (case 239) in agreement with the study by Conte et al. (2008). The major thalamic inputs to ACC are known to arise from intralaminar and midline thalamic nuclei (Berendse and Groenewegen, 1991; Wang and Shyu, 2004), but the connections between LP, ACC and AGm have not been described in depth. Our results also showed that ACC and the dorsal fmLPMR are reciprocally connected. Although the separation of ventral versus dorsal fmLPMR was not entirely clear (most likely due to the relatively large injection site in Cg1 in case 286), we conclude that Cg1 projects primarily to dorsal fmLPMR. These findings support the conclusion that ventral and dorsal fmLPMR and ACC are in line with our recent retrograde tracing study focusing on the differential topography between ACC, AGm, and LP (Conte et al., 2008). Because ACC has reciprocal connections with AGm and RSG (Vogt and Miller, 1983), it is possible that ACC participates in the network for directed attention in addition to its known role of processing nociception (Wang and Shyu, 2004, Conte et al., 2008). This is supported by previous reports of neglect following lesions confined to ACC in humans (Heilman and Valenstein, 1998; Leibovitch et al., 1998; Bukлина, 2002). Similar to ventral fmLPMR, dorsal fmLPMR projects only sparsely to PPC and visual cortical areas.

The central LPMR has significantly heavier projections to PPC and Oc2M rather than to AGm (cases 297 and 238). PPC in rats is interconnected with several associative cortical areas including AGm, Oc2M, Oc2L, orbitofrontal cortices, somatosensory areas parietal cortex, area 1 (Par1) and Par2, as well as with thalamic nuclei LD, LP, and Po (Reep et al., 1994). Further, behavioral testing after damaging PPC or its connections with AGm demonstrated the presence of neglect (Burcham et al., 1997; Corwin and Reep, 1998). These anatomical and behavioral findings as well as electrophysiological evidence (Chen et al., 1994) indicate that PPC is one component of the circuitry directly involved in directed spatial attention. In our previous study, we found that the central LPMR projects to the dorsal periphery of DCS, the target region of corticostriatal projections from PPC and visual areas (Kamishina et al., 2008). Thus, again there is a complete loop linking central LPMR, dorsal DCS, and cortical areas PPC, Oc2M, as well as Oc2L.

The lateral LPMR (case 295) has a different cortical connection pattern from central or medial LPMR. Lateral LPMR projects most heavily to RSD and RSGc, and to visual areas Oc2M, Oc2L, and Oc1. It also has robust connection with AGl and caudal AGm. Although we did not cover all cortical areas, corticothalamic projection patterns in general exhibit reciprocal connections between these cortical areas and lateral LPMR. For example, caudal AGm (a−p = −2.7) (case 100) and AGl (case 340) both project to lateral LPMR. Oc2M and Oc2L project to a wide region encompassing central LPMR medially and central LPLR laterally (case 101). In sharp contrast to LPMR cortical connectivity patterns, LPLR projects sparsely to AGm, AGl, and ACC (case 326). Heavier projections were found in more caudal cortical areas including PPC, Oc2M, Oc1 and PtPD, but not in RSD or RSGc.

In addition to the topographic organization of cortical projections from LP, we found that their cortical labeling exhibits distinct laminar patterns. Cortical projections from ventral fmLPMR to AGm (cases 297 and 238) terminate most heavily in layers I and III with additional scattered labeling in layer V (Fig. 9A). A similar laminar pattern of terminal axons in PPC following lateral LPMR injection was also observed and showed dense labeling in layer I and III with additional scattered labeling in layer V of medial PPC and more spread labeling in layer V of lateral PPC (Fig. 9B).

Fig. 9 – Cortical projections from LP terminate in laminar patterns. (A) In case 238, projections to areas AGm and Cg were focused in bands in layers I and III, and were scattered in layer V. (B) Labeling in case 240 was dense in layers I and III of areas RSD and medial PPC, with additional scattered labeling in layer V of medial PPC. More laterally in PPC, terminal labeling was prominent in layer V and was also present in layer I. Arrowheads indicate boundaries between labeled cortical areas; cg = cingulum bundle.
3.2. Cortical connections of lateral dorsal thalamic nucleus

Thalamic nucleus LD is known to have intimate connections with cortical areas that form the cortical network for spatial processing and directed attention. For instance, LD has reciprocal projections with cortical areas PPC and Oc2M (Chandler et al., 1992; Reep et al., 1994), which are interconnected with caudal AGm (Takahashi, 1985; Reep et al., 1994; Reep and Corwin, 1999). Nucleus LD also has reciprocal connections with caudal AGm, ACC, and retrosplenial cortex (Hicks and Huerta, 1991; Reep and Corwin, 1999; Shibata, 2000; Guandalini, 2001; Shibata and Naito, 2005). Our finding that LD has reciprocal connections mainly with caudal cortical areas PPC, Oc2M, Oc2L, RSD, and RSGc was consistent with these previous studies. We found that mid and caudal AGm also connects reciprocally with LDLG and LDVL based on anterograde (cases 109 and 100) and retrograde (cases 290 and 291) tracing cases. Our previous anterograde and retrograde studies demonstrated that cortical areas PPC and Oc2M provide strong corticostriatal projections to the dorsal region of DCS (Cheatwood et al., 2003; Reep et al., 2003). Injection of retrograde tracer in the dorsal periphery of DCS produced strong LD labeling contrary to LP labeling following tracer injection in central DCS (Cheatwood et al., 2003). These findings collectively suggest that LD has intimate connections with PPC and Oc2M and projects strongly to the dorsal peripheral region of DCS where there may be a regional convergence with corticostriatal projections from these cortical areas.

3.3. Cortical connections of central lateral thalamic nucleus

The thalamocortical projection pattern from CL can be characterized by dense labeling in ACC similar to that from dorsal fMLPMR. In AGm, however, CL projects to a rostral part of AGm whereas dorsal fMLPMR projects to a more caudal part of AGm. Case 297 had the injection center in ventral fMLPMR but seemed to have slight involvement of caudal part of AGm. Case 297 had the injection center in ventral fMLPMR and also medially adjacent CL. Consequently, this case also had labeling in ACC in a similar topographical organization as previously reported (Conte et al., 2008). Other previous studies identified that thalamic projections to ACC originate from AM, VM, CL, PC, and MD (Shibata, 1993; Shibata and Kato, 1993; Vertes, 2002; Hoover and Vertes, 2007). In the present study, 10k BDA injection in Cgl resulted in dense labeling in dorsal CL in addition to other intralaminar and midline thalamic nuclei AM, MD, AV, PC, CM (case 286). Therefore, cortical area ACC is reciprocally connected with CL, especially with its dorsal part. Since lesion of ACC has been shown to cause hemispatial neglect in humans (Heilman and Valenstein, 1998; Leibovitch et al., 1998; Buklina, 2002), interconnected CL may participate in the network of spatial processing as well. We found that dorsal CL is a focus not only of projections from ACC, but is also the source of projections from CL to ACC. Therefore, if CL participates in the network of spatial processing and directed attention, it is possible that the dorsal part of CL represents the most important component.

3.4. Cortical projection of posterior thalamic nucleus

Injection of 10k BDA in Po produced dense labeling in AGm and AGl in rostral sections and in PPC, Oc2M, and Oc2L in caudal sections (case 296). There was also labeling in RSD and RSGc. Injection of 10k BDA in various cortical areas resulted in labeling in Po which was consistently located in dorsal Po regardless of the injection sites in the cortex. The nucleus Po is known to have reciprocal connections with various cortical areas including rostral AGm (Reep et al., 1984, 1987), caudal AGm (Sukekawa, 1988), and PPC (Reep et al., 1994). Our findings on the connectivity of Po and cortical areas were consistent with these previous studies.

3.5. Functional considerations

Four lines of anatomical evidence suggest that nucleus LP plays a pivotal role in the circuitry for spatial attention and neglect in rats, similar to the central role suggested for the pulvinar in primate attention. First, LP has reciprocal connections with AGm and PPC, as well as with visual areas Oc1, Oc2L and Oc2M, (Reep et al., 1990; 1994; see Sefton et al., 2004; Sukekawa, 1988). Second, LP projections to DCS overlap with similarly organized striatal projections from cortical areas PPC and Oc2M (Cheatwood et al., 2005). Third, LP receives input from the basal ganglia, specifically the ventral substantia nigra pars reticulata, to which DCS projects (Reep and Corwin, 2008). Fourth, LP has reciprocal connections with the superior colliculus (see Sefton et al., 2004), a major center for multimodal integration and directed motor activation.

It has become clear in the present study that different regions of LP connect with different sets of cortical areas. The subregions in LPMR also exhibit specific connection patterns with striatum including its nodal region DCS (Kamishina et al., 2008). These findings suggest the existence of multiple cortical–thalamic–striatal loops, composing as a whole the complex network mediating spatial processing and directed attention. These observations indicate that each component of this network may play a distinct functional role in spatial processing and directed attention, as supported by functional studies showing different manifestation of spatial deficits with lesioning of different anatomical components of this neural circuitry (Vargo et al., 1988; Aggleton et al., 1995, 1996; Alexinsky, 2001).

3.6. Conclusion

Our findings suggest that, based on its connectivity with cortical areas, LPMR is the nodal nucleus in the thalamus mediating spatial processing and directed attention in rats, and that subregions of LPMR may play different functional roles. The ventral fMLPMR, a source of input to central DCS, has prominent connections with AGm whereas the cortical projections of the dorsal fMLPMR, a source of input to dorsal DCS, are focused in ACC. The central LPMR, a source of input to dorsal DCS, has intimate connections with PPC and Oc2M, both of which have strong projections to dorsal and central DCS. The main components of the neural circuitry of directed attention involving fMLPMR and
and posterior nucleus (Po) in 1 case. Injections of these cases were made through glass pipettes with a tip diameter of 30–40 μm, using constant current pulses of 5 μA delivered from an iontophoresis unit (Kation Scientific, Minneapolis, MN, Model BAB-350). This was performed in a 5 s on/5 s off pattern for 20 min. After the injection, the skull hole was filled with gelform, and antibiotic ointment was placed between the scalp and skull. The scalp wound was closed using wound clips.

In 7 cases, the fluorescent-conjugated retrograde axonal tracer 3k DA (3k mol wt dextran, Invitrogen, Inc.) was injected in various cortical areas. Tracer was mixed at 10% in phosphate buffer at pH 4.0–4.4 and injected in the cortex through glass pipettes, using Picospritzer (General Valve) with an average cycle of 8 injections per injection site using 20–30 p.s.i., 5–20 ms duration. Two cases received double injections; tetramethylrhodamine-conjugated 3k DA was injected into AGm and fluorescein-conjugated retrograde tracer was injected into PPC. A single injection of tetramethylrhodamine-conjugated 3k DA was made in AGm in 4 cases and in AGl in 1 case.

In 24 cases, the anterograde axonal tracer 10k BDA was injected into one of the following cortical areas: AGm, PPC, Oc2M, Par 1, hindlimb cortex (HL), and FL.

After ten (for anterograde tracer) or five (for retrograde tracer) days postsurgery, rats were sacrificed and the brains were fixed by transcardial perfusion with phosphate-buffered saline at 37 °C followed by phosphate-buffered 4% paraformaldehyde of pH 7.2–7.4. The brain was extracted and postfixed in 0.4% paraformaldehyde containing 30% sucrose for 2–3 days. The brain was cut coronally on a freezing sliding microtome in 40 μm sections.

Three sets of spaced 40 μm thick coronal frozen sections were mounted and coverslipped for analysis. For sections with anterograde tracer injections, the first two sets were processed for 10k BDA using avidin–biotin horseradish peroxidase histochemistry. For sections with retrograde tracer injections, the first two sets were used for viewing on a fluorescent microscope. The third set for both tracer types was processed with cresyl violet (CV) staining for cytoarchitectural orientation.

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