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Projections to the rostral reticular thalamic nucleus in the rat

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Summary. Afferent pathways to the rostral reticular thalamic nucleus (Rt) in the rat were studied using anterograde and retrograde lectin tracing techniques, with sensitive immunocytochemical methods. The analysis was carried out to further investigate previously described subregions of the reticular thalamic nucleus, which are related to subdivisions of the dorsal thalamus, in the paraventricular and midline nuclei and three segments of the mediodorsal thalamic nucleus. Cortical inputs to the rostral reticular nucleus were found from lamina VI of cingulate, orbital and infralimbic cortex. These projected with a clear topography to lateral, intermediate and medial reticular nucleus respectively. Thalamic inputs were found from lateral and central segments of the mediodorsal nucleus to the lateral and intermediate rostral reticular nucleus respectively and heavy paraventricular thalamic inputs were found to the medial reticular nucleus. In the basal forebrain, afferents were found from the vertical and horizontal limbs of the diagonal band, substantia innominata, ventral pallidum and medial globus pallidus. Brainstem projections were identified from ventrolateral periaqueductal grey and adjacent sites in the mesencephalic reticular formation, laterodorsal tegmental nucleus, pedunculopontine nucleus, medial pretectum and ventral tegmental area. The results suggest a general similarity in the organisation of some brainstem Rt afferents in rat and cat, but also show previously unsuspected inputs. Furthermore, there appear to be at least two functional subdivisions of rostral Rt which is reflected by their connections with cortex and thalamus. The studies also extend recent findings that the ventral striatum, via inputs from the paraventricular thalamic nucleus, is included in the circuitry of the rostral Rt, providing further evidence that basal ganglia may function in concert with Rt. Evidence is also outlined with regard to the possibility that rostral Rt plays a significant role in visuomotor functions.

Key words: Reticular thalamic nucleus – Neuroanatomical tracing – Mediodorsal nucleus – Rat

Abbreviations: ac: anterior commissure; aca: anterior commissure, anterior; Acb: accumbens nucleus; AI: agranular insular cortex; AM: anteromedial thalamic nucleus; AV: anteroventral thalamic nucleus; BST: bed nucleus of stria terminalis; Cg: cingulate cortex; CG: central gray; CL: centrolateral thalamic nucleus; CM: central medial thalamic nucleus; CPu: caudate putamen; DR: dorsal raphe nucleus; DTg: dorsal tegmental nucleus; EP: entopeduncular nucleus; f: fornix; Fr2: Frontal cortex, area 2; G: gelatinosus thalamic nucleus; GP: globus pallidus; Hb: habenula; HDB: horizontal limb of diagonal band; IAM: interanterodorsal thalamic nucleus; ic: internal capsule; INC: interstitial nucleus of Cajal; IF: interfascicular nucleus; IL: infralimbic cortex; IP: interpeduncular nucleus; LC: locus coeruleus; LDTg: laterodorsal tegmental nucleus; LH: lateral hypothalamus; LHb: lateral habenular nucleus; ll: lateral lemniscus; LO: lateral orbital cortex; LPB: lateral parabrachial nucleus; MD: mediodorsal thalamic nucleus; MDL: mediodorsal thalamic nucleus, lateral segment; Me5: mesencephalic trigeminal nucleus; MHb: medial habenular nucleus; mlf: medial longitudinal fasciculus; MnR: median raphe nucleus; MO: medial orbital cortex; mt: mammillothalamic tract; OPT: olivary pretectal nucleus; pc: posterior commissure; PC: paracentral thalamic nucleus; PF: parafascicular thalamic nucleus; PPTg: pedunculopontine tegmental nucleus; PrC: precommissural nucleus; PT: paratenial thalamic nucleus; PV: paraventricular thalamic nucleus; PVA: paraventricular thalamic nucleus, anterior; R: red nucleus; Re: reuniens thalamic nucleus; RRF: retrorubral field; Rt: reticular thalamic nucleus; Scp: superior cerebellar peduncle; SI: substantia innominata; sm: stria medullaris; SNR: substantia nigra, reticular; st: stria terminalis; TT: tenia tecta; VL: ventrolateral thalamic nucleus; VO: ventral orbital cortex; VP: ventral pallidum; VPL: ventral posterolateral thalamic nucleus; VTA: ventral tegmental area; 3: oculomotor nucleus; 3V: 3rd ventricle; 4: trochlear nucleus

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Introduction

General interest in the neurobiology of the reticular thalamic nucleus arises from evidence that it lies in a key position to influence many aspects of forebrain function. Earlier studies have established that its connections with

thalamus and cortex are organised in a topographic manner. Furthermore each Rt subregion receives collaterals of specific cortico-thalamic projections, and is linked to the thalamus by specific reciprocal reticulothalamic projections (Scheibel and Scheibel 1966; Jones 1975; Montero et al. 1977; Ohara and Lieberman 1985). Rt may also regulate activity, not only in specific thalamo-cortical relay nuclei (Singer 1979), but in several "non-specific" thalamic nuclei. For example recent investigations of the paraventricular and mediodorsal thalamic nuclei in the rat justify their subdivision into at least four subnuclei (Leonard 1969; Krettek and Price 1977; Groenewegen 1988) each of which appears to receive a specific input from the rostral Rt (Cornwall and Phillipson 1988a, b) suggesting a precise organisation of this region of Rt in relation to thalamus and cortex. Furthermore, by these anatomical routes, Rt may also be in a position to regulate the function of both dorsal and ventral striatum in the basal ganglia, and the prefrontal cortex which respectively receive a topographically specific set of projections from the parafascicular, midline and mediodorsal (MD) thalamic nuclei (Cornwall and Phillipson 1988a–c).

Brainstem connections of Rt have received less attention, although in the cat physiological and anatomical studies indicate some pathways that project to Rt (Singer 1979; Steriade and Deschenes 1984; Steriade et al. 1986; Pare et al. 1988). However, in the rat, anatomical evidence for the existence of brainstem connections has been controversial (Berry et al. 1986; Hallanger et al. 1987), and we have also reexamined this question in the present report.

Methods

Male Wistar derived rats (250–300 g) were used throughout and stereotaxic injections made under chloral hydrate anaesthesia (400 mg/kg i.p.) with coordinates derived from the atlas of Paxinos and Watson (1986). Selection of optimal coordinates was achieved by preliminary pressure injections of pontamine sky blue (2% w/v in 0.5 M sodium acetate) followed immediately by perfusion fixation and examination of frozen sections. Lectin tracing methods using immunocytochemistry were preferred over the relatively less sensitive HRP methods so that positive labelling could be achieved from extremely small injection sites. An additional advantage of using lectin is the limited spread of tracer at the injection site, which is an important advantage when studying Rt.

Retrograde tracing

Unconjugated wheat germ agglutinin (WGA; Sigma; 8 μg/μl in 0.05 M Tris HCl saline pH 8.6) was injected microelectrophoretically into selected sites in the rostral Rt with glass micropipettes (internal diameter 20–40 μm), using a positive current of 4 μA applied intermittently over 20 min.

The results presented are selected from a total of 14 animals and represent cases where the centres of the injection sites were optimal with minimal contamination of adjacent structures and in the pipette track. After short survival times of no more than 24 h, animals were perfused under deep chloral hydrate anaesthesia with 50–100 ml isotonic saline followed by 500 ml modified Bouin's fixative (326 ml saturated picric acid, 108 ml 10% formaldehyde, 44 ml saturated mercuric chloride, 22 ml glacial acetic acid). Brains were removed immediately and either post fixed overnight in the same solution or sectioned. Frozen 40 μm sections were collected into 0.05 M Tris-HCl buffer pH 7.8 and washed for 20 min in alcoholic iodine and potassium iodide to control background. After washing in Tris buffer, sections were incubated overnight in the first antibody (polyclonal rabbit anti-WGA diluted 1:2000 in Tris buffer, 0.7% carageenan, and 0.5% Triton X-100) and processed by immunocytochemistry as previously described (Cornwall and Phillipson 1988a). Controls were run to exclude endogenous binding sites; and sections reacted by substituting non-immune rabbit serum for 1st antibody, showed no staining.

Anterograde tracing

Selected projections identified by the retrograde method were also studied with the anterograde lectin tracer Phaseolus vulgaris-leucoagglutinin (PHA-L). The method was based on Gerfen and Sawchenko (1984) and the materials and protocol supplied by Vector (Vectastain, ABC method). Number of animals studied was as follows: intralaminar thalamus (4); basal forebrain (3); medial pretectum (9); laterodorsal tegmental nucleus (11); paraoculomotor cell groups (6). Injections were made using a 2.5% solution of PHA-L in 10 mM sodium phosphate buffered saline pH 8.0, from glass micropipettes with a tip diameter 10–15 μm. A 5 μA positive intermittent current was applied 7 s on, 7 s off for 10 min. Animals were allowed for survive 7–14 days, after which they were deeply anaesthetised, cooled on ice and perfused through the heart with 100 ml normal saline followed by 500 ml 0.1 M phosphate buffer containing 4% paraformaldehyde, 0.05% glutaraldehyde and 0.2% saturated picric acid at 4° C. Brains were removed, post-fixed for 2–4 h and transferred to 10% sucrose in phosphate buffer overnight. Sections were cut at 30 μm on a freezing microtome and collected into ice-cold 0.02 M potassium phosphate buffer pH 7.4 (KPB) and given 3, 15 min washes at room temperature in KPB. Prior to immunocytochemistry sections were incubated overnight in KPB containing 0.3% Triton X-100, 0.5 M NaCl and 2% normal rabbit serum (KPBST/NRS) followed by 20 min in 10% normal rabbit serum in KPBST. Sections were then incubated in 1:1000 dilution of affinity purified goat anti-PHA (E+L form) in KPBST/NRS for 48 h at 4° C. All subsequent steps were performed at room temperature. Sections were rinsed in three 10 min changes of KPBST followed by 20 min in 10% NRS in KPBST, and then reacted with biotinylated affinity purified anti-goat IgG in KPBST/NRS (second antibody) for 50 min. Following two 10 min washes in KPBS, sections were incubated in a preformed avidin: biotinylated horseradish peroxidase macromolecular complex (Vectastain ABC) solution in KPBST for 45 min and then given three 10 min washes with KPBST. Sections were then recycled through the second antibody, wash, ABC, and wash steps and then incubated with freshly prepared filtered diaminobenzidine 0.05% and hydrogen peroxide 0.0025% in KPB for 5–20 min, washed in ice-cold phosphate buffer and mounted on gelatin coated slides. Slides were either stained with light green or defatted prior to thionin staining, dehydrated and coverslipped in the usual manner. In our hands, this method does not appear to label fibres of passage, nor does it label neurones retrogradely, except in possibly a few exceptional cases, in which there is no difficulty in distinguishing from normal anterograde transport. Endogenous binding sites are often detected by this method but these are always clearly distinguishable from normal anterograde label.

Immunocytochemistry for dopamine

Rats were anaesthetised with chloral hydrate and perfused through the heart with 50 ml 0.2% $NaNO_2$ in 0.9% NaCl, followed by 400 ml 5% glutaraldehyde in 0.1 M cacodylate buffer pH 4. Brains

were removed and immersed in the same fixative for 45 min at 4° C. Sections were cut at 30 μm on a freezing microtome and washed in 0.05 M Tris-HCl pH 7.2 in 1% $Na_2S_2O_5$ for 30 min. Immunoperoxidase staining was performed with a dopamine antiserum generously supplied by Dr. Buijs. The characterisation of this antiserum is reported by Geffard et al. (1984). Sections were reacted according to the method of Buijs et al. (1984).

Results

Injection sites

Deposits of WGA in the rostral Rt are shown in Fig. 1. As far as is possible the centre of injections is in register with previously described cell groups projecting to MD. It is not possible practically to confine the spread to those groups only, however, particularly since the cells of origin of Rt-MD pathways appear to be organised in discontinuous patches or strips (Cornwall and Phillipson 1988a). In addition, it is possible that we have not covered the entire region of Rt which provides Rt afferents to the MD. (Throughout the text reference to 'Rt' is confined to the rostral sector as defined by its thalamic inputs, see below.)

Controls for detecting transneuronal transport of lectin (for example Fabian and Coulter 1985) have been reported earlier (Cornwall and Phillipson 1988a), and indicate that, using the low quantities of WGA injected and the short survival times, transneuronal transport could not be detected.

Cortical afferents (Fig. 2)

The terminology follows Zilles (1985) and Paxinos and Watson (1986). Thus, only two subregions on the medial surface of cortex, cingulate (Cg) and infralimbic (IL); and three areas of orbital cortex, medial orbital (MO), ventral orbital (VO) and lateral orbital (LO), are referred to. Cg1 and Cg3 (Zilles) are together referred to here as Cg. Following WGA injection of furthest lateral regions of Rt (RT4), where cells are known to project to lateral MD, retrograde label was found exclusively within lamina VI of cingulate cortex. In case RT1, where the WGA deposit lay medial to that found in RT4, and included cell groups known to project to central MD, cells were found further rostral and dorsal in lamina VI of cingulate cortex, in rostral sections on the dorsolateral surface of cortex, and also in medial, ventral and lateral orbital cortex, and in further caudal sections in the deepest layers of orbital cortex referred to as claustrum by Paxinos and Watson (1986). Occasional cells were found in the infralimbic cortex. In case RT5 label was almost entirely confined to the deepest layer of infralimbic cortex, although occasional cells were also found in the adjacent cingulate cortex, medial orbital cortex as well as in the adjacent medial nucleus accumbens. In case RT3 Cg label similar to that in RT4 was found and clear extension of label was seen in Fr2 (PrCm of Krettek and Price 1977) immediately adjacent to the dorsolateral margins of Cg1.

Thalamic afferents (Fig. 3)

Following WGA injection of the lateral rostral Rt (RT4) retrogradely labelled cells were found in subhabenular regions in the rostral MD and occasional cells in the midline.

However, much stronger label was found ventral and lateral to MD in intralaminar cells of the centromedian, paracentral and centrolateral thalamus. These cells were restricted to a specific region within the intralaminar group. Surprisingly however, this finding could not be replicated in anterograde studies using the PHA-L method, in several cases in which PHA-L was injected only to this specific region of intralaminar thalamus, although clear heavy anterograde labelling of striatum was seen and some label was distributed widely in the deep layers of cerebral cortex.

In case RT1, a different pattern of retrograde thalamic WGA label reflected the further medial Rt injection site, and MD labelling appeared in the central segment. As in RT4, additional much stronger label appeared in intralaminar nuclei but in further medial and ventral sites compared to that found in RT4. In addition a few cells appeared further ventral in rhomboid thalamus. In RT5, very extensive retrograde label was found to extend over the whole anteroposterior extent of dorsal midline thalamus. Most cells lay in the paraventricular thalamic nucleus (PV) and rostrally encircled the parataenial nucleus which was largely unlabelled. In caudal thalamus label extended beyond the paraventricular nucleus and was found also below medial habenula and medial to the parafascicular nucleus in a precommissural transition zone between pretectum and thalamus. Occasional labelled cells were also found in the medial part of the lateral habenula and in the medial habenula. The topographic order of midline thalamus input to rostral Rt was further demonstrated in a further case following caudal medial Rt injections of WGA (results not shown). In this case retrograde cell label was found in the midline in nucleus reuniens, but in caudal-ventral sites compared to those described for RT5.

Confirmation of links between midline thalamus and rostral Rt was obtained with the PHA-L method, in which a localised deposit into mid PV resulted in localized axonal terminations in rostral medial Rt with no extension to further lateral Rt (Fig. 4) and heavy innervation in the nucleus accumbens.

Basal forebrain afferents

In all cases, retrograde label was found in large multipolar neurones in septum, basal forebrain and globus pallidus. Case RT3 most clearly illustrated these results (Fig. 5), and showed cells in the vertical and horizontal limb of the diagonal band, substantia innominata, ven-

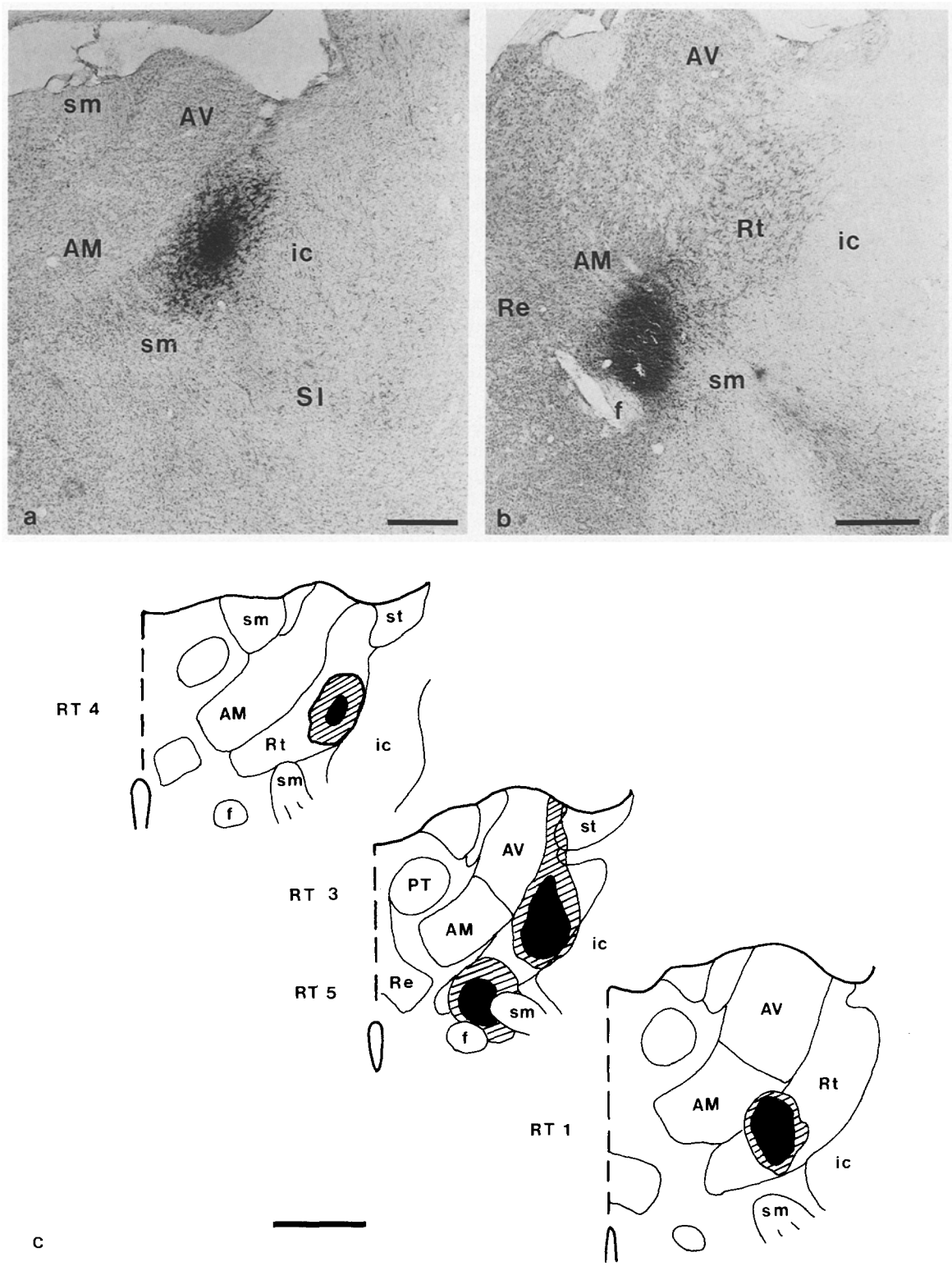

Fig. 1. a, b Injection sites in two examples in relation to the detailed cytoarchitecture of the region. These show that in selected cases it is possible to confine the WGA deposit almost entirely within the limits of rostral Rt, although there is inevitably some track label in most cases. **a** RT4; bar = 500 μm; **b** RT5; bar = 500 μm. **c** Comparison of injection sites for cases reported in detail for retrograde tracing with WGA. The solid centre represents undifferentiated WGA reaction product and the hatched surround a halo of stained cell bodies and lighter staining of neuropil. In the case with the largest WGA deposit (RT3), the dense centre of the injection site was entirely confined to the lateral part of the nucleus with no spread into the adjacent internal capsule or the overlying anterior thalamus. However, an area of light staining surrounding the centre and in the pipette track showed contamination of the anteroventral thalamic nucleus, the stria terminalis, corpus callosum and cortex. In case RT4 (**a**), a very small injection of the rostral and lateral Rt was entirely confined within the nucleus and there was no spread of the surround halo of stained cells outside its margins apart

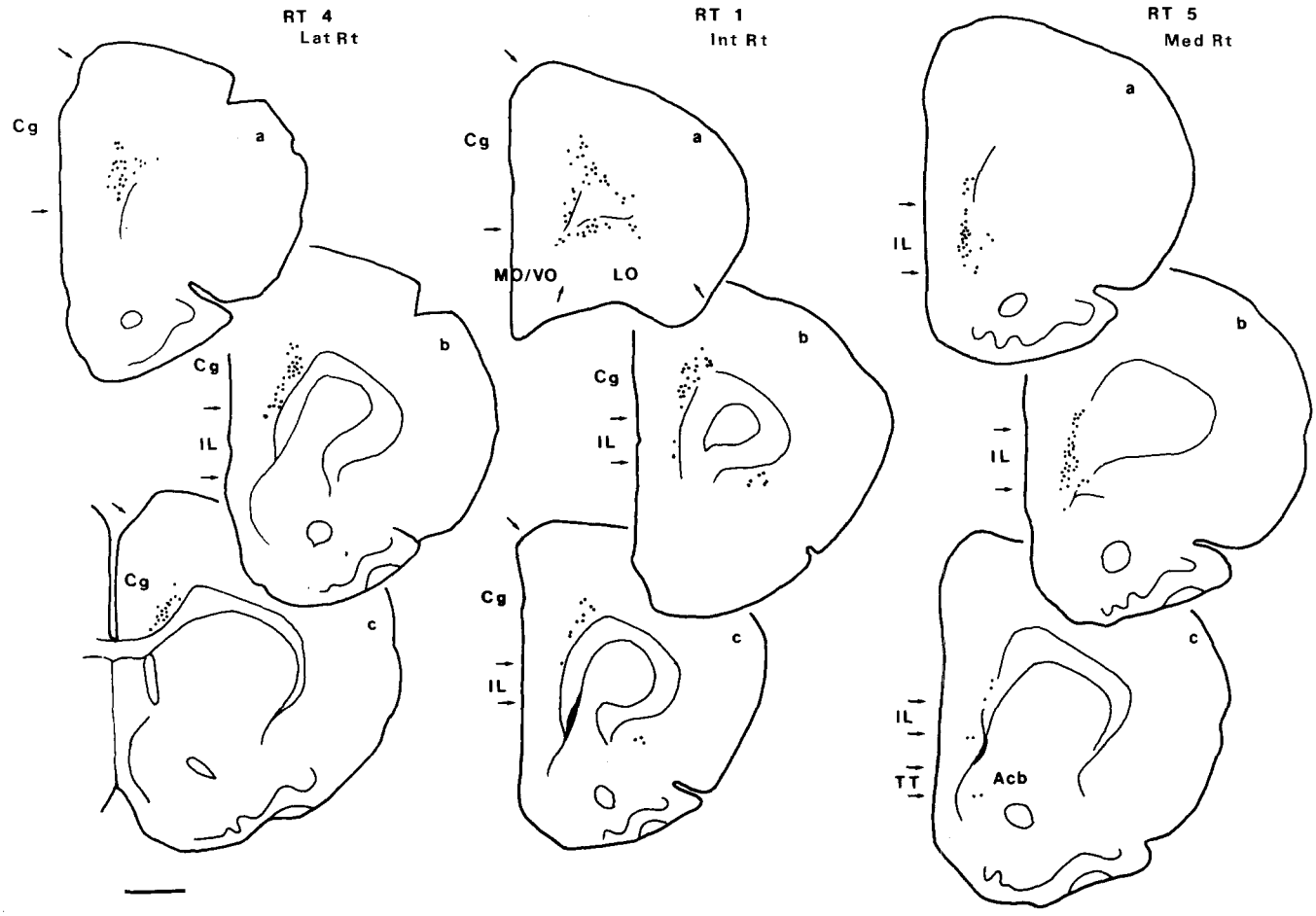

Fig. 2. Pattern of retrograde cell label in the frontal cortex after WGA injection of lateral (RT4), intermediate (RT1) and medial Rt (RT5). The plots are positional, not quantitative. Well labelled sections contained up to 300 labelled cells; others 50–100. Bar = 1 mm

tral pallidum and in the medial globus pallidus adjacent to the internal capsule. Some of these cell groups appeared to correspond to the positions of cells of Ch 1–4 (Mesulam et al. 1983). Cells were rarely found in the magnocellular preoptic area. Since cholinergic basal forebrain neurones are well known to project to cortex, probably via the internal capsule, and some of these cells, particularly the Ch4 group of the globus pallidus, were near the WGA injection site, anterograde tracing from globus pallidus was carried out to confirm this projection. The results are shown in Fig. 6. Spread of PHA-L at the injection site was limited, as is characteristic for this tracer, and clearly did not extend into Rt. Heavy anterograde label was found in axons leaving the injection site in medial globus pallidus and coursing ventromedial to the medial tip of the internal capsule where they entered the rostral Rt. Apparent termination patterns were observed in patches (Fig. 6a, b, d), and were more frequent in the dorsal part of Rt, especially in more caudal sections, and extremely heavy uniform termination patterns were found in the most medial rostral tip of Rt. Labelling was also found in Rt areas outside

from inevitable light contamination of the pipette track. In case RT1 the injection centre was significantly medial to cases RT4 and 3, but excluded the most medial tip of Rt. There was a minor degree of spread to the overlying anteromedial thalamic nucleus. In RT1, 3 and 4, injections appeared to lie approximately within those districts of Rt previously identified as projecting to the mediodorsal thalamic nucleus (MD) (Cornwall and Phillipson 1988a) i.e., the region injected in RT4 projects to lateral segment of MD, and in RT1 to medial MD. In case RT5 the injection was centred on the medial tip of Rt, previously shown to project to midline paraventricular thalamic nucleus (Cornwall and Phillipson 1988b). The borders of this injection lay adjacent to, but did not appear to invade the stria medullaris or the fornix, and there was no apparent spread into the overlying anteromedial thalamic nucleus (**b**, Fig. 2). Sections through the mamillary nuclei were checked in each case to assess spread of the injections to the overlying anterior thalamic nuclei. Only in RT3 was significant retrograde label found (58 cells in the lateral part of the medial mamillary nucleus). All other cases were essentially free of label (case RT5 showed one clearly labelled cell in the lateral nucleus, RT1 one faintly labelled cell, and RT4 no label in any mamillary nucleus). No caudal spread at the injection site to VM thalamus was found in RT4 or RT5, although minor spread was observed in RT3 and 1. This was considered insignificant in view of absence of label from recognised VM input sites other than nigra (labelled also in RT4). Bar = 1 mm

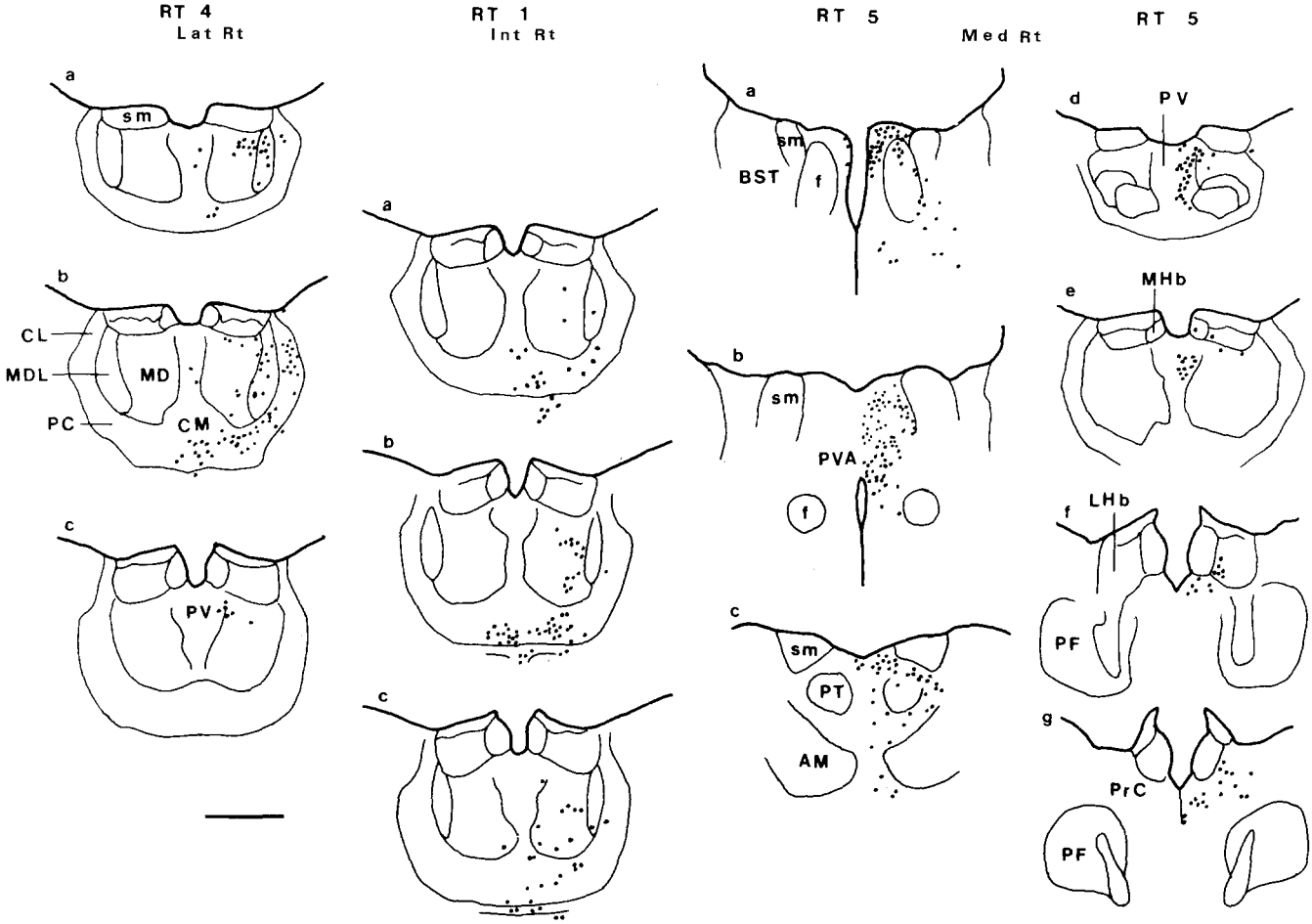

Fig. 3. Retrograde cell labelling in thalamus following WGA injection of lateral (RT4) intermediate (RT1) and medial Rt (RT5). Bar = 1 mm

the rostral Rt, but description of this complex distribution lies outside the scope of the present study.

Brainstem afferents (Fig. 7; Figs. 8–9)

Results were most clearly demonstrated in RT3 with a laterally placed injection, and similar results were found following a smaller laterally placed injection in RT4. RT1 also showed similar brainstem label, although less clearly than for other cases. A different pattern of label was seen, however, in RT5.

In RT3 (Fig. 7; RT3 a–d) bilateral retrograde label was seen in the mesencephalic reticular formation dorsolateral to the red nucleus, and in substantia nigra and retrorubral fields. Nigral label was seen regardless of contamination of VM nucleus at the injection site (absent in RT 4 and 5; light contamination in RT 3 and 1). In both RT3 and 4, small clusters of cells were seen at the ventrolateral margins of periaqueductal grey dorsal and lateral to the somatic motor oculomotor nucleus (in supraoculomotor central gray and the parvocellular division of the oculomotor nucleus, Su3 and 3PC, according to Paxinos and Watson (1986)) and in the paratrochlear region. Some of these extended ventral and lateral to the medial longitudinal fasciculus to lie in the adjacent pontine reticular formation, although they appeared to form a continuous group with the Su3/3PC group. Cells were also found at this level dorsomedial to the oculomotor nucleus in or near the dorsal raphe nucleus and midline central grey. Pedunculopontine nucleus (PPTg) and laterodorsal tegmental nucleus (LDTg) were both labelled, although more clearly in RT3 than RT4.

Confirmation of the LDTg-Rt projection was obtained with the PHA-L anterograde technique. Figure 8a, b shows that an injection, which clearly did not label the adjacent locus coeruleus, or extend to the midline raphe, resulted in labelling of a few very thin fibres and boutons scattered throughout the rostral Rt. The density of label was less than that obtained following globus pallidus injections. Terminal-like label was also found in further caudal sections of the most medial Rt adjacent to zona incerta, which received heavy innervation.

Confirmation of paraoculomotor and paratrochlear projection to rostral Rt was obtained with the PHA-L technique (Fig. 8c, d). The injection site was located in the ventral lateral margin of periaqueductal grey above the caudal end of the oculomotor nucleus (supraoculo-

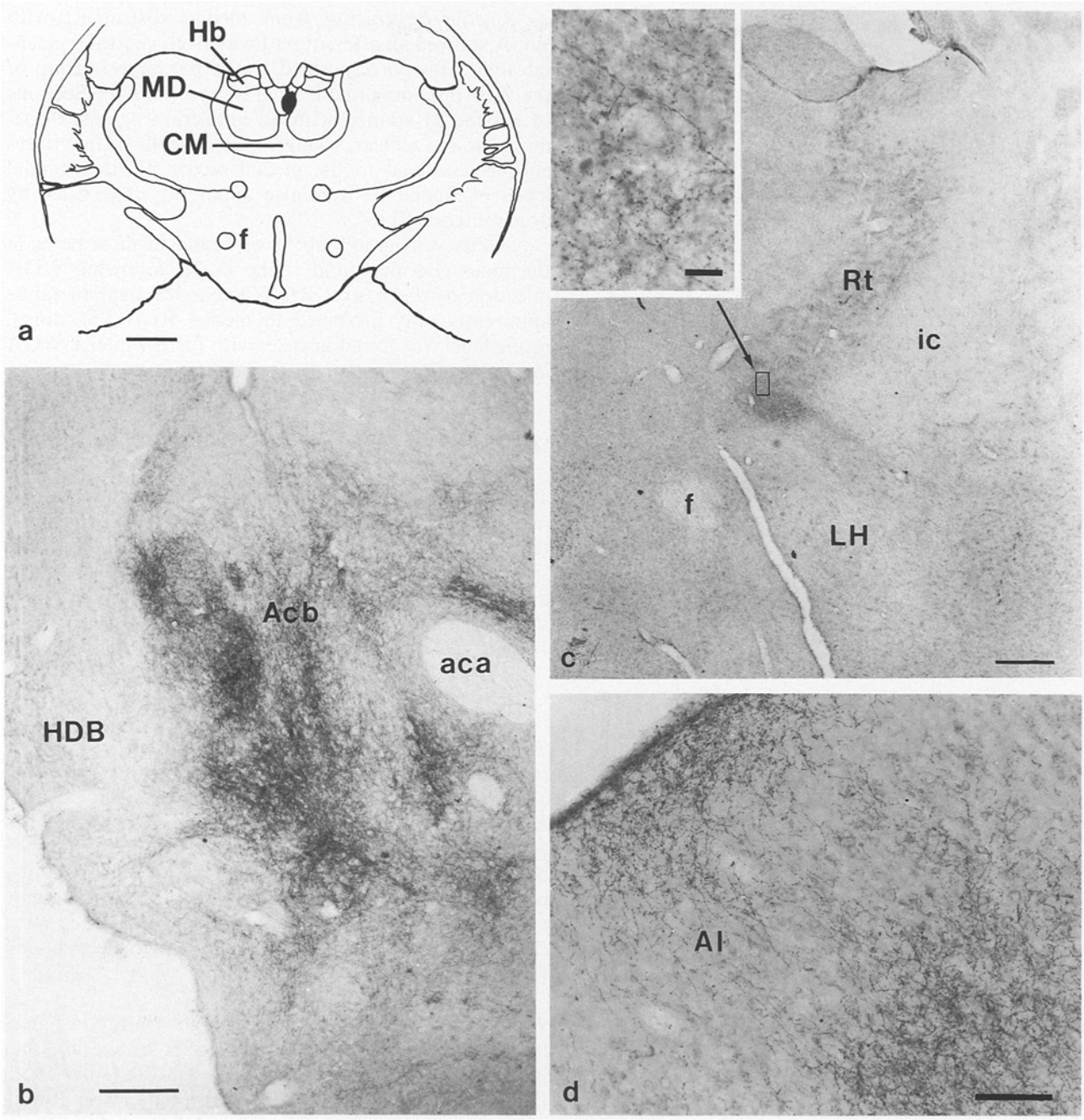

Fig. 4a–d. Anterograde transport of PHA-L from the paraventricular (PV) thalamic nucleus. **a** Injection site shows that the bulk of PHA-L is confined to PV, although the lateral edge invades a small area of the medial segment of mediodorsal (MD) thalamic nucleus as indicated by label found in agranular insular cortex (see Fig. 9d). Bar = 1 mm. **b** Heavy anterograde labelling with a patchy distribution in the nucleus accumbens. Bar = 400 μm. **c** Low power photomicrograph shows the rostral reticular thalamic nucleus (Rt) outlined by some background staining. Bar = 500 μm. At its ventromedial tip a stream of labelled axons enters Rt from the base of the internal capsule (ic). Insert shows high power view of ventromedial Rt (box) with anterograde label. Bar = 15 μm. **d** Anterograde labelling in the agranular insular cortex. Bar = 100 μm

motor central grey) and above the trochlear nucleus. There was some inclusion of the lateral margin of dorsal raphe and the paratrochlear nucleus laterally. Axon terminals were observed in the rostral Rt.

In case RT5 the only retrograde cell label in the midbrain was found in the interfascicular nucleus and ventral tegmental area. In pons, the median raphe was heavily labelled, but only an occasional cell found in PPTg. Additional scattered label was found in further lateral tegmental regions, LDTg and a localised cluster of cells in the lateral parabrachial region.

In RT5 but not in other cases, clear retrograde cell label was also found in the medial pretectal nucleus (MPN) at the mesodiencephalic junction at the level of the posterior commissure. This label appeared to be a caudal extension of the midline thalamic label reported

Fig. 5a–d. Retrograde cell labelling of septal region (**a**), basal forebrain (**b, c**) and globus pallidus (**d**) following WGA injection of lateral Rt (RT3). Bar = 1 mm

above. This pathway was confirmed in anterograde tracing experiments in which PHA-L was injected into the MPN (Fig. 9a, b). A clear plexus of fine terminal fibres was found restricted to the medial Rt. Fibres also appeared to extend laterally from this position, but they did so in small numbers and at the margin of lateral Rt in between the dorsal margin of Rt and the overlying anterior thalamus, where a few terminal boutons were seen. MPN was unlabelled in retrograde experiments in cases RT1, 3 and 4.

Since MPN projects to the interstitial nucleus of the medial longitudinal fasciculus (Cajal) (INC) (Cooper and Phillipson 1988), an important premotor station for oculomotor activity, and our retrograde tracing suggested Rt inputs to the lateral Rt arose from paraoculomotor neurones of the pons which were near the caudal extreme of the interstitial nucleus (Rutherford and Gwyn 1982), anterograde tracing of the interstitial nucleus was carried out with the PHA-L method. Although the injection sites were not entirely confined to the interstitial nucleus, clear, though sparse terminal patterns were found in both medial and lateral sectors of the rostral Rt.

Retrograde tracing from medial rostral Rt with WGA showed an afferent pathway from ventral tegmental area. This corresponded to the paranigral group of the A10 dopaminergic cells (Phillipson 1979). Sections of rostral Rt stained with an antiserum against dopamine showed a clear, though sparse plexus of fine fibres entirely confined to the medial sector. Further caudal levels of medial Rt were also apparently innervated by dopaminergic fibres.

Scanty retrograde label was found in most cases in the locus coeruleus and raphe nuclei following WGA injection of the rostral Rt. Whereas the median raphe apparently only projected to medial Rt (RT5), dorsal raphe label was found in cases with further lateral WGA injections (Fig. 7).

Discussion

The main findings of the present study are summarised in Fig. 10 and show topographically ordered cortical and thalamic projections related to medial and lateral divisions in the rostral reticular thalamic nucleus, as well as inputs from the basal forebrain and a variety of brainstem sites.

Cortical inputs

Terminology. Our definition of subregions of the prefrontal cortex, differs from that described by Krettek and Price (1977). The chief difference is the position and extent of lamina VI of infralimbic cortex which appears to extend into the region defined as "prelimbic" by Krettek and Price (Eden and Uylings 1985). We use here the simpler definitions of two regions, cingulate and infralimbic, since our connectivity data fitted most closely these distinctions and seem to correlate well with function (see below).

Topography. The present results in the rat are in agreement with earlier studies in a number of species, that prefrontal cortex projects to the rostral Rt (Auer 1956; De Vito and Smith 1964; Carman et al. 1964; Rinvik 1968; Leonard 1969; Leichnetz and Astruc 1976; Beckstead 1979). In addition, however, the fine grain organisation of this projection to subregions of rostral Rt is now apparent. The cytoarchitectural distinctions between Cg1 and 3 (or the PrCm, ACd, PL distinctions of Krettek and Price 1977) suggest that further studies with techniques of higher resolution could determine whether subregions of Cg might project to even more highly specific subregions within rostral lateral Rt. This point is of interest since in case RT3 the PrCm cortex of Krettek and Price, which is related to the paralamellar segment of MD, was clearly labelled although this was not found in RT4. Previous work investigating the cortical input to the visual part of Rt in the rabbit, has shown a highly specific fine grain organisation defined by visual field coordinates, a finding supported by physiological

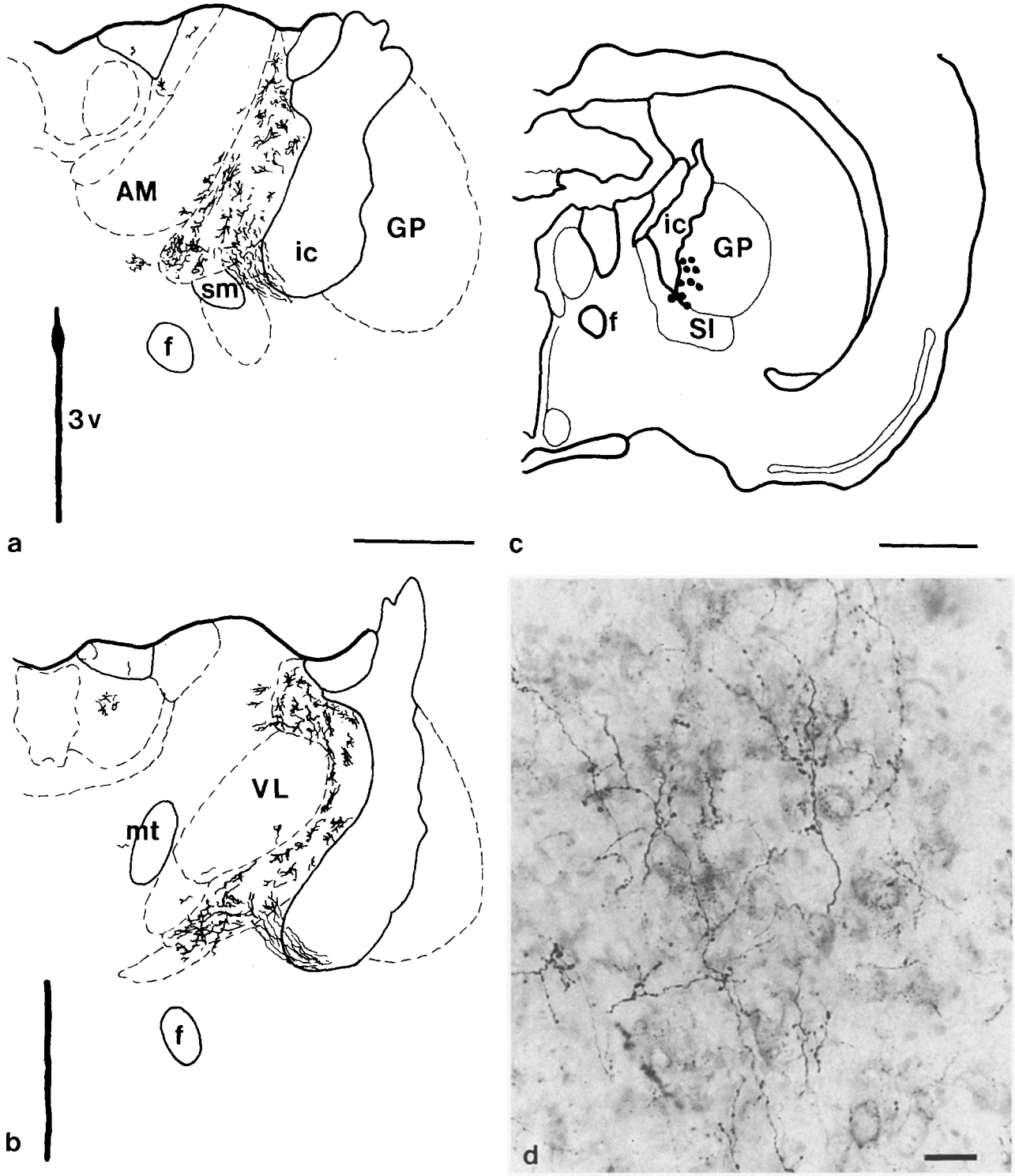

Fig. 6a–d. Anterograde transport of PHA-L from globus pallidus (GP) to rostral reticular thalamic nucleus (Rt). **a** Distribution of anterograde label in both medial and lateral Rt. Bar = 1 mm. **b** At further caudal levels, the label is less dense and concentrated in dorsal and medial Rt. Mag. as in (**a**). **c** Injection site shows the distribution of labelled cell bodies in the medial GP and the caudal adjacent substantia innominata (SI). Bar = 1 mm. **d** Axon terminal like patterns in Rt appear to be concentrated in clusters. Bar = 20 μm

evidence in the rat (Montero et al. 1977; Hale et al. 1982; Crabtree and Killackey 1989).

The retrograde label in cingulate, orbital and infralimbic cortex clearly reflects the different injection sites in rostral Rt and appears to register precisely via the thalamo-cortical projections with regions of thalamus receiving Rt inputs (Cornwall and Phillipson 1988a; Cornwall and Phillipson 1988b). Thus, medial rostral Rt projects to midline thalamus, which in turn projects to nucleus accumbens. Further lateral cells in rostral Rt

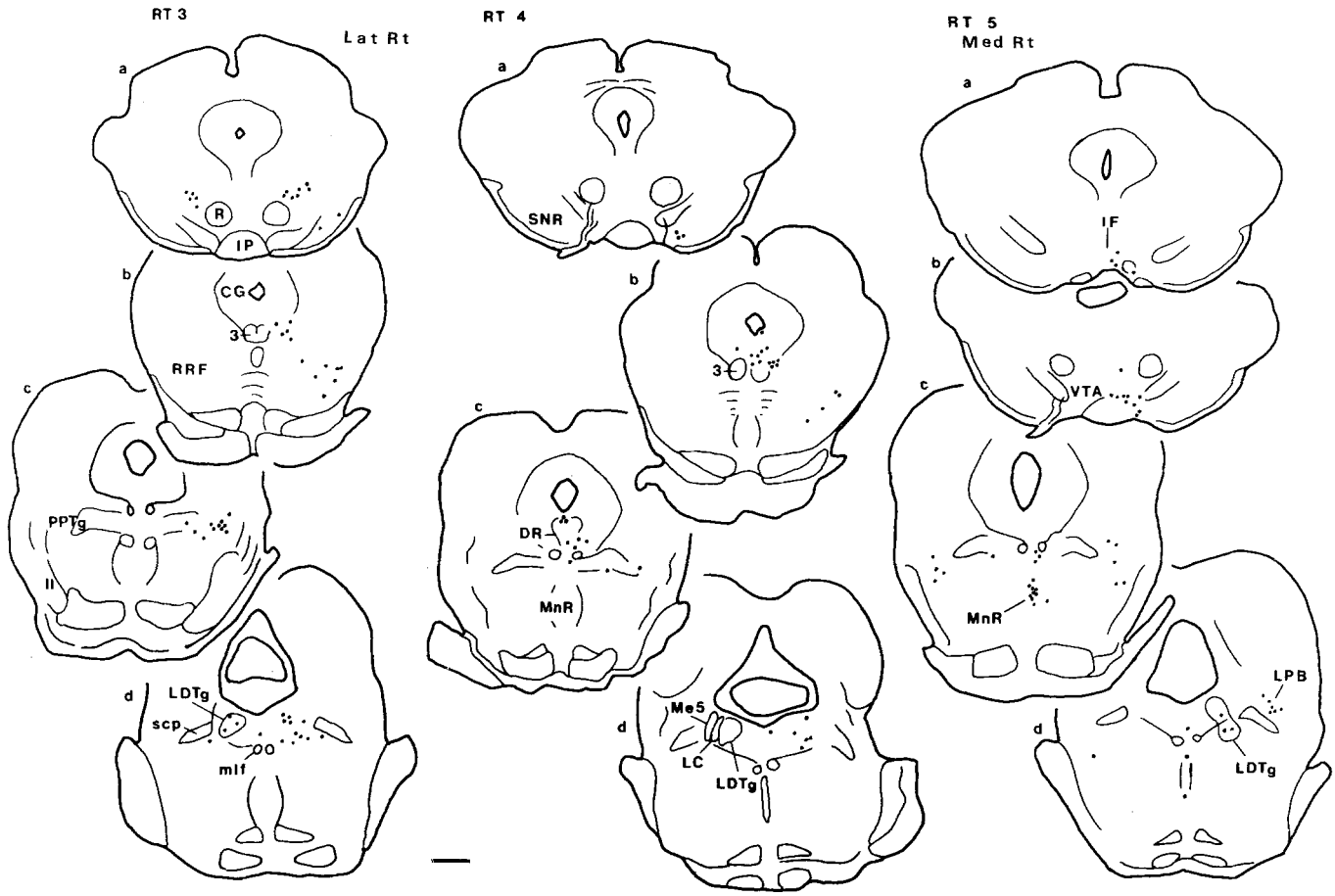

Fig. 7. Retrograde cell labelling in the brainstem following WGA injection of lateral (RT3, RT4) or medial (RT5) Rt. Bar = 1 mm

project to medial MD, and furthest lateral Rt projects to lateral MD. Reciprocal thalamo-cortical connections link different segments of MD to orbital, insular and cingulate cortex (Leonard 1969; Krettek and Price 1977; Price and Slotnick 1983; Groenewegen 1988; Cornwall and Phillipson 1988a). For the case of the medial rostral Rt, the corresponding cortical input registration appears to be IL cortex. These connections mark out the IL cortex as a distinct structure in the organisation of the prefrontal area which may, via links with medial rostral Rt have connections with midline thalamus and the nucleus accumbens. The general pattern of these results therefore resembles the general topographic organisation of other regions of Rt described by earlier workers (Jones 1975; Shosaku et al. 1984; Sefton et al. 1981; Ohara and Lieberman 1985). In addition, many of the cortical output fibres to Rt appear to provide collateral axons to the corresponding thalamic relay nucleus (Sefton et al. 1981; Cornwall and Phillipson 1988c), a finding in agreement with suggestions from earlier Golgi studies (Scheibel and Scheibel 1966).

Thalamus

The present findings, taken together with earlier results (Cornwall and Phillipson 1988a, b) show that in general there are reciprocal links between the rostral Rt and midline and MD thalamic nuclei. Furthermore, the reticulo-thalamic projections appear to be far heavier than the thalamo-reticular links. This was also the conclusion of Scheibel and Scheibel (1966) using a different technique. However, this was not a uniform finding, since thalamo-reticular projections from midline thalamus were far more prominent than those from MD. In addition, we could not demonstrate any thalamo-reticular projections from the medial MD, which is the segment related to insular cortex (Saper 1984). Further information is necessary on this apparent exception.

The finding of intralaminar thalamic projections to the rostral Rt by retrograde techniques was not confirmed by the PHA-L method. Intralaminar PHA-L injection resulted in clear cortical label of lamina VI. It seems possible therefore, that retrograde intralaminar label was obtained by cortical contamination with WGA. This may explain earlier data recording intralaminar-Rt projections by retrograde techniques (Sotgiu et al. 1981). However, recent findings with the autoradiographic technique (Beckstead 1984; Royce and Mourey 1985) strongly suggest a parafascicular intralaminar-Rt projection in the cat. Since we did not find positive evidence for such a pathway in the rat with a technique which clearly identifies terminals as distinct from fibres of passage, further evidence is needed on this point in the cat.

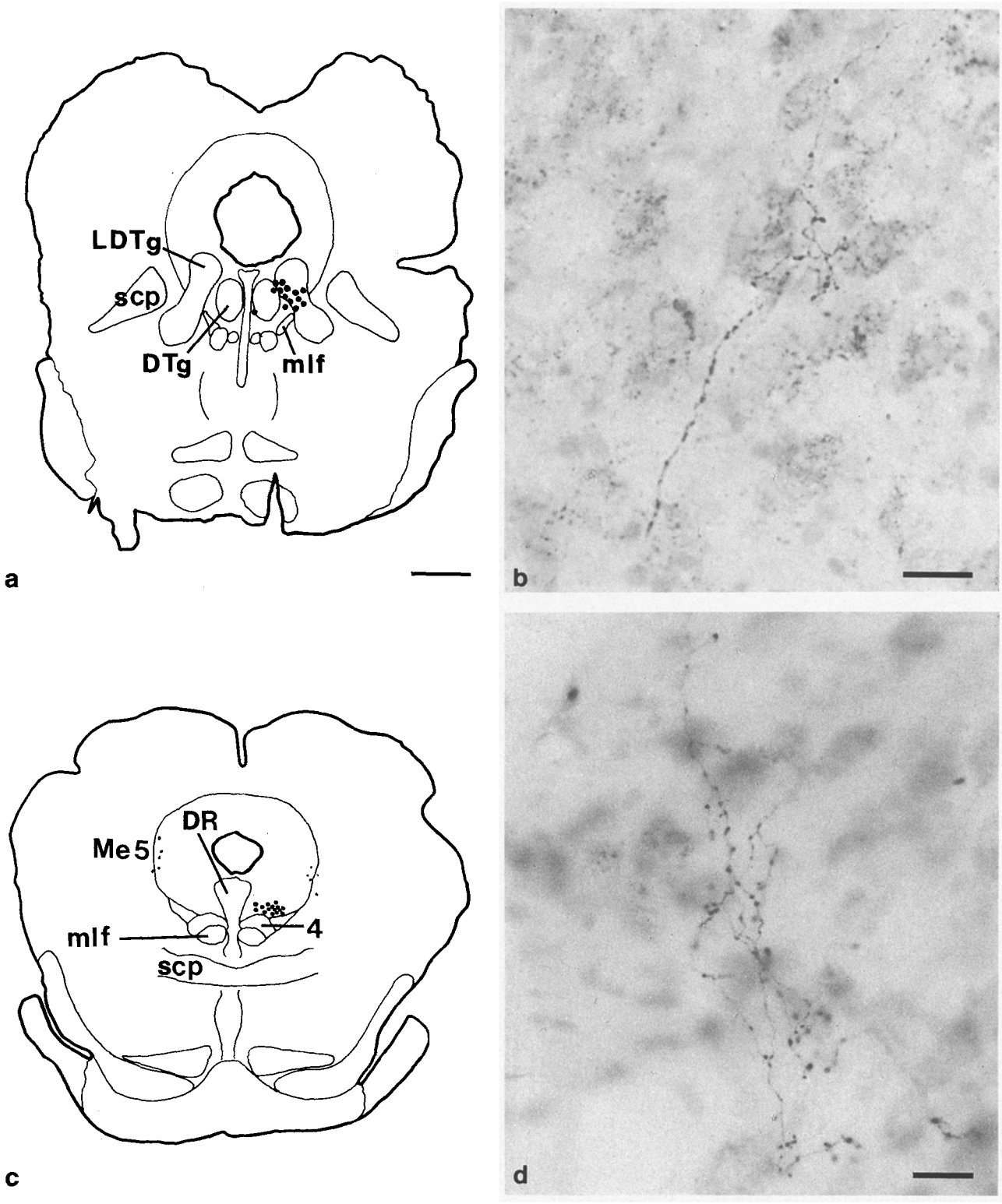

Fig. 8. a, b Anterograde transport of PHA-L from laterodorsal tegmental nucleus (LDTg) to rostral reticular thalamic nucleus (Rt). Injection site (**a**) is largely confined to the rostral portion of LDTg within the periaqueductal grey. Bar = 1 mm. Terminal-like labelling (**b**) was scattered throughout Rt. Bar = 25 μm. **c, d** Anterograde transport of PHA-L from ventrolateral periaqueductal grey dorsal to the trochlear, and caudal oculomotor nuclei, to rostral reticular thalamic nucleus. Injection site (**c**) was centred above the trochlear nucleus, but extended to supraoculomotor regions in adjacent sections. Magn. as in (**a**). Fine axonal labelling in Rt (**d**). Bar = 20 μm

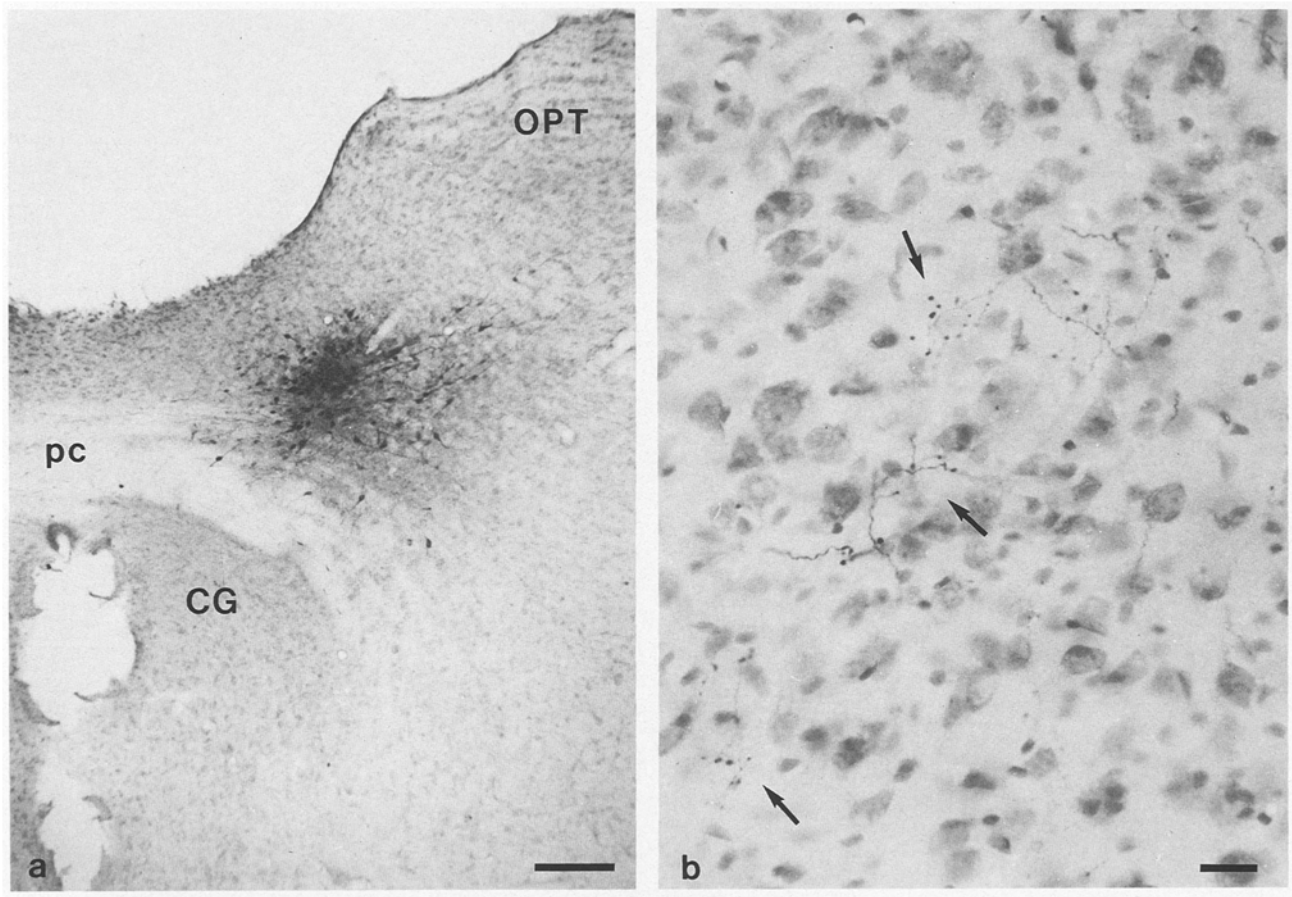

Fig. 9a, b. Anterograde transport of PHA-L from the medial pretectum to rostral medial reticular thalamic nucleus (Rt). **a** Injection site showing position of labelled cell bodies dorsal to the posterior commissure (pc) at the medial extreme of the pretectum. Bar = 200 μm. **b** Fine labelled axons in medial Rt. Bar = 20 μm

In contrast to the findings on the intralaminar thalamus, PHA-L injection of the midline thalamus showed clear evidence of terminal labelling confined to the most medial rostral Rt, as well as terminal label in nucleus accumbens. Cortical input to nucleus accumbens appears to arise principally from CA1 field of the hippocampus (Phillipson and Griffiths 1985). Thus it seems clear that hippocampus, nucleus accumbens, midline thalamus and IL cortex form a distinct set of links compared to those relating the different regions of frontal cortex, and the mediodorsal nucleus, suggesting distinct functional roles for medial Rt as compared to lateral Rt (see below). These mediolateral distinctions in Rt probably reflect specific functional properties of IL cortex which relate to gustatory autonomic and visceral information processing in nucleus of the tractus solitarius and parabrachial nuclei (Terreberry and Neafsey 1987), whilst the cortex related to the MD thalamus may relate in part to olfactory (Price and Slotnick 1983) and visuomotor functions (see below).

Brainstem and basal forebrain

The brainstem reticular formation is known to be involved in the regulation of different stages of cortical activity via pathways which, in some cases, include Rt (see Steriade and Deschenes 1984 for review) and specific influences on defined aspects of visual functions via visual Rt have been suggested (Singer 1979). In the rat, however, anatomical substrates for such effects have been difficult to confirm (Berry et al. 1986). Our findings show, not only that several brainstem projections to Rt do exist in the rat, but that some of these are similar to those reported in cat and monkey (Berman 1977; Beneveneto et al. 1977; Moon-Edley and Graybiel 1983; Pare et al. 1988).

The finding that mesencephalic reticular formation projects to rostral Rt is in agreement with the results of Edwards and deOlmos (1976) using the autoradiographic technique and Hallanger et al. (1987) using WGA-HRP. Furthermore, this region projects generally to all regions of Rt, as well as to midline and intralaminar nuclei; a pattern which is similar to that seen with other brainstem components of the reticular activating system (see below). A further finding of interest in the study of Edwards and deOlmos is that of a strong input from mesencephalic reticular formation to the ventrolateral geniculate nucleus, a finding reported also by Mackay-Sim et al. (1983) in the rat. The emerging evidence for links between the lateral rostral Rt, frontal

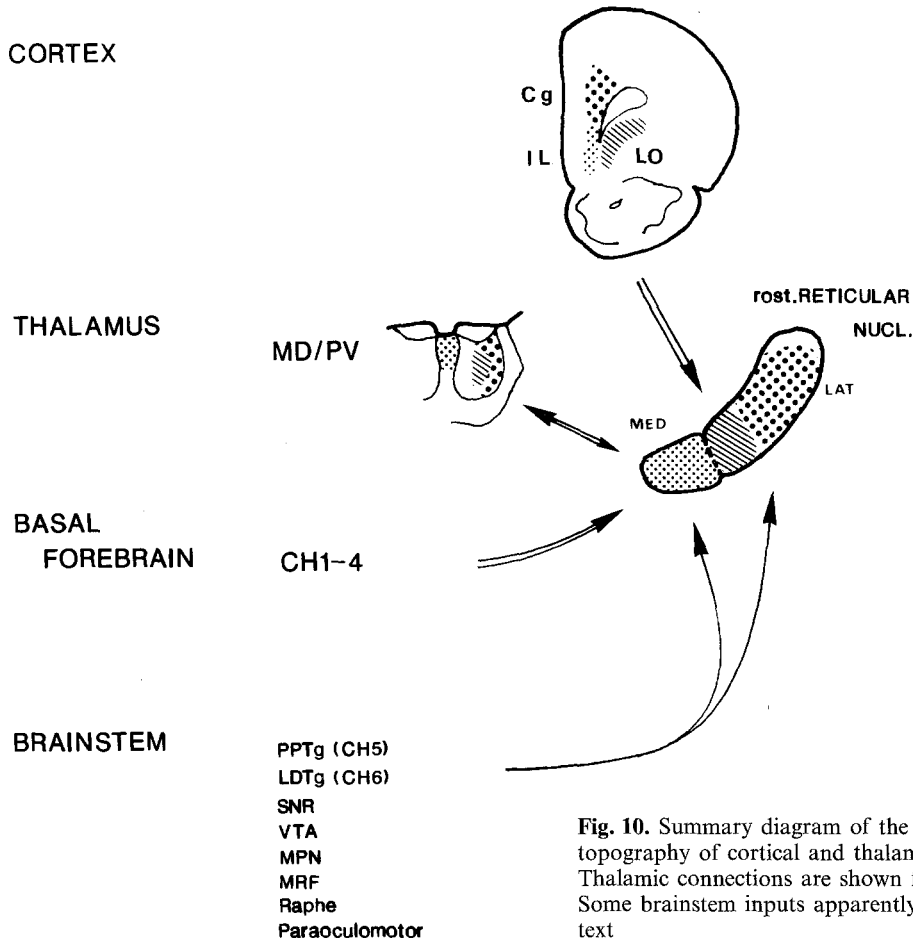

Fig. 10. Summary diagram of the principal rostral Rt inputs. Mediolateral topography of cortical and thalamic inputs is indicated by matching codes. Thalamic connections are shown from MD (lateral) and PV (midline) nuclei. Some brainstem inputs apparently specific for medial Rt are referred to in the text

cortex and preoculomotor sites, suggests that these visual relations of this part of the reticular formation may have particular functional significance in mechanisms of visual attention.

In further caudal regions of brainstem, groups of cells were identified in the pedunculopontine tegmental nucleus and laterodorsal tegmental nucleus a result similar to that reported in the cat (Moon-Edley and Graybiel 1983; Pare et al. 1988). This is also consistent with anterograde PHA-L tracing of PPTg efferents in the rat, which shows that PPTg provides Rt innervation which extends outside rostral Rt as well (Hallanger and Wainer 1988). Double labelling evidence indicates this projection (Ch6), and that from PPTg (Ch5) to Rt is at least in part cholinergic (Woolf and Butcher 1986; Hallanger et al. 1987; Rye et al. 1987). Additional cholinergic and non-cholinergic inputs from Ch1–4 groups of the basal forebrain have been described by other workers in rat and cat (Levey et al. 1987; Hallanger et al. 1987; Smith et al. 1988) and these projections are confirmed by the present findings.

Only the medial rostral Rt received projections from a restricted region of the rostral parabrachial area, further supporting the suggestion of links between medial Rt and gustatory, autonomic and visceral relays. Thus, parabrachial neurones are known to be strongly linked to outputs from nucleus of the tractus solitarius (Ricardo and Koh 1978; Norgren 1978) and to have reciprocal links with cortical areas subserving gustatory functions and with the central nucleus of the amygdala (Saper and Loewy 1980; Saper 1982b; Veening et al. 1984; Schwaber et al. 1982).

Scattered innervation of the rostral Rt from locus coeruleus and dorsal raphe cell groups found in the present study is consistent with previous work (Pechanski et al. 1984) and with evidence for a noradrenergic and a serotoninergic innervation of the rostral Rt (Swanson and Hartman 1975; Cropper et al. 1984).

In addition, substantia nigra and retrorubral fields projected to lateral Rt, apparently from non-dopaminergic cells, whereas ventral tegmental area and interfascicular nucleus appeared to project dopaminergic fibres solely to the medial Rt.

Projections from the pretectum to the reticular thalamic nucleus have been described earlier in the cat (Berman 1977) and monkey (Benevento et al. 1977). The present results show that these connections also exist in rat and that the medial pretectum appears to project only to the medial rostral Rt. Since additional pretectal projections to the interstitial nucleus of Cajal were also found (Cooper and Phillipson 1988), this suggests that the rostral Rt may play a role in preoculomotor mechanisms (see below).

Taking the results of all brainstem sites together, it

seems that the mediolateral organisation of Rt, so clearly reflected in its thalamic and cortical connections (Fig. 10), is also in part suggested by some brainstem inputs. Thus medial pretectum, VTA and parabrachial areas apparently project only to medial rostral Rt.

The results also reveal the presence of so far unrecognised Rt afferents in the rat from groups of cells lying dorsal and lateral to the oculomotor and trochlear nuclei. Earlier work in the cat (Ruda 1976) has shown ventral periaqueductal grey projections to the reticular thalamic nucleus, although at further caudal levels of Rt. The cells found in our study lay at the margins of the central grey matter and adjacent reticular formation and, at some levels, could be interpreted as lying within the caudal tip of the interstitial nucleus of Cajal. Anterograde studies with PHA-L, in which the injection site included these paraoculomotor regions and the caudal INC (Cooper et al. 1989) confirmed these projections to Rt.

Functional considerations. It seems well established that reticular activation is associated with alerting, orienting, and attentional states (Hobson and Scheibel 1980). These effects may be mediated in part by MRF projections to Rt. (Steriade et al. 1986), but are more likely to act in concert with recently described brainstem cholinergic groups CH5/6, which have at least some characteristics associated with the ascending reticular activating system (Rye et al. 1987; Pare et al. 1988; Steriade et al. 1988). CH5/6 may also be directly responsible for the onset of rapid eye movements in REM sleep via cholinergic projections to preoculomotor centres of the paramedian pontine tegmental region (Mitani et al. 1988). Furthermore, CH5/6 neurones project to visual relay and association nuclei and send collaterals to the visual reticular nucleus (Smith et al. 1988). These links with the visual system are of interest in the context of the present findings of paraoculomotor, paratrochlear, medial pretectal and ventral periaqueductal grey (PAG) projections to the rostral Rt. For example, ventral PAG has many links with visuomotor circuits (Gonzalo-Ruiz et al. 1988) and the medial pretectum sends strong contralateral input to the interstitial nucleus (Cooper and Phillipson 1988). The rostral Rt may, therefore, be one region involved in the higher regulation of eye movements and in mechanisms underlying visual attention.

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