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### Circuits through prefrontal cortex, basal ganglia, and ventral anterior nucleus map pathways beyond motor control

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#### Abstract

The ventral anterior (VA) nucleus of the thalamus is connected with prefrontal and premotor cortices and with the basal ganglia. Although classically associated with motor functions, recent evidence implicates the basal ganglia in cognition and emotion as well. Here, we used two complementary approaches to investigate whether the VA is a key link for pathways underlying cognitive and emotional processes through prefrontal cortices and the basal ganglia. After application of bidirectional tracers in functionally distinct lateral, medial, and orbitofrontal cortices, we found that projection neurons were embedded in much larger patches of axonal terminations found in the magnocellular part of VA (VAmc), and in the principal part of VA. Connections from medial prefrontal cortices occupied the dorsomedial and ventromedial VA, and orbitofrontal connections were found in ventrolateral VAmc. Moreover, about half of all projection neurons in orbitofrontal areas directed to the VA or VAmc were positive for calbindin but not parvalbumin, even though comparable populations of neurons were positive for each marker in the VA. We then applied tracers in VA and investigated simultaneously projections from all prefrontal areas, the internal segment of the globus pallidus (GPi), the substantia nigra reticulata (SNr), and the thalamic reticular nucleus. Projection neurons were most densely distributed in anterior cingulate areas 24 and 32, and dorsolateral areas 9 and 8, innervating the same VA sites that received projections from a large part of GPi and dorsal SNr. Nearly as many projection neurons originated from cortical layer V as from layer VI. There is evidence that cortical layer VI neurons innervate thalamic neurons that project focally to the middle cortical layers, whereas layer V neurons synapse with thalamic neurons projecting widely to cortical layer I. Projections from layer V to the VA may facilitate cortical recruitment for executive functions within a cognitive context through lateral prefrontal areas, and autonomic responses within an emotional context through anterior cingulate areas.

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Abbreviations: A, arcuate sulcus; AM, anterior medial nucleus; BDA, biotinylated dextran amine; Caud, caudate; CB, calbindin; Cdc, central densocellular nucleus; Cg, cingulate sulcus; GPe, external segment of globus pallidus; GPi, internal segment of globus pallidus; HRP-WGA, horseradish peroxidase-wheat germ agglutinin; LF, lateral fissure; LO, lateral orbital sulcus; MD, mediodorsal nucleus; MDmc, mediodorsal nucleus, magnocellular sector; MO, medial orbital sulcus; MPAll, medial periallocortex (agranular cortex); OLF, olfactory area; OPAll, orbital periallocortex (agranular cortex); OPro, orbital proisocortex (dysgranular cortex); P, principal sulcus; Pcn, paracentral nucleus; ProM, promotor area; Pu, putamen; PV, parvalbumin; R, reticular nucleus; Re, reuniens nucleus; Rf, rhinal fissure; Ro, rostral sulcus; SNc, substantia nigra compacta; SNr, substantia nigra reticulata; STh, subthalamic nucleus; VA, ventral anterior nucleus, principal part; VAmc, ventral anterior nucleus, magnocellular sector; VLa, anterior ventral lateral nucleus (also known as VLo); VLm, ventral lateral medial nucleus (also known as VM); VLo, ventral lateral oral nucleus (also known as VLa); VM, ventromedial nucleus (also known as VLm; Letters before architectonic areas designated by numbers indicate:; D, dorsal; L, lateral; M, medial; O, orbital; V, ventral

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#### 1. Introduction

The ventral anterior (VA) nucleus of the thalamus in primates is innervated by the output of the basal ganglia, the substantia nigra reticulata (SNr) and the internal segment of the globus pallidus (GPi), in circuits classically implicated in motor functions (for reviews see Ilinsky et al., 1985; Goldman-Rakic, 1987; Graybiel, 1996, 2000; Haber and McFarland, 2001; Anderson, 2001). However, the VA is connected with the prefrontal cortex as well, whose principal thalamic nucleus, the mediodorsal, is a key link of the prefrontal cortex with the basal ganglia (Groenewegen et al., 1990). Thus, the prefrontal cortex has a special relationship with the basal ganglia, because it not only projects to the neostriatum like the rest of the cortex, but its thalamic interactions are modulated by signals from the output of the basal ganglia (for reviews see Alexander et al., 1986; Strick et al., 1995; Haber and McFarland, 2001). In addition, we recently provided evidence for another parallel circuit, through a projection from the anterior medial tip of GPi to the anterior medial thalamic nucleus (Xiao and Barbas, 2002b), which is robustly linked with orbitofrontal and medial prefrontal cortices (Barbas et al., 1991; Dermon and Barbas, 1994; Xiao and Barbas, 2002a), two prefrontal sectors with a pivotal role in emotions (for reviews see Barbas, 1995; Price et al., 1996; Barbas et al., 2002).

The linkage of thalamic nuclei with the basal ganglia and prefrontal cortices is consistent with recent findings implicating the basal ganglia in functions beyond motor control, including cognition, emotion, learning, and memory (Hikosaka et al., 1999; Middleton and Strick, 2000; Graybiel, 2000; Sato and Hikosaka, 2002; Toni et al., 2002). These functions may be mediated through parallel pathways linking functionally distinct prefrontal areas with the basal ganglia through the thalamus. The complex emotional and behavioral changes in Parkinson's and Huntington's disease and in neuropsychiatric disorders (for reviews see Joel and Weiner, 1997; Graybiel, 2000) may be traced, in part, to linkage of the basal ganglia with functionally distinct sectors of the prefrontal cortex, engaged in executive control and goal directed behavior (for review see Barbas, 2000a).

The connections of the mediodorsal thalamic nucleus with the prefrontal cortex and the basal ganglia have been described in considerable detail (for reviews see Groenewegen et al., 1990; Steriade et al., 1997). Information on the interactions of the VA with the prefrontal cortex and the basal ganglia, dates back to studies using ablation-degeneration procedures (Carmel, 1970; Tanaka, 1976) and subsequent tract-tracing studies in cats and monkeys (Kievit and Kuypers, 1975; Jacobson et al., 1978; Kunzle, 1978; Ilinsky et al., 1985; Preuss and Goldman-Rakic, 1987; Yeterian and Pandya, 1988; Barbas et al., 1991; Musil and Olson, 1991; Morecraft et al., 1992; Dermon and Barbas, 1994; Chiba et al., 2001; McFarland and Haber, 2002). However, since the publication of the above studies considerable amount of evidence has been produced on the structural and functional specialization of prefrontal cortices and the VA nucleus (e.g. Ilinsky and Kultas-Ilinsky, 1987; Fuster, 2001; Barbas et al., 2002), as well as the specificity and parallel processing of circuits linking the thalamus with different cortical layers (for reviews see Jones, 1998b; Rouiller and Welker, 2000; Sherman and Guillery, 2002).

Here, we addressed several issues within the context of the above conceptual advances. First, we investigated the relationship of input and output pathways in the VA nucleus that link it with functionally distinct lateral, medial, and orbitofrontal cortices. Second, by injecting retrograde tracers directly in the VA we investigated simultaneously projections to the VA from three key structures involved in circuits for executive control: prefrontal cortices, the GPi/SNr, and the reticular nucleus of the thalamus. This approach made it possible to simultaneously identify preferential projections to the VA from specific layers of functionally distinct prefrontal cortices and the associated internal pallidal and nigral segments. The combined approaches provide anatomic specificity for a system which has been a target for therapeutic interventions to relieve the symptoms of Parkinson's disease (e.g. Svennilson et al., 1960; Bakay et al., 1992; Jahanshahi et al., 2000; Graybiel, 2000; Baron et al., 2000).

#### 2. Methods and techniques

#### 2.1. Surgical procedures

Experiments were conducted on 24 adult rhesus monkeys (*Macaca mulatta*) under sterile procedure, according to the NIH guide for the Care and Use of Laboratory Animals (DHEW Publication no. [NIH] 80-22, revised 1996, Bethesda, MD). Procedures were designed to minimize animal suffering and reduce their number.

To inject tracers in the VA nucleus it was necessary to first obtain a map of the thalamus using magnetic resonance imaging (MRI). To mark the interaural line, hollow ear bars of the stereotaxic apparatus were filled with betadine salve which is visible in MRI. Brain scans were obtained from monkeys sedated with ketamine hydrochloride (10 mg/kg, intramuscularly) and then anesthetized with sodium pentobarbital, administered intravenously through a femoral catheter (to effect). The stereotaxic coordinates for the VA injection were calculated in three dimensions using the interaural line as reference.

To inject neural tracers, 1 week later the monkeys were sedated with ketamine hydrochloride (10 mg/kg, intramuscularly), and given a general anesthetic (sodium pentobarbital, intravenously, to effect) or gas anesthetic (isoflurane) after intubation, until a surgical level of anesthesia was achieved. Overall physiological condition was monitored, including heart rate and temperature. A craniotomy was made, the dura was retracted to expose the cortex and the needle was lowered to the desired location under microscopic guidance.

Cortical injections were made 1.5 mm below the pial surface with a microsyringe (5 or 10 µl; Hamilton) mounted on a microdrive. In all cases, we used a direct cortical approach, penetrating only the cortex of the injection site. We gained direct access to the orbitofrontal cortex through a retro-orbital approach, sparing the zygomatic arch and the eve. We injected prefrontal cortices with distinct tracers: the bidirectional tracers horseradish peroxidase conjugated to wheat germ agglutinin (HRP-WGA, Sigma, St. Louis, MO; 8% solution, volume of 0.05-0.1 µl); biotinylated dextran amine (BDA, Molecular Probes; 10% solution, volume of  $6-8 \mu l$ ; fluoroemerald (Molecular Probes; 10%) solution, volume of 3-4 µl); fluororuby (dextran tetramethylrhodamine, Molecular Probes; 10% solution, volume of  $3-4 \mu l$ ), the retrograde tracer fast blue (Sigma, St. Louis, MO; 1% solution, volume of  $0.8-2 \mu l$ ), or the anterograde tracers tritiated amino acids  $[^{3}H]$  leucine and  $[^{3}H]$  proline (New England Nuclear, Boston, MA, now Perkin-Elmer; specific activity 40–80  $\mu$ Ci, volume of 0.4–1.0  $\mu$ l).

To inject tracers in the VA a small hole was made above the injection site for penetration of the injection needle. We injected fast blue (0.3 µl), fluororuby (3 or 4 µl) or fluoroemerald (5 µl) in the VA. Subcortical injections must traverse the white matter and other structures en route to the injection site. Several precautions were taken to prevent leakage of the tracer along the needle tract. First, we simulated injections outside the brain to determine when tracer first filled the needle bevel. The tracer was then withdrawn until the needle was empty, and the bevel was rinsed in sterile saline several times before it was inserted in the brain. After injection of tracers the needle was left in place for 10-15 min to avoid diffusion of label up the needle tract. The needle was then withdrawn, the wound was closed in anatomic layers and the skin sutured. The animals were monitored until recovery from anesthesia, they were given antibiotics and analgesic (Buprenex, intramuscularly) every 12h, or as needed.

#### 2.2. Perfusion and tissue processing

Animals were given an overdose of anesthetic (sodium pentobarbital, >50 mg/kg, to effect) and perfused through the heart with a fixative after a survival period that depended on the tracers injected.

In HRP-WGA experiments, animals were perfused 2 days after injection (40–48 h) with 21 of fixative (1.25% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffered saline (PBS, pH 7.4), followed by 21 of phosphate buffer (0.1 M, pH 7.4). The brain was then removed from the skull, photographed, and cryoprotected in glycerol phosphate buffer (10% glycerol, Sigma; 2% dimethyl sulfoxide, Sigma, in 0.1 M phosphate buffer, pH 7.4) for 1 day, and then in 20% glycerol phosphate buffer for two additional days. The brain was then frozen in -75 °C, and cut coronally at 40 µm in ten matched series on a freezing microtome.

In experiments with injection of  $[^{3}H]$ -labeled amino acids, after a 10 day survival period animals were perfused with saline followed by 10% paraformaldehyde. The brain was removed, processed for embedding in paraffin and cut into 10  $\mu$ m thick coronal sections. The autoradiographic procedure was based on the method of Cowan et al. (1972). Tissue sections were counterstained with thionin and coverslipped.

In experiments with injection of BDA or fluorescent tracers, animals were perfused 18 days after injection with 2–41 of fixative (4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4). The brain was then removed, photographed and placed in graded series of sucrose solutions for cryoprotection (10, 15, 20, 25 and 30% in 0.1 M PBS with 0.05% azide), frozen in -75 °C isopentane for 2 h (Rosene et al., 1986) and cut coronally at 40 or 50 µm in 10 matched series. In experiments with fluorescent dyes, two matched series of sections were mounted, dried under darkness, and stored at 4 °C. One series was used to map labeled neurons and terminals. The other series was coverslipped with Krystalon (EM Science) and placed in cold storage  $(4 \,^{\circ}C)$  for photography.

## 2.3. Histochemical/immunocytochemical procedures and staining

One series of sections was treated to visualize HRP, according to the method of Mesulam et al. (1980). In experiments with BDA injection, tissue sections were washed in 0.1 M PBS and placed overnight in avidin–biotin–peroxidase complex solution (Vector Labs, cat. # PK 6100, Burlingame, CA). The sections were then washed and processed for immunoperoxidase reaction (3,3'-diaminobenzidine tetrahydrochloride (DAB, plus kit), Zymed Lab, cat. # 00-2020) to visualize the transported dextran. The tissue was mounted, dried, and counterstained with neutral red (for HRP) or thionin (for BDA). In all cases, adjacent series of sections were stained for Nissl, and myelin or AChE to aid in delineating architectonic borders in thalamic nuclei and the prefrontal cortex.

To study whether neurons from the VA projecting to the prefrontal cortex, or from the reticular nucleus projecting to the VA, were positive for the calcium binding proteins parvalbumin (PV) or calbindin (CB), or the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), we performed immunocytochemical procedures in matched series of sections. The tissue was washed with 0.1 M PBS (pH 7.4) and preblocked with 10% goat serum (with 0.2% Triton-X) for 1 h, and incubated for 2-3 days in primary antibody for PV (1:2000; mouse monoclonal, Chemicon), or CB (1:2000; mouse monoclonal, Accurate Chemical and Scientific Corp.), or GABA (1:1000; rabbit polyclonal, DiaSorin Inc., Stillwater, MN) for 2-3 days. The tissue was then placed overnight in goat-anti-mouse IgG (for CB or PV) or goat anti-rabbit IgG (for GABA) conjugated with the fluorescent probe Cyanoindocarbocyanine (Cy3, Chemicon, 1:800), or Alexa 488 (Molecular Probes, 1:200) with 0.1% Triton-X and 1% normal goat serum) and rinsed in PBS.

#### 2.4. Data analysis

## 2.4.1. Stereologic procedures to estimate the prevalence of CB and PV neurons in VA

We used stereologic procedures to estimate the proportion of Nissl stained neurons that were positive for CB or PV in the VA (cases BC, BF and BA), according to standard procedures (for a review see Howard and Reed, 1998). We used a commercial system (StereoInvestigator, Microbrightfield, Colchester, VT), and set the counting frame at 130  $\mu$ m × 130  $\mu$ m for estimating the population of CB or PV positive neurons, and at 50  $\mu$ m × 50  $\mu$ m for the total neuronal population. Grid size was set at 1000  $\mu$ m × 1000  $\mu$ m for both populations, according to the optimal size obtained after a pilot study, as described previously (Xiao and Barbas, 2002b).

#### 2.4.2. Mapping anterograde label in the VA nucleus

We mapped the distribution of terminals in the VA nucleus after prefrontal cortical injections of bidirectional or anterograde tracers using a commercial tracing system (Neurolucida, Microbrightfield, Colchester, VT). We evaluated the density of anterograde label by optical density analysis of terminals using an image analysis system (MetaMorph, Universal Imaging Corp., West Chester, PA), as described previously (Xiao and Barbas, 2002a).

#### 2.4.3. Mapping the distribution of labeled neurons

After injection of fluorescent tracers in the VA nucleus, we used one series of sections to conduct exhaustive mapping of labeled neurons on the ipsilateral side in prefrontal cortices, the thalamic reticular nucleus, and the basal ganglia (GPi and SNr). We used the same approach to map projection neurons in the VA after injection of fluorescent dyes or HRP-WGA in prefrontal cortices. We viewed brain sections with a fluorescence microscope, or under brightfield illumination (Nikon, Optiphot), equipped with an encoded stage and coupled electronically to a PC computer. This system makes it possible to map labeled neurons or terminals in precise register with anatomic landmarks. Software developed in our laboratory ensured that each neuron was counted only once, as described previously (e.g. Barbas and De Olmos, 1990). In some cases, we plotted labeled neurons using a commercial system (Neurolucida, Microbrightfield, Colchester, VT), coupled to a microscope (Olympus, BX 60). After plotting, sections were counterstained with thionin and returned to the microscope to delineate layers, count labeled neurons and measure the area occupied by each layer in individual prefrontal areas.

## 2.4.4. Normalized density of projection neurons in prefrontal areas

We normalized the data to compare the areal distribution of labeled neurons in prefrontal areas directed to the VA among cases. This was accomplished by expressing the density in each prefrontal area as a percentage of the total density in all prefrontal areas in each case. Density of labeled neurons was estimated by dividing the total number of labeled neurons by the volume of the tissue examined in each area, expressed as neurons per mm<sup>3</sup>. Density of labeled neurons was comparable among cases (case AX and BG overall had a higher density of labeled neurons than cases BD and BE). The size of the injection, precise location of the injection site, and tracer used, may have contributed to the differences.

#### 2.4.5. Photography

Photographs through the thalamus were captured with a CCD camera using a software system (Neurolucida, Virtual slice, Colchester, VT). Images were transferred into Adobe Photoshop (Adobe Systems Inc., San Jose, CA) to adjust contrast and overall brightness, but were not retouched. Images of PV or CB positive neurons labeled with different

fluorescent probes, and projection neurons labeled with fluorescent tracers were captured using a confocal microscope (Olympus, Fluoview).

#### 3. Results

## 3.1. Experiments with tracer injections in prefrontal cortices

#### 3.1.1. Injection sites

We first studied bidirectional connections in the VA nuclei after injecting neural tracers in prefrontal cortices in 21 animals. Fig. 1 shows the sites and type of tracer injected in medial (A), lateral (B) and orbitofrontal areas (C). Injection sites were confined to the cortical mantle, and included all cortical layers except for case BC (fluoroemerald), where the injection site was centered in the deep cortical layers of area 13. The injection sites were reconstructed as described previously (e.g. Barbas, 1988). Most of the cases with injections in prefrontal cortices were used previously to study other connections, unrelated to the present study, and are identified by the same names (Barbas and Blatt, 1995; Rempel-Clower and Barbas, 1998; Barbas et al., 1999; Rempel-Clower and Barbas, 2000; Ghashghaei and Barbas, 2001, 2002). Analysis of connections with the thalamus previously was restricted to retrogradely labeled thalamic neurons projecting to prefrontal cortices (Barbas et al., 1991; Dermon and Barbas, 1994), and a detailed analysis of prefrontal connections with the anterior thalamic nuclei (Xiao and Barbas, 2002a). Two cases were not previously described. In one of these, the injection site was in the caudal and dorsal part of area 10 (case BF, Fig. 1B). In another case, the only one with injections on both sides, had an injection in the right hemisphere in area 32 (case  $BG_r$ ), and in the left hemisphere (case  $BG_l$ ) in the rostral part of medial area 9 (Fig. 1A). The pattern of labeling was similar to another area 9 case, or three other area 10 cases.

## 3.2. Input–output zones in the VA nucleus connected with prefrontal areas

The nomenclature of the thalamus is according to the map of Jones (1985), which is largely consistent with the classic map of Olszewski (1952). In both maps, VA consists of a principal part (VA), and a magnocellular part. Another recent map (Ilinsky and Kultas-Ilinsky, 1987), recognizes these divisions as well (the principal part is VA parvicellular), as well as a densicellular division (VAdc), which corresponds to the ventral lateral oralis (VLo) of Olszewski (1952), and the anterior ventral lateral nucleus of Jones (1985). The connections linking the VA with prefrontal cortices are shown in Figs. 2–4, and described for areas within each sector of the prefrontal cortex below.

#### 3.2.1. Lateral cases

Axons from area 8 (cases BFg, BFr, AD) terminated as distinct patches of label in VA and at the lateral part of VAmc, and labeled neurons were distributed more sparsely within the axonal terminals (Fig. 2 top). We saw a similar pattern in cases with injection of several tracers in area 46 (Fig. 1B; ventral area 46: cases MAV, MBH, MFF, AA; and dorsal area 46: case BFb). For example, axonal terminals



from ventral area 46 formed 2-4 patches in VAmc, with some label extending slightly into VA, but not as far as for area 8. Small clusters of neurons were embedded within the axonal terminals (case MAV). Label alternated from moderate to dense in the rostrocaudal direction (not shown). A similar pattern was seen for connections with area 10 (n =4; Fig. 1B), although the patches were more diffuse than for areas 8 or 46. Connections of the central and ventral parts of area 10 were arranged in several loose clusters of axonal terminals and a few projection neurons in VAmc, extending to the ventral part of VA (case SF, Fig. 2, bottom; case BA, not shown). Axonal terminations from the dorsal and caudal parts of area 10 were arranged in diffuse clusters as well, which were dense dorsally in VAmc and the adjacent part of VA, suggesting a certain degree of topographic specificity (cases BF, BC, not shown).

#### 3.2.2. Medial cases

Like lateral areas, axonal terminations from medial prefrontal areas occupied larger zones in the VA than projection neurons. In addition, in comparison with lateral cases, the connections of medial areas extended more rostrally and occupied more medial parts of VAmc. This pattern was seen after HRP injection in medial area 9, where labeled axonal terminals and projection neurons were found at the dorsal border of VAmc and VA (case AO, Fig. 3, top). Another case for medial area 9 showed a similar pattern of connection with the VA (case BG<sub>1</sub>, not shown). The medially situated area 32 showed a similar pattern of connection with the VA (n = 4; Fig. 1A), where dense to light terminal label was concentrated in VAmc and the dorsal parts of VA. Label in the area 32 cases extended over a larger area of the VA than for area 9 or lateral areas (Fig. 3, center, case, AE; cases AY, MDQ and BG<sub>r</sub>, are not shown).

#### 3.2.3. Orbital cases

The zones of connections of rostral orbitofrontal area 11 (n = 3; Fig. 1C; cases AM; MBJ; MFT) overlapped with zones for medial areas in VAmc. In VA, however, antero-

Fig. 1. Composite of injection sites shown on the medial (A), lateral (B) and orbital (C) surfaces of the prefrontal cortex in the rhesus monkey. The temporal pole is depicted transparent on the orbital view (C, large dashed line) to show the posterior orbitofrontal cortex. Small dashed lines delineate areas indicated by numbers based on an architectonic map of the prefrontal cortex (Barbas and Pandya, 1989). MPAll, OPAll, OPro and OLF refer to architectonic areas. Other letter combinations refer to cases. The injection site patterns refer to the type of tracer used: striped area, HRP-WGA; black area, BDA; black outline, [<sup>3</sup>H] amino acids; light grey area, fluorescent tracers. Injections were in the left hemisphere in cases: AM (HRP), MFT ([<sup>3</sup>H] amino acids); AF (HRP), MAR ([<sup>3</sup>H] amino acids), AO (HRP), BG1 (BDA), AE (HRP), AY (BDA), MDQ ([<sup>3</sup>H] amino acids), BC (BDA), and BF (BDA); and in the right hemisphere in cases: MBJ (HRP), MBY (HRP), BAb (fast blue), BCg (fluoroemerald), AG (HRP), BCb (fast blue), BAg (fluoroemerald), BGr (BDA; only bilateral case), BFb (fast blue), AA (HRP), MBH (HRP), MAV (HRP), MFF ([<sup>3</sup>H] amino acids), AD (HRP), BFg (fluoroemerald), BFr (fluororuby), BA (BDA), SF (HRP).



Fig. 2. Connections of lateral prefrontal areas with the VA nucleus. Distribution of labeled neurons (big dots) and terminals (small dots) shown in a series of coronal sections in rostral (left) to caudal (right) thalamic levels. Top: (A–C) bidirectional connections mapped in VAmc and VA. (D) The injection of HRP-WGA was in area 8, shown on the lateral surface of the brain (case AD, black area). Bottom: connections of lateral area 10 with the VA. (A–C) Bidirectional connections mapped in VAmc and VA. (D) The injection of HRP-WGA was in lateral area 10 (case SF, black area).

grade label did not extend dorsally as for medial areas. In a case with HRP injection in the rostral part of area 11, most label was seen in VAmc. As in all other cases, a few projection neurons were embedded in much larger patches of anterograde label (case AM, Fig. 3, bottom, A; case MBJ, not shown). In a case with HRP injection in adjacent orbital area 12 (Fig. 1C, case MBY), patches of axonal terminals were found in VAmc and several clusters of terminals and labeled neurons were seen in VA (not shown).

The connections of caudal orbitofrontal cortex (area OPro, cases AF, AG, BCb, MAR and BAg) differed from rostral orbitofrontal areas by stronger connections with the VA and VAmc, and tighter organization of label in distinct patches. Clusters of axonal terminals and labeled neurons occupied a ventromedial position in VA, at sites not connected with lateral areas, and only sparsely connected with medial prefrontal areas. In case AF, well-delineated clusters of label were seen in VA along the border with VAmc, and within the ventral half of VAmc (Fig. 4A–C). Labeled neurons were arranged in clusters of circular arrays (Fig. 4D), and most clusters were embedded within larger patches of anterograde label (Fig. 4E–F).

#### 3.2.4. Summary of input–output zones in VA

Fig. 5 summarizes the topography of connections of lateral (A), medial (B) and orbitofrontal cortices (C) in the VA. Overall, connections were densest in VAmc, where they overlapped extensively for different prefrontal areas, but appeared more segregated in VA. In all cases, labeled neurons occupied restricted sites within the VA (Fig. 5, circles with black outline), and frequently they were embedded within considerably larger zones of anterograde label (Fig. 5, uniform color).

On the lateral surface, axonal terminations from area 46 were organized into discrete clusters mainly in VAmc, and to a lesser extent in VA; this pattern was more accentuated for terminations from area 8, where the clusters were more distinct and extended further in VA than for other lateral cases (Fig. 5A, case AD). Axons from area 10 terminated in more diffuse patches in VAmc than for areas 8 and 46 (Fig. 5A). By comparison, axons from medial areas 9 and 32 were distributed in loose patches, occupying the entire medial flank of VAmc, and extending into the dorsal part of VA, at sites not occupied by other prefrontal areas studied (Fig. 5B). Axonal terminations from rostral orbitofrontal areas 11 and 12 overlapped extensively in VAmc with terminations from medial prefrontal areas, but extended into the lateral border of VAmc (Fig. 5C), at a site also occupied by connections for area 10 (Fig. 5A). Axons from caudal orbitofrontal cortex were distinguished by their organization into tight patches, found predominantly in the ventral half of VA and VAmc (Figs. 4 and 5C, area OPro).



Fig. 3. Connections of medial and rostral orbital prefrontal areas with the VA nucleus. Top: (A–C) bidirectional connections mapped in the VA and VAmc. (D) The injection of HRP-WGA was in area 9, shown on the medial surface of the brain (case AO, black area). Center: (A–C) bidirectional connections mapped in VA and VAmc (found medial to dotted line through the VA nucleus). (D) The injection of HRP-WGA was in area 32 (case AE, black area). Bottom: Bidirectional connections of anterior orbitofrontal area 11 with the VA nucleus. (D) The injection of HRP-WGA was in rostral area 11, shown on the basal surface of the brain (case AM, black area).

## 3.3. Afferent projections to orbitofrontal cortex from CB positive neurons in the VA

It has been shown that populations of neurons positive for the calcium binding proteins CB and PV represent two parallel thalamocortical systems in sensory relay thalamic systems. CB positive thalamic neurons project widely to cortical layer I, whereas PV positive neurons issue restricted projections to the middle cortical layers (Jones, 1998a,b). Here, we investigated whether projection neurons from the thalamic VA nucleus to prefrontal cortices were positive for CB and PV. We first estimated the normal distribution of CB and PV positive neurons in VA. Stereologic analysis showed comparable proportions of neurons labeled with each marker, with PV positive neurons constituting an average of 17.2% of all Nissl stained neurons in the VA (14.8 % in case BC; 17% in case BF; and 20.5% in case BA). CB positive neurons made up an average of 18.4% of the entire neuronal population in VA (13.2% in case BA; 16.9% in case BC; 25% in case BF). These results are consistent with previous findings that PV and CB positive neurons are equally distributed in the VA (Jones and Hendry, 1989; Jones, 2001). In double-labeling experiments, we found no evidence that either CB or PV positive neurons were GABAergic, which is consistent with evidence that they project to the cortex (Jones, 1998a).

We next investigated to what extent neurons in the VA that projected to prefrontal cortices were positive for CB or PV in cases with injections in orbitofrontal areas (n = 3; area OPro, cases BCb; BAg; area O12, case BAb) and lateral prefrontal areas (n = 3: dorsal area 46, case BFb; and area 8, cases BFr; BFg; Fig. 1). Calbindin positive neurons in the VAmc and VA constituted about half of all projection neurons directed to orbitofrontal areas O12 and OPro (51.4–60%; not shown). None of the projection neurons were positive for PV. In cases with tracer injections in lateral pre-



Fig. 4. Connections of posterior orbitofrontal cortex with the VA nucleus. (A–C) Darkfield photomicrographs taken from representative coronal sections from rostral to caudal extent of the thalamic VA nucleus showing anterograde label in VA (yellow grain) in case AF with an injection of HRP-WGA in orbitofrontal area OPro. (D) Projection neurons arranged in circular array in the VA; the light grain (arrowheads) shows anterograde label. (E and F) Projection neurons (arrows), embedded in large patches of anterograde label (arrowheads). Scale bar in D–F =  $100 \,\mu$ m.

frontal cortices, the number of projection neurons in the VA was too small to evaluate quantitatively.

#### 3.4. Experiments with injections in the VA

#### 3.4.1. Injection sites

In four animals, we injected retrograde or bidirectional tracers in VA (five distinct injection sites). The needle tract for thalamic injections must traverse several structures, including the cortex and white matter, and leakage of dye along the needle tract can potentially complicate interpretation of results. We saw no leakage of tracer along the needle tract, suggesting that structures were not labeled en route to the thalamic injection site. This interpretation is supported by the pattern of labeling in the cortex, where labeled neurons were restricted to the deep layers (V and VI), matching the pattern of corticothalamic projections, but not corticocortical projections which originate in large numbers

in the supragranular layers as well as in the deep layers in all prefrontal areas (e.g. Barbas, 1986; Barbas and Rempel-Clower, 1997).

In all cases, the injection included parts of the VA nucleus, and in two of these, it was confined to the VA (case AX; Fig. 6F), or to the VA and VAmc (case BD; Fig. 7F). In case AX, the core of the injection of fast blue was restricted to the dorsomedial part of the VA (Fig. 6F), and the halo impinged on the rostral parts of VA. In case BD, the core of the injection of fluoroemerald was in VAmc and the ventral part of VA (Fig. 7F). The halo of the injection extended to the ventral part of the ventral lateral oral nucleus (VLo of Olszewski, 1952). In three cases, the injection involved parts of the VA as well as parts of adjacent ventral nuclei. In case BG, an injection of fluororuby covered the caudal quarter of VA, the adjacent part of VLa (VLo of Olszewski, 1952) and a small part of VM (VLm of Olszewski, 1952). In case BE, an injection of fluororuby covered the caudal half of VAmc



Fig. 5. Summary of the input and output zones of the VA nucleus with prefrontal areas. The injection sites are shown on photographs of the cerebral hemisphere on the left, and the connections in VA are summarized on the right. Uniform color patches on the right represent the territory of axonal terminals from prefrontal cortex, and small patches with black outline represent mixed clusters of projection neurons and axonal terminals in VA. (A) Lateral areas and their connections with VA; (B) medial areas and their connections with VA; (C) orbitofrontal areas and their connections with VA.

and the ventral part of VLa (not shown). The dye impinged on a small part of the VM (VLm) and the ventral part of area X. In the same case, an injection site of fast blue was in the ventral part of VAmc and VA, but also included a part of VLa, and nucleus X (not shown).

## 3.4.2. Topography and density of projection neurons in prefrontal areas directed to VA

We studied the distribution and density of projection neurons in the entire prefrontal cortex after injection of tracers in VA or VAmc. Dorsomedial and lateral prefrontal cortices



Fig. 6. Projection neurons in prefrontal cortices directed to the VA nucleus. (A–E) Distribution of projection neurons (black dots) shown in a series of coronal sections in rostral (A) to caudal (E) prefrontal cortices after injection of fast blue in the central part of VA. (F) Injection site in VA is shown in coronal section through the VA (black area, case AX). Dotted line in A–E shows the upper border of cortical layer V. (G) Density of projection neurons in prefrontal cortices directed to the VA nucleus in case AX.

(areas 9, 24, 32 and 8) had significantly higher densities of projection neurons directed to the VA (average density range,  $132-297/\text{mm}^3$ ) than ventromedial and orbitofrontal cortices (average density range,  $7-88/\text{mm}^3$ ; P < 0.01).

In a case where the core of the injection was restricted to the principal part of VA (Fig. 6F, case AX), the highest density of projection neurons was found in area 9 and anterior cingulate area 24, followed by dorsal areas 8 and 32. Lower densities were found in orbitofrontal, and other medial and lateral prefrontal areas (Fig. 6G). In another case, the tracer injection was in the caudal and ventral part of VA (not shown). In this case, however, the injection invaded the ventral part of VLa (VLo of Olszewski, 1952), which does not appear to have connections with prefrontal areas (Barbas et al., 1991; Dermon and Barbas, 1994). The pattern of labeling was similar to case AX, with the densest distributions of projection neurons found in area 24, followed by areas 9 and 8. The lowest densities were noted in orbitofrontal and several other medial and lateral prefrontal areas (not shown).

In two cases, where the tracer injections involved VAmc (cases BD, BE), the pattern of labeling in prefrontal cortices was similar to the previous two cases with injection in the



Fig. 7. Projection neurons in prefrontal cortices directed to the VA/VAmc. (A–E) Distribution of projection neurons (black dots) shown in a series of coronal sections through the prefrontal cortex. The dotted lines demarcate the upper border of layer V. (F) The injection of fluoroemerald was in the VAmc and VA (black area, case BD). (G) Histogram showing the density of projection neurons in the prefrontal cortices in case BD.

principal part of VA, but also showed some differences. In case BD, injection of both VAmc and VA (Fig. 7F) resulted in retrograde labeling in all prefrontal areas (Fig. 7A–E). As in the previous cases, a high density of projection neurons was found in areas 32, 9 and 8. Area 24 also contained labeled neurons but it was not evaluated quantitatively because

part of it was obscured with injection of a retrograde tracer for an unrelated experiment. The injection of diamidino yellow in a part of area 24 did not otherwise affect the results, since the tracer was distinct from the tracer injected in the VA nucleus in the same case (fluoroemerald). A moderate density of projection neurons was found in areas O12, 10 and L12, and lower densities were seen in areas 25, M14, OPro, 13, 11, 14 and 46 (Fig. 7G). This case differed from case AX by having more labeled neurons in orbitofrontal areas, particularly area O12 (Fig. 7C-D). However, labeling in orbitofrontal areas was considerably more robust after injection in the caudal part of VAmc, which also involved VM (VLm of Olszewski), VLa, and a small part of area X (case BE, fluororuby injection). In this case, the highest densities of labeled neurons were recorded in cingulate area 24 and orbitofrontal area OPro (not shown). In previous studies, we found that VLm of Olszewski (1952) sends a moderate projection to prefrontal cortices, particularly orbitofrontal areas (Dermon and Barbas, 1994), suggesting that the higher labeling in orbitofrontal areas may be attributed to involvement of VLm (VM). Moderate densities were seen in medial area 9 and lateral area 12, and considerably lower densities were seen in all other prefrontal areas. In the same case, an injection of fast blue in VAmc/VA, VLa and area X (case BE, fast blue), resulted in a similar pattern of labeling as in the other cases, with the highest densities of projection neurons found in area 24, followed by areas M9, 12 and V8 (not shown).

To compare the relative distribution of projection neurons across prefrontal areas, the density of labeled neurons in each area was normalized to the total density in all prefrontal areas for each case (n = 4; cases AX, BD, BE (fluororuby), BG). In spite of the differences in the injection sites, the distribution of projection neurons was remarkably similar across cases, being most dense in area 24 and medial area 9, followed by dorsal area 9, area 8, and area 32 (Fig. 8A). Projection neurons were comparatively less dense in ventromedial areas M14 and M25, most orbitofrontal areas, and lateral areas 10 and 46. These findings are consistent with corticothalamic studies using anterograde tracers in prefrontal cortices, as described above.

# 3.5. Laminar origin of projection neurons in prefrontal cortices and some observations on termination of VA axons in prefrontal areas

Projection neurons from prefrontal cortices directed to the VA originated from layers V and VI. Unlike the common pattern of corticothalamic projection neurons to principal thalamic nuclei, which originate overwhelmingly from layer VI (Jones, 1985), about half of the projection neurons in prefrontal cortices directed to VA/VAmc originated from layer V (V = 44%; VI = 56%), regardless of whether VLa (VLo) or VM (VLm) were also involved. The laminar distribution of projection neurons is shown in Fig. 8B for areas with consistent projections across cases. Dorsal area 8 and OPro issued the highest percentage of projection neurons from layer V.

In cases with bidirectional fluorescent dyes in the VA/VAmc (case BD), or in VA with involvement of VLa (VLo of Olszewski, 1952; case BG), we made some observations on the laminar distribution of thalamocortical



Fig. 8. (A) Normalized density of projection neurons in the prefrontal cortices directed to the VA. (B) Laminar origin of projection neurons in prefrontal cortices directed to the VA in areas that consistently showed projections in four cases (cases AX, BD, BE (fluororuby), BG. Vertical lines on bars show standard error.

terminal fields in the prefrontal cortex, and their relationship to projection neurons. Detailed analyses of thalamocortical terminations in frontal cortices, their relationship to corticothalamic neurons and reciprocity of connections were provided in other studies (Shinoda et al., 1993; McFarland and Haber, 2002). In this study, we found that axons from VA formed patches in both superficial and deep layers of prefrontal cortices, targeting prominently medial and dorsal prefrontal cortices that had the highest density of projection neurons (areas 24, 32, 9, and 8; cases BD and BG). These findings are consistent with previous studies of connections in frontal cortices after injections in VA and sectors of VL (Shinoda et al., 1993; McFarland and Haber, 2002).

Axonal projections from VA/VAmc terminated in the superficial layers (layers I–III) and in layer IV, a pattern seen particularly in area 9 (case BD). The terminals were situated above projection neurons, which were found in the deep layers. Similarly, projections from VA (involving also VLa) terminated in both superficial and deep layers (case BG). The axonal terminals were densely distributed in the deep layers of areas 9, 24 and dorsal area 8, in areas with densely distributed corticothalamic projection neurons (not shown).

## 3.6. Projections from the basal ganglia and the reticular thalamic nucleus to the VA

In cases with thalamic injections, we found a large number of projection neurons in the internal segment of the globus pallidus and the substantia nigra reticulata, which make up the output of the basal ganglia (cases BD; BG; BE, fluororuby dye). This finding is consistent with previous studies (Kuo and Carpenter, 1973; Kim et al., 1976; Ilinsky et al., 1985; Ilinsky and Kultas-Ilinsky, 1987; Fenelon et al., 1990; for review see Parent and Hazrati, 1995). Labeling was similar in the GPi whether the injection site included VA/VAmc alone (case BD; Fig. 9, top), or one or both parts of the VA along with VM (case BE, fluororuby dye), or VLa (case BG, not shown). Labeled neurons were arranged in circu-



Fig. 9. Projections from the basal ganglia and reticular neurons to VA/VAmc. Top: (A–D) distribution of labeled neurons in GPi directed to VA and VAmc, shown in a series of rostral (A) to caudal (D) coronal sections, mapped after injection of fluoroemerald in VA/VAmc (case BD, inset, panel F). Center: distribution of labeled neurons in the dorsolateral part of the SNr directed to VA and VAmc, shown in rostral (A) to progressively more caudal (E) coronal sections in case BD (inset, panel F). Bottom: distribution of labeled neurons in the rostral part of the reticular nucleus, shown in rostral (A) to more caudal (C) coronal sections in case BD (inset, panel F).

lar arrays, stretching over 4 mm in the rostral to caudal extent of the GPi, but excluding the very rostral and caudal tips (Fig. 9, top). Small areas at the dorsolateral and medial edges of GPi were devoid of labeled neurons following injection in VAmc/VA (case BD), or in VAmc along with VM or VLa (case BE, fluororuby dye; case BG). Projection neurons were also found in patches throughout the rostrocaudal extent of the SNr, and were concentrated dorsally, while the ventral part of the nucleus had few, if any, labeled neurons (Fig. 9, center).

The output of thalamic nuclei is modulated by projections from the thalamic reticular nucleus (for a review see Steriade et al., 1997), as shown for the VA in this study (Fig. 9, bottom, case BD; and cases BE (fluororuby dye) and BG, not shown). Prefrontal projections to the VA originated from the rostral fifth of the reticular nucleus. Labeled projection neurons in the reticular nucleus were most prevalent in the dorsal half of the nucleus, but some were also seen in its ventral sector. Many projection neurons were positive for PV, a marker for a large population of GABAergic neurons in the reticular nucleus (for review see Steriade et al., 1997).

#### 4. Discussion

#### 4.1. Topography

The association of the VA thalamic nucleus with the prefrontal cortex has been shown in previous studies (e.g. Kievit and Kuypers, 1977; Kunzle, 1978; Ilinsky et al., 1985; Preuss and Goldman-Rakic, 1987; Yeterian and Pandya, 1988; Chiba et al., 2001; Middleton and Strick, 2002; McFarland and Haber, 2002; for review see Cavada et al., 2000). There is general agreement that projection neurons directed to prefrontal areas are found mostly in the magnocellular part of VA (Kievit and Kuypers, 1975; Dermon and Barbas, 1994), arranged in crude topography, with preferential projection of the ventral sector of VA to orbitofrontal cortices, and the dorsal sector to area 46 (Goldman-Rakic and Porrino, 1985; Barbas et al., 1991). Based on two complementary neural tracing approaches, our findings confirm and extend previous studies in several ways. First, the connections of the prefrontal cortex with the VA were more extensive than previously thought, involving all prefrontal areas. The widespread projections from prefrontal cortices to the VA cannot be attributed to involvement of thalamic nuclei besides VA, since this was noted in cases where the injections were restricted to VA and VAmc (cases AX, BD). Moreover, the adjacent motor-related nucleus VLo does not have connections with prefrontal cortices (Barbas et al., 1991; Dermon and Barbas, 1994), so its involvement in some of the injections could not have affected the distribution of prefrontal projection neurons. In accordance with previous findings, we found overlap in the connections of different prefrontal areas in the VA, but also identified sites occupied preferentially by different prefrontal regions. Thus, dorsomedial and ventromedial VA are the predominant domain of medial prefrontal cortices, whereas the ventrolateral VAmc is innervated preferentially by orbitofrontal cortices (Fig. 5).

## 4.2. Preferential projections from lateral areas 8 and 9 and medial areas 24 and 32 to the VA

Medial areas 24 and 32 in the anterior cingulate, and dorsolateral areas 9 and 8 stood apart from the rest by more robust projections to the VA, regardless of the specific part of the VA injected, or involvement of adjacent nuclei, including VLa and VM (Fig. 8A). These robust projections were revealed after injection of retrograde tracers in VA and corroborated after focal injection of bidirectional tracers in areas 9, 8 and 32. Evidence from the combined approaches indicated that the populations of projection neurons in prefrontal areas, and their terminal fields in VA were extensive. In contrast, projection neurons directed to the same prefrontal cortices occupied a comparatively smaller territory in the VA.

Lateral prefrontal cortices that have robust connections with the VA are distinguished for their role in cognitive processes linked to action. These posterior lateral prefrontal areas guide behavior by directing attention to behaviorally relevant stimuli, selecting appropriate responses, suppressing inappropriate responses, and holding temporarily a series of short-term events in memory (for reviews see Goldman-Rakic, 1988; Fuster, 1993; Petrides, 1996; Barbas, 2000a,b). In human and non-human primates, there is evidence from functional imaging and lesion studies that dorsolateral prefrontal areas 9, 46 and 8 are implicated in integration of temporal, spatial and visuomotor information (Petrides, 1995, 1996; Cohen et al., 1997; Braver et al., 1997; Levy and Goldman-Rakic, 2000; Schiller and Tehovnik, 2001; Pochon et al., 2002). In the classically defined frontal eye fields (area 8), oculomotor responses are tightly linked to action (Wurtz et al., 1980; Schiller, 1998), and area 9 is implicated in monitoring performance in human and non-human primates (Petrides, 2000).

The VA nucleus also received dense projections from anterior cingulate areas 24, and 32, which have robust connections with the amygdala and a key role in emotions (for reviews see Price et al., 1987; Barbas et al., 2002). Functional imaging studies in humans implicate the anterior cingulate in monitoring performance in tasks involving conflict (Carter et al., 2000; Milham et al., 2001), a hypothesis that is consistent with the connections. Area 32, in particular, is distinguished by diverse connections with central autonomic structures, including robust excitatory synaptic connections with hypothalamic autonomic centers (Rempel-Clower and Barbas, 1998; Ongur et al., 1998; Barbas et al., 2003). In addition, area 32 projects to two other key autonomic-related structures, the extended amygdala (Ghashghaei and Barbas, 2001), and nuclei of the amygdala (Ghashghaei and Barbas, 2002) that innervate central autonomic structures (for review The intricate relationship of the dorsolateral and anterior cingulate areas with structures associated with action-oriented behavior is further substantiated by the robust innervation of the same VA sites by the GPi and SNr. This evidence confirms and extends classic findings indicating that several thalamic nuclei that project to the frontal lobe, including the mediodorsal and intralaminar, are modulated by projections from the output nuclei of the basal ganglia (e.g. Rosvold, 1972; Alexander et al., 1986; Groenewegen et al., 1990; Joel and Weiner, 1994; Haber and McFarland, 2001; Parent et al., 2001; Anderson, 2001; Middleton and Strick, 2002).

Evidence from transneuronal transport of tracers injected in restricted frontal cortices, demonstrated segregated output channels in the GPi in monkeys (for reviews see Strick et al., 1995; Middleton and Strick, 2000). Thus, projection neurons associated with prefrontal areas 9 and 46 were found at a dorsal strip of GPi, those linked to the ventral premotor cortex occupied the most ventral strip, while projections associated with the primary motor and supplementary motor areas occupied the middle part of GPi. Moreover, a large territory of GPi was associated with 'projections' to prefrontal areas 46, 9 and 12, comparable in volume (though not in topography) to the territory associated with motor areas (Middleton and Strick, 2002).

Using a parallel approach, we found equally strong, or stronger, projections directed from GPi to VA, which, in turn, received robust projections from prefrontal cortices. However, it is not possible to provide an accurate estimate of the territory of GPi associated with prefrontal areas, since the VA is connected with premotor areas as well as with prefrontal areas (for review see Steriade et al., 1997). Nevertheless, even our most restricted injections, involving only part of VA, resulted in widespread projections in GPi, which likely reflects the pattern of innervation of the VA by GPi neurons (Ilinsky and Kultas-Ilinsky, 1987; Parent et al., 2001). Conversely, we found that even large injections in VA, which also invaded parts of VLa and VM, labeled projection neurons in circular arrays in GPi, with the core devoid of label. This pattern suggests the presence of interdigitated modules in GPi, occupied by premotor, motor and prefrontal systems. This idea is consistent with the topographic specificity of projection neurons in GPi in transneuronal mapping by viral tracers injected in premotor areas and the primary motor cortex (Hoover and Strick, 1993).

Our data also showed that the medial edge of GPi was devoid of labeled neurons, at sites occupied by projection neurons directed to the anterior medial nucleus (AM) of the thalamus (Xiao and Barbas, 2002b). The AM, in turn, is connected most densely with orbitofrontal and medial prefrontal cortices, two areas with a documented role in emotions (for review see Barbas et al., 2002). Projection neurons at the medial tip of GPi may represent an output channel linking the basal ganglia with the limbic component of the prefrontal cortex through AM, which is distinct from channels directed to lateral prefrontal, premotor and motor cortices through the VA and VL nuclei (Hoover and Strick, 1993; Middleton and Strick, 2002).

## 4.3. Laminar organization of corticothalamic projections directed to the VA nucleus

Projection neurons from layer V to the VA or VA along with adjacent parts of VLa and VM, accounted for about half of all prefrontal projection neurons, a pattern that differs from corticothalamic projections to principal thalamic nuclei, which originate mostly, or entirely, in layer VI (Gilbert and Kelly, 1975; Robson and Hall, 1975; Lund et al., 1976; Jones and Wise, 1977; for reviews see Jones, 1985; Steriade et al., 1997). The significance of this finding is based on evidence from sensory systems that corticothalamic neurons originating in layer V differ substantially from those in layer VI (for review see Rouiller and Welker, 2000). Thus, axons of layer VI neurons extend small and continuous terminals to the distal dendrites of thalamic neurons which project to cortical layer IV (Rouiller and Welker, 1991; Ojima, 1994; Rockland, 1996). In contrast, layer V pyramidal neurons give rise to large and discontinuous axonal terminals in the thalamus, forming both non-reciprocal and reciprocal projections on the dendrites of thalamocortical projection neurons which terminate widely in cortical layer I (for reviews see Jones, 1985; Steriade et al., 1997; Castro-Alamancos and Connors, 1997; Rouiller and Welker, 2000; Haber and McFarland, 2001; Jones, 2002). The patchy distribution of connections seen in the VA here, is consistent with the high distribution of projections from layer V.

Projections from VA to layer I of the frontal cortex have been described in detail previously (McFarland and Haber, 2002). However, further studies are necessary to determine if cortical layer I receives projections from thalamic neurons innervated by neurons from layer V of the same cortical area. Our results provide indirect support for this circuit, by showing that about half of the neurons in the VA that projected to orbitofrontal cortices were positive for calbindin, which labels a projection system with widespread terminations to cortical layer I (Jones, 1998a, 2001). Calbindin positive neurons in the thalamus may have a role in recruiting the thalamus and cortex in synchrony (for review see Jones, 1998b). Axons from the VA terminating in layer I of prefrontal cortices may further excite the apical dendrites of layer V neurons (for review see Castro-Alamancos and Connors, 1997), triggering widespread excitation across cortical areas and back to the thalamus. Circuits linking the prefrontal cortex with the VA may have a prominent role in recruiting other cortical areas in behavior, in view of the substantial projections from layer V of prefrontal areas, shown here. Over excitation of the pathway from layer V may trigger epileptiform activity (Castro-Alamancos and Connors, 1996; Telfeian and Connors, 1998; Connors and Telfeian, 2000).

#### 4.4. Projections to the VA from the reticular nucleus

The VA nucleus is also modulated by projections from the rostral part of the reticular nucleus, which are entirely inhibitory (e.g. Steriade et al., 1984, 1997; Ilinsky et al., 1999). The reticular nucleus appears to have an important role in modulating the state of vigilance, by switching on specific groups of thalamocortical relay neurons (for reviews see Crick, 1984; Steriade, 2000; Jones, 2002). The patchy connectional architecture of the VA demonstrated here (e.g. Figs. 2 and 4), may facilitate the selection of modules for specific tasks. Additional control within this system is exercised by inhibitory projections from the external segment of the globus pallidus and SNr to the reticular nucleus (Hazrati and Parent, 1991; Kayahara and Nakano, 1998; Tsumori et al., 2000). This pattern of innervation leads to disinhibition and opening the gate of thalamocortical outputs from the VA nucleus, facilitating the initiation and continuation of motor behavior. The robust projections from prefrontal layer V to the VA may help initiate or sustain specific actions, within the domain of specialization of distinct sectors of the prefrontal cortex. These functions likely include executive control within a cognitive context in lateral prefrontal areas, and autonomic responses within an emotional context in medial prefrontal areas in the anterior cingulate.

#### 4.5. Implications for neuropathology

Dysfunction of output channels linking functionally distinct prefrontal areas with the basal ganglia may contribute to cognitive and emotional deficits associated with Parkinson's disease (e.g. Hoover and Strick, 1993; Sabatini et al., 2000). Cognitive deficits may be traced to disruption of a strong linkage of lateral prefrontal areas with the VA nucleus through the basal ganglia. Emotional dysfunction may be due to disruption of an equally strong pathway through the anterior cingulate. Clinical studies have provided evidence that the anterior cingulate cortex has a role in motivated actions, and its damage results in akinetic mutism (for review see Devinsky et al., 1995). This syndrome is akin to the classically described neglect syndrome (e.g. Mesulam, 1981, 1999) within the motor domain. The inability of Parkinsonian patients to initiate speech, or readily change facial expression in emotional situations, may have its root in disruption of a loop through the VA, the basal ganglia and the anterior cingulate. An additional loop that likely has a role in responses within an emotional context, is the medial tip of GPi which appears to be dedicated to projection to the AM nucleus of the limbic thalamus (Xiao and Barbas, 2002b), which then projects robustly to the anterior cingulate.

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