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Thalamus & Related Systems 2 (2002) 33–48

Thalamus & Related Systems

www.elsevier.com/locate/tharel

Pathways for emotions and memory
II. Afferent input to the anterior thalamic nuclei from prefrontal, temporal, hypothalamic areas and the basal ganglia in the rhesus monkey

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Accepted 25 May 2002

Abstract

The anterior thalamic nuclei are a key link in pathways associated with emotions and memory. In the preceding study we found that one of the anterior nuclei, the anterior medial (AM), had particularly robust connections with specific medial prefrontal and orbitofrontal cortices and moderate connections with frontal polar cortices. The goal of this study was to use a direct approach to determine the sources of projections to the AM nucleus from all prefrontal cortices, as well as from temporal structures and the hypothalamic mammillary body, known for their role in distinct aspects of memory and emotion. We addressed this issue with targeted injections of retrograde fluorescent tracers in the AM nucleus to determine its sources of input.

Projection neurons directed to the AM nucleus were found in the deep layers of most prefrontal cortices (layers V and VI), and were most densely distributed in medial areas 24, 32 and 25, orbitofrontal areas 13 and 25, and lateral areas 10 and 46. Most projection neurons were found in layer VI, though in medial prefrontal cortices and dorsal area 9 about a third were found in layer V, a significantly higher proportion than in lateral and orbitofrontal cortices. In the temporal lobe, projection neurons originated mostly from the hippocampal formation (ammonic field CA3 and subicular complex), and the amygdala (basolateral, lateral, and basomedial nuclei). In the hypothalamus, a significant number of neurons in the ipsilateral medial mammillary body projected to the AM nucleus, some of which were positive for calbindin (CB) or parvalbumin (PV), markers expressed, respectively, in "diffuse" and "specific" pathways in the thalamus [Adv. Neurol. 77 (1998a) 49]. As recipient of diverse signals, the AM nucleus is in a key position to link pathways associated with emotions, and may be an important interface for systems associated with retrieval of information from long-term memory in the process of solving problems within working memory. Finally, the internal segment of the globus pallidus (GPi) issued projections to AM, suggesting direct linkage with executive systems through the basal ganglia. The diverse connections of the AM nucleus may help explain the varied deficits in memory and emotions seen in neurodegenerative and psychiatric diseases affecting the anterior thalamic nuclei.

Keywords: Corticothalamic connections; Layer V connections; Layer VI connections; Hippocampus; Amygdala

Abbreviations: A, arcuate sulcus; AD, anterior dorsal nucleus; AM, anterior medial nucleus; Amy, amygdala; AV, anterior ventral nucleus; BL, basolateral nucleus; BM, basomedial nucleus (also known as accessory basal); CA1–CA4, cornu Ammonis hippocampal fields of Lorente de Nó (Lorente de Nó, 1934); CB, calbindin; Cdc, central densocellular nucleus; Ce, central nucleus; Clc, central latocellular nucleus; Csl, central superior lateral nucleus; Cg, cingulate sulcus; GPe, external segment of globus pallidus; GPi, internal segment of globus pallidus; HATA, hippocampal–amygdaloid transition area; Hf, hippocampal fissure; Hipp, hippocampal formation; L, lateral nucleus of amygdala; LF, lateral fissure; LO, lateral orbital sulcus; MD, mediodorsal nucleus; Me, medial nucleus; MM, mammillary body; MO, medial orbital sulcus; OLF, olfactory cortex: olfactory tubercle, anterior olfactory nucleus; OPAll, orbital periallocortex (agranular cortex); OPro, orbital proisocortex (dysgranular cortex); P, principal sulcus; Pa, paraventricular nucleus; ParaS, parasubiculum; Pcn, paracentral nucleus; PreS, presubiculum; ProS, prosubiculum; Pu, putamen; PV, parvalbumin; R, reticular nucleus; Re, reuniens nucleus; Rf, rhinal fissure; Ro, rostral sulcus; S, subiculum; Sm, stria medullaris; ST, superior temporal sulcus; TH, temporal cortical area of Von Bonin and Bailey (1947); VA, ventral anterior nucleus; VCo, ventral cortical nucleus; VL, ventral lateral nucleus

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1472-9288/02/$ – see front matter
PII: S1472-9288(02)00030-4

1. Introduction

The anterior thalamic nuclei are an integral component of the classic Papez circuit for emotions (for reviews see Papez, 1937; Ploog, 1989; Armstrong, 1990). In addition, the anterior thalamic nuclei play an important role in memory (Mair et al., 1979; Mayes et al., 1988; Kopelman, 1995; Parker and Gaffan, 1997a; Gaffan and Parker, 2000), suggested by strong inputs from the hippocampal formation and the hypothalamic mammillary body in various species, including rats, cats and monkeys (for reviews see Jones, 1985; Steriade et al., 1997). Distinct anterior thalamic nuclei are topographically connected with the subicular complex (Meibach and Siegel, 1977; Somogyi et al., 1978; DeVito, 1980; Amaral and Cowan, 1980; Robertson and Kaitz, 1981; Aggleton et al., 1986; Yanagihara et al., 1987; Van Groen and Wyss, 1990; Shibata, 1993). Subcortical projections to the anterior medial (AM) and anterior ventral (AV) nuclei of the anterior thalamic complex originate from the ipsilateral medial mammillary body and projections to the anterior dorsal (AD) nucleus originate from the lateral mammillary body bilaterally (for review see Steriade et al., 1997). Moreover, lesions or pathology that include the anterior nuclei have been described in Alzheimer's patients who have poor episodic memory (Braak and Braak, 1991), and in rats and monkeys exhibiting spatial and anterograde amnesia (Aggleton and Mishkin, 1983; Aggleton et al., 1991; Aggleton and Sahgal, 1993; Warburton et al., 1997). Lesions of the mammillary body, its afferent fornix and its efferent mammillothalamic tract, impair spatial memory in rats and episodic memory in monkeys (Thomas and Gash, 1985; Sutherland and Rodriguez, 1989; Gaffan and Harrison, 1989; Gaffan, 1993; Parker and Gaffan, 1997a,b; Warburton and Aggleton, 1999; Sziklas and Petrides, 2000; Gaffan et al., 2001).

In the preceding paper, we showed that distinct orbitofrontal, medial prefrontal, and frontal polar cortices are preferentially connected with the anterior nuclei. However, the involvement of the anterior nuclei in emotions and memory suggests extended connections with several other cortices and subcortical structures, including the amygdala and the hippocampus. Here, we sought to determine the sources of the entire complement of prefrontal as well as other cortical and hypothalamic projections to the anterior nuclei. We used a direct approach to address this issue through targeted injections of retrograde tracers in one of the anterior nuclei, the anterior medial, which had the strongest connections with prefrontal cortices. The results provide evidence that the anterior medial nucleus is a common link for distinct orbitofrontal and medial prefrontal areas, medial temporal cortices and the hypothalamic mammillary body in pathways processing both emotional and mnemonic information, as well as frontal polar area 10 and lateral area 46 associated with distinct aspects of working memory.

2. Methods and techniques

Experiments were conducted on four 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 1987, Office of Science and Health Reports, DRR/NIH, Bethesda, MD). All procedures used were designed to minimize animal suffering and reduce their number.

2.1. Magnetic resonance imaging

To inject tracers in the AM nucleus it was necessary to first obtain a map of the thalamus using magnetic resonance imaging (MRI). Hollow ear bars of the stereotaxic apparatus were filled with betadine salve (containing polymyxin B sulfate, bacitracin zinc and pramoxine HCl, Purdue Frederick Company, Norwalk, CT), which is visible in MRI indicating the interaural line. MRI scans were obtained from monkeys anesthetized with a mixture of ketamine hydrochloride (10 mg/kg, intramuscularly) followed by sodium pentobarbital, administered intravenously through a femoral catheter (to effect). The stereotaxic coordinates for the AM nucleus were calculated in three dimensions using the interaural line as reference.

2.2. Surgical procedures

One week after the MRI, the monkeys were anesthetized with ketamine hydrochloride (10 mg/kg, intramuscularly), intubated and anesthetized with gas anesthetic (isoflurane) until a surgical level of anesthesia was achieved. Overall physiological condition was monitored, including heart rate and temperature. Injections of neural tracers were made with a microsyringe (5 or 10 μl; Hamilton) mounted on a microdrive. A small hole was made above the injection site for penetration of the injection needle. Small amounts of diamidino yellow (Sigma; 3% solution, volume of 0.3–1.6 μl), or fluoro-ruby (dextran tetramethylrhodamine, Molecular Probes; 10% solution, volume of 3–4 μl) were delivered to the AM nucleus. After injection of tracers the wound was closed in anatomic layers and the skin sutured. At the completion of surgical procedures the animals were monitored until recovery from anesthesia, they were given antibiotics and analgesic (Buprenex, intramuscularly) every 12 h, or as needed.

Eighteen days after injection of tracers each animal was given an overdose of anesthetic (sodium pentobarbital, intravenously, to effect) and perfused with 2–4 l 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). The brain then was frozen in −75 °C isopentane and cut on a freezing microtome coronally at 50 μm. Two matched series of sections were mounted, dried under darkness, and

stored at 4 °C. One series of sections was used to map labeled neurons in prefrontal cortices and temporal structures. One series was coverslipped with Fluoromount (Fischer) and placed in cold storage (4 °C) for photography.

2.3. Immunocytochemical procedures and staining

To study whether neurons in the mammillary body which projected to the AM nucleus were positive for the calcium binding proteins parvalbumin (PV), calbindin (CB), or the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), we performed immunocytochemical procedures. 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. The tissue was then incubated for 2–3 days in a solution containing the 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 a solution containing 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. After several rinses in PBS, sections were mounted on gelatin-coated slides.

To visualize labeled neurons projecting to the AM nucleus among all neurons in the mammillary body, sections were counterstained with a red fluorescent Nissl stain (NeuroTrace 530/615, Molecular Probes) for 1–3 h, washed and mounted on glass slides for photography. After plotting projection neurons in the mammillary body directed to AM, sections were counterstained with thionin to calculate the proportion of projection neurons to all neurons in the mammillary body using stereologic procedures. Sections through the prefrontal and temporal cortices were stained with thionin to aid in delineating architectonic borders of thalamic nuclei and areas and layers in the cortex (Olszewski, 1952; Olivier et al., 1969; Jones, 1985; Rosene and Van Hoesen, 1987; Barbas and Pandya, 1989; Suzuki and Amaral, 1994).

2.4. Data analysis

2.4.1. Mapping labeled neurons

After injection of retrograde tracers in the AM nucleus we mapped all labeled neurons in prefrontal cortices ipsilateral to the injection side from one series of sections in three cases (cases AY, AZ, BD). Coronal sections through the ipsilateral prefrontal cortex were viewed with a microscope under fluorescence illumination (Nikon, Optiphot). Section outlines and the location of labeled neurons were plotted on paper using a digital plotter (Hewlett-Packard, 7475A), which was electronically coupled to the stage of the microscope and to a PC computer. Movement of the stage was recorded through linear potentiometers (Vernitech, Axsys, San Diego, CA) mounted on the *X*- and *Y*-axes of the microscope stage. Analog signals were converted to digital signals via an analog-to-digital converter (Data Translation, Marlboro, MA). Software designed in our laboratory ensured that each neuron was counted only once, as described previously (e.g. Barbas and De Olmos, 1990). After plotting, sections were counterstained with thionin and returned to the microscope to delineate layers, count labeled neurons and measure the area by layer in individual prefrontal areas. The same system was used to map labeled neurons in the hippocampal formation and adjacent temporal cortices in two cases (cases BB and BD) and in the basal ganglia (cases BB, AY, BD).

The density of labeled projection neurons in prefrontal areas was estimated by dividing the total number of labeled neurons by the volume of the tissue examined in each area and was expressed as neurons per mm^3.

2.4.2. Stereologic procedures to estimate the total number of labeled neurons

We also performed stereologic procedures to estimate the total number of projection neurons (for a review see Howard and Reed, 1998) using a commercial system (StereoInvestigator, Microbrightfield, Colchester, VT). We used this approach to estimate the density of projection neurons in prefrontal cortices in one case (case BB) and the total number of labeled neurons in the mammillary body (cases BB, AZ, BD). In the ipsilateral mammillary body we also estimated the proportion of projection neurons from the total population of neurons, as well as the proportion of projection neurons that were positive for CB or PV. PV estimates were obtained for only one case (BB) because of tissue availability.

We considered several parameters in estimating the total number of neurons, including the coefficient of error (CE, which was set to less than 10%), target cell counts, section interval, counting frame size, grid size (the distance along the *X*-axis, ΔX, and *Y*-axis, ΔY, between counting frames), section thickness and guard zone size (West and Gundersen, 1990; West et al., 1991; Gundersen et al., 1999; for review see Howard and Reed, 1998). Section thickness was adequate for using the optical disector, where counting is restricted to a fraction of the tissue height, and includes a guard zone to avoid error due to cell plucking or cell splitting during tissue sectioning. The total estimated number of cells was calculated as

$$N = \sum Q^{-} \cdot \frac{t}{h} \cdot \frac{1}{\text{asf}} \cdot \frac{1}{\text{ssf}}$$

where $\sum Q^{-}$ represents the total number of counted cells, t is the mean section thickness (μm), h the height of the optical disector (μm), asf the areal sampling fraction (the ratio of the counting frame area to the sampling grid area), and ssf is the slice sampling fraction, which was one in every 10 sections, at 500 μm intervals. Section thickness was 50 μm after cutting, which shrank to 12 μm after mounting on gelatin-coated slides. The guard zone was set at 2 μm

for the top and bottom of the tissue section, leaving 8 μm in the counting brick zone. After the pilot study, the counting frame was set at 150 μm × 150 μm and grid size at 500 μm × 500 μm for counting projection neurons in the prefrontal cortices. To count neurons in the hypothalamic mammillary body, the counting frame size was set at 100 μm × 100 μm and grid size at 300 μm × 300 μm.

As in the other three cases described earlier, the density of labeled projection neurons in different prefrontal cortices was estimated by dividing the total number of labeled neurons by the volume of the tissue examined, and expressed as neurons per mm^3 for each area.

2.4.3. Normalized density of projection neurons in prefrontal areas

The density of labeled neurons in ipsilateral prefrontal cortices varied among cases (BB overall had the highest number of labeled neurons, BD the lowest, and cases AY and AZ an intermediate number). The size of the injection and precise location of the injection site within the AM may have contributed to the differences. To compare the areal distribution of labeled neurons among cases, we normalized the data by expressing the density in each prefrontal area as a percentage of the total density in all prefrontal areas in each case.

2.4.4. Photography

Photographs through the thalamus and the mammillary body 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) for arrangement and adjusting of contrast and overall brightness, but were not retouched. Images obtained using different fluorescence filters were superimposed in Photoshop by adjusting the opacity of one layer to show double-labeled neurons.

3. Results

3.1. Injection sites in the AM nucleus

In four cases, we injected retrograde tracers in the AM, because it had the strongest connections with prefrontal cortices among the anterior thalamic nuclei, as determined in the preceding study. In three cases, the injection site included the anterior two thirds of the AM nucleus (cases AY, BB and BD) and in the fourth case the core of the injection was in the posterior third of AM (case AZ). Specifically, in case AY the core of the injection was restricted to the central portion of the AM nucleus (Fig. 1F). The halo of the dye invaded

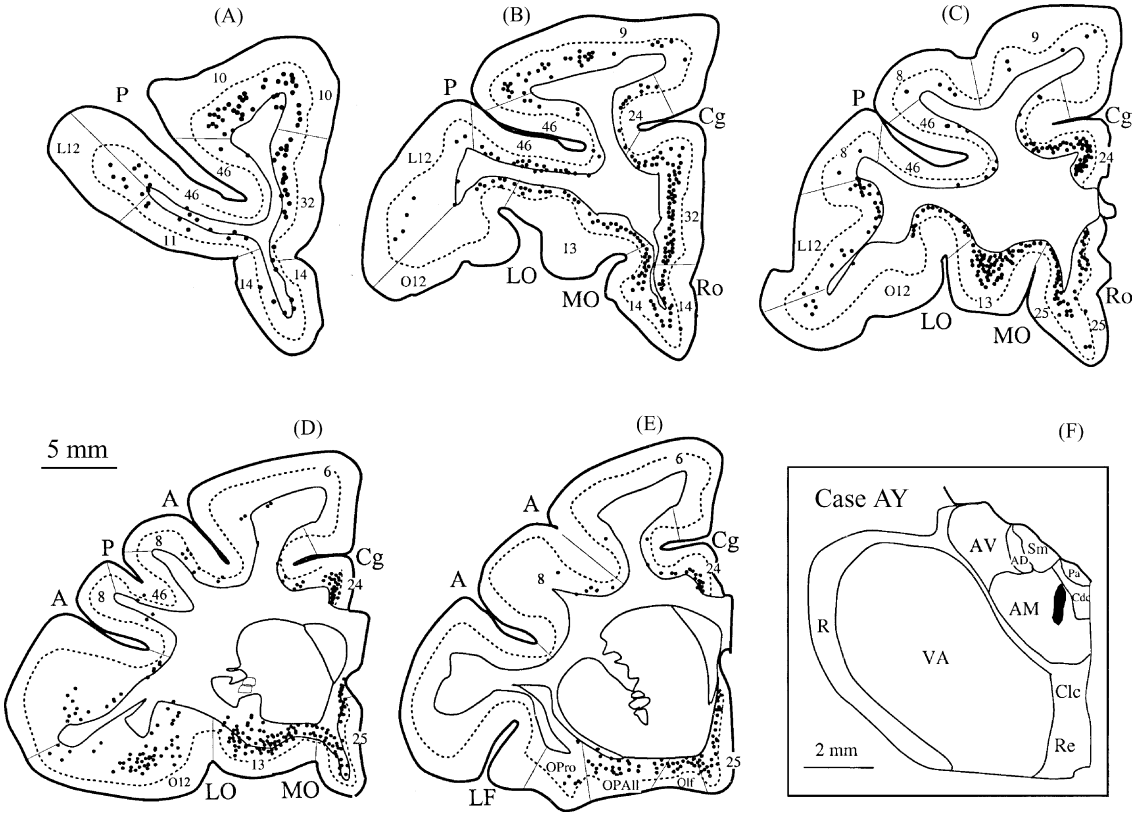


Fig. 1. Projection neurons in prefrontal cortices directed to the anterior medial nucleus. (A–E) Distribution of projection neurons in a series of coronal sections in rostral (A) to caudal (E) prefrontal cortices after injection of diamidino yellow in the central part of AM. (F) Injection site in AM shown in coronal section through the anterior thalamus (black area, case AY). Dotted line in A–E shows the bottom of cortical layer 4.

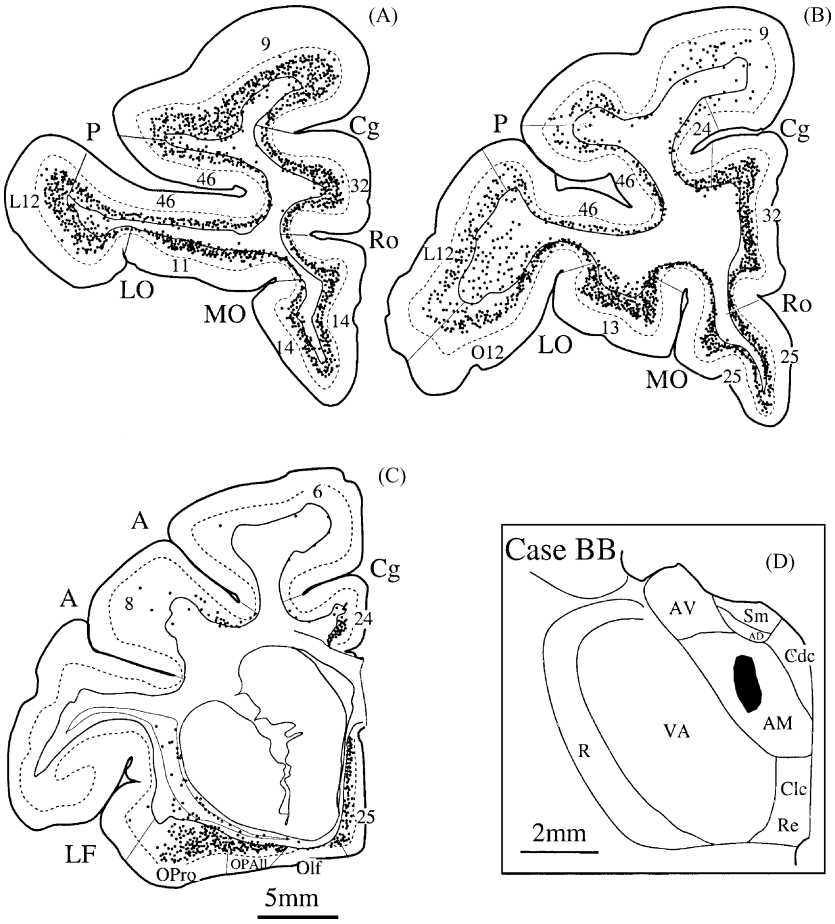


Fig. 2. Projection neurons in prefrontal cortices directed to the anterior medial nucleus. (A–C) Distribution of projection neurons in a series of coronal sections in rostral (A) to caudal (C) prefrontal cortices after injection of diamidino yellow in the central part of AM. (D) Injection site in AM shown in coronal section through the anterior thalamus (black area, case BB). Dotted line in A–C shows the bottom of cortical layer 4.

the intralaminar nucleus central densocellular (Cdc) and the stria medullaris (Sm). In case BB, the core of the injection was restricted to the central portion of the AM nucleus as well (Fig. 2D). The halo of the injection impinged on the dorsal part of the mammillothalamic tract, and the needle tract passed through the ventral part of the fornix. In case BD, the injection of fluoro-ruby was restricted to the ventral edge of the nucleus (Fig. 3F). In case AZ, the core of the injection was in caudal AM and spread to a small portion of the anterior part of the mediodorsal nucleus (MD), the AV, Cdc and central superior lateral (Csl) nuclei, and the stria medullaris (Fig. 4D; Table 1).

3.2. *Afferent projections to AM from prefrontal cortices*

We investigated the distribution and density of corticothalamic neurons in prefrontal cortices projecting to AM. In general, all ipsilateral prefrontal areas issued some projections to AM. The highest density of projection neurons was noted in several medial and orbitofrontal cortices (areas 24, 25, 32, 13; Figs. 1–4). In the case with an injection at the ventral rim of AM, the density of projection neurons in area 25 was lower than in the rest (Fig. 3D and E, case BD). Moderate numbers of projection neurons were seen in the medial and orbital parts of area 14, and in most other orbitofrontal areas (areas OPAll, area 11, O12;[1] Figs. 1, 2A–C and 3B–D), and in lateral areas 10 and 46 (Figs. 1A and B, 3A and B and 4A). In cases where the core of the injection was restricted to AM (cases AY, BB and BD), only a few

Table 1
Injection sites, cases, tracer types and types of analysis

Injection site	Case	Tracer type and injection side[a]
Thalamus AM		
AM (central)	AY	Diamidino yellow (L)
AM (caudal), Cdc, rostral MD	AZ	Diamidino yellow (L)
AM (central)	BB	Diamidino yellow (L)
AM (ventral)	BD	Fluoro-ruby (L)

[a] Retrograde analysis; (L): left side.

[1] Letters preceding numbers designating prefrontal architectonic areas refer to: D, dorsal; L, lateral; M, medial; O, orbital; R, rostral; V, ventral.

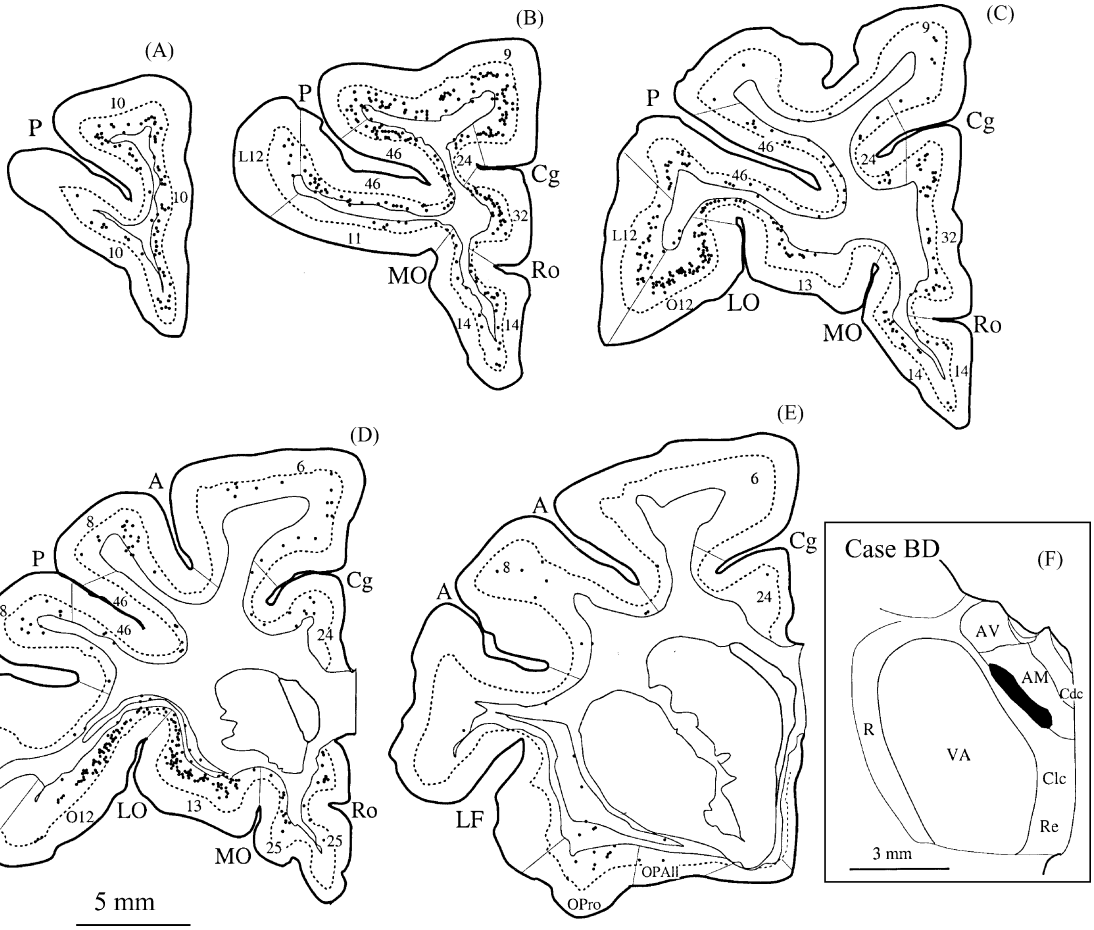


Fig. 3. Projection neurons in prefrontal cortices directed to the anterior medial nucleus. (A–E) Distribution of projection neurons in a series of coronal sections in rostral (A) to caudal (E) prefrontal cortices after injection of fluoro-ruby in the ventral part of AM. (F) Injection site in AM shown in coronal section through the anterior thalamus (black area, case BD). Dotted line in A–E shows the bottom of layer 4.

projection neurons were noted in the caudal part of area 46 or area 8 (Figs. 1C–E, 2C and 3D and E), but the density was somewhat higher when the injection spread to the anterior part of MD (Fig. 4). The latter finding is consistent with the topographic connections of areas 46 and 8 with anterior MD (Arikuni et al., 1983; Goldman-Rakic and Porrino, 1985; Barbas et al., 1991).

The density of labeled neurons in each area was normalized to the total density in all prefrontal areas in each case and the group data for the four cases are shown in Fig. 5. Medial prefrontal (24, 25, 32) and orbitofrontal area 13 had the highest density, followed by most other orbitofrontal (areas OPAll, O25, O12, 11, O14) areas and medial area 14, which had a moderate density of projection neurons. Areas OPro and M9 were the exception among orbitofrontal and medial prefrontal areas, having a lower density than the rest. Among lateral cortices, areas 10 and 46 stood out with moderate distributions of projection neurons, in comparison with areas 9, L12 and 8, where the density was lower. The regional differences among medial, orbital and lateral prefrontal cortices were significant [$F(2, 16) = 4.59$, $P < 0.05$], and could be specifically traced to a significantly higher density in medial than in lateral prefrontal cortices ($P < 0.05$). These findings are consistent with data obtained using anterograde tracers in prefrontal cortices (described in the preceding paper). The adjacent premotor area 6 had only a few labeled neurons after injections in AM (Figs. 1D and E, 2C, 3D and E and 4C).

3.2.1. Laminar origin of projection neurons in prefrontal cortices directed to AM

Prefrontal projection neurons directed to the AM originated from the deep layers, though there was regional variation in their distribution within layers V and VI (Fig. 6). Thus, in all orbital areas (11–14, except area OPro) and lateral areas (areas 10, 12, 46, 8), 5–20% of projection neurons originated from layer V, and the rest were found in layer VI (Fig. 6A). However, a significantly higher proportion of projection neurons (30–35%) from medial prefrontal areas (areas 24, 25, 32, 14, M9) and the adjacent dorsal area 9 (D9) originated from layer V and the rest originated from layer VI (Fig. 6B; $P < 0.001$).

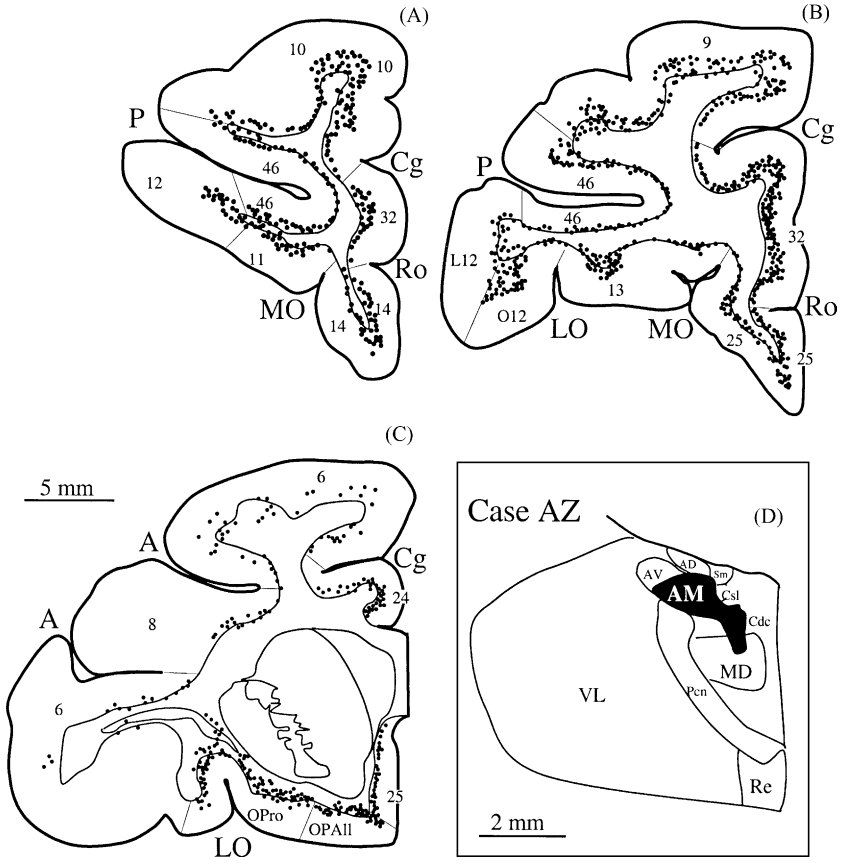


Fig. 4. Projection neurons in prefrontal cortices directed to the anterior medial nucleus. (A–C) Distribution of projection neurons shown in a series of coronal sections in rostral (A) to caudal (C) prefrontal cortices after injection of diamidino yellow in the caudal part of AM. (D) Injection site in the posterior third of AM, impinged on the anterior part of MD, AV, Cdc, Csl and the stria medullaris (black area, case AZ).

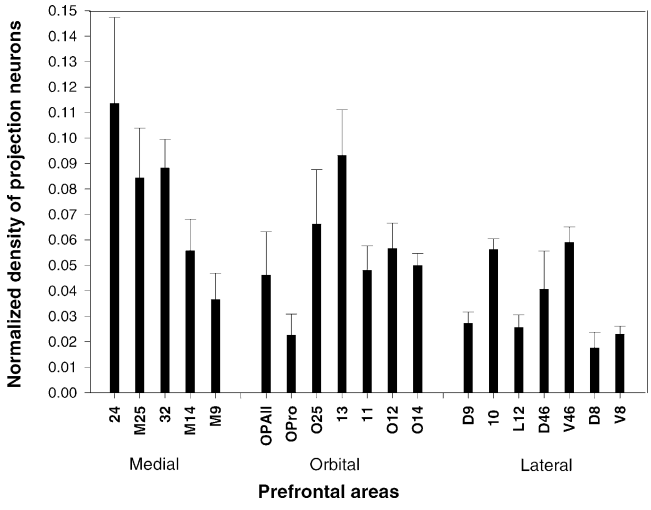


Fig. 5. Normalized density of projection neurons in the prefrontal cortices directed to the anterior medial nucleus. Medial and orbital areas issued dense projections to the AM nucleus (except areas M9 and OPro). Lateral areas (except areas 10 and V46) issued light and moderate projections to the AM nucleus (cases AY, AZ, BB, BD; vertical lines on bars show standard error).

3.3. Afferent projections to AM from temporal structures

In addition to connections with prefrontal areas, the AM received projections from several ipsilateral temporal structures. The nomenclature for the hippocampal formation (ammonic fields, subicular complex and entorhinal cortex), the perirhinal cortex and parahippocampal cortex is based on previous studies (Rosene and Van Hoesen, 1987; Suzuki and Amaral, 1994). Dense populations of projection neurons directed to the AM were noted in the subicular complex of the hippocampal formation. In addition, projection neurons were found in the basolateral, basomedial (also known as accessory basal) and lateral nuclei of the amygdala, the hippocampal–amygdaloid transition area (HATA) and the hippocampal ammonic fields (mostly in CA3). Projection neurons were sparsely distributed in the entorhinal cortex (area 28), the perirhinal cortices (areas 35 and 36) and parahippocampal area TH (Fig. 7, case BD). However, in a case where the injection spread to the fornix, large populations of projection neurons were observed in the entorhinal cortex and the hippocampal ammonic fields (CA2–CA4 and a few in CA1) as well, throughout their anterior to posterior extent (Fig. 8A–D, case BB).

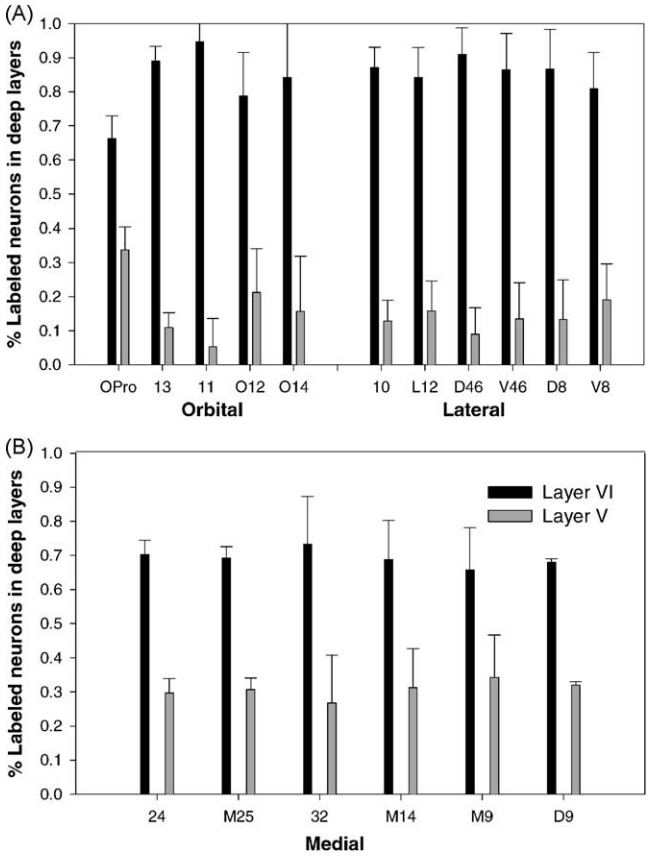


Fig. 6. Laminar origin of projection neurons in prefrontal cortices directed to the anterior medial nucleus. (A) The laminar distribution of projection neurons in orbitofrontal and lateral prefrontal areas. (B) The laminar distribution of projection neurons in medial prefrontal areas and adjacent dorsal area 9, which included a substantial proportion in layer V (cases AY, AZ, BD; vertical lines on bars show standard error).

In the presubiculum (PreS) and parasubiculum (ParaS) pyramidal and fusiform neurons issued projections mostly from the deep layers (PreS, 89% and ParaS, 78%). In the caudal entorhinal cortex, projection neurons originated mostly from the deep layers (Fig. 8B). Only a few projection neurons were found in the rostral extent of the entorhinal cortex (area 28, Figs. 7A and B and 8A). Projection neurons were also noted in posterior cingulate cortex (area 23, not shown).

3.4. Afferent projections to the AM from the hypothalamic mammillary body

The injections in AM made it possible to study the connections with its hypothalamic relay, the mammillary body, located at the posterior extent of the hypothalamus. In a case where the injection was in caudal AM (case AZ), projection neurons were noted in all parts of the ipsilateral mammillary body, but were most densely distributed in its medial and dorsal parts (Fig. 9A). In cases where the injection was in the central (case BB) and ventral (case BD) parts of AM, projection neurons were noted in all parts of the ipsilateral medial mammillary body (Fig. 9B and C). Stereologic analysis revealed that projection neurons accounted for a significant proportion of all Nissl stained neurons, constituting the majority in a case with injection in the rostral two thirds of AM (65%, case BB; Fig. 9D), or in the caudal part of AM (67%, case AZ). The proportion of projection neurons was lower in a case with a small injection in the ventral edge of AM (20%, case BD).

3.4.1. Afferent projections to AM from PV or CB positive neurons in the mammillary body

It has been shown that PV and CB are expressed in two distinct thalamocortical pathways in the sensory relay thalamic systems in monkeys (for reviews see Jones, 1998a,b). We investigated whether projection neurons from the mammillary body directed to AM were also positive for PV or CB, and found a population of each in the medial mammillary body, noted mostly medial to the mammillothalamic tract (Fig. 10A). Stereologic analysis showed that a subpopulation of projection neurons was positive for PV (13%, case BB; Fig. 10B), and another subpopulation was positive for CB (17%, case BB, Fig. 10C and D; 13%, case AZ). Double-labeling immunocytochemical procedures indicated that projection neurons and PV or CB positive neurons did not overlap with GABAergic neurons in the medial mammillary body (not shown).

3.5. Afferent projections to AM from the basal ganglia

Finally, in order to explore the possibility that the AM nucleus may be involved in the circuit through the basal ganglia, we investigated whether it received projections from the basal ganglia in the three cases where the core of the injection was restricted to the AM nucleus. We found that the internal segment of the globus pallidus (GPi) sent projections to the AM (Fig. 11). The labeled neurons were found mostly in the medial part of GPi in clusters of 3–5 and decreased in density caudally (case BB). The same pattern, though sparser in density, was noted in another case with tracer injection in the central part of AM (case AY, data not shown). In a case with a restricted injection in the ventral part of AM there was no evidence of labeled neurons in GPi (case BD).

4. Discussion

Targeted injections of retrograde tracers in the AM nucleus enabled us to determine the extent of feedback projections from prefrontal areas to the AM nucleus. Our results extend previous findings by showing that dense descending projections to the AM nucleus arose from some orbitofrontal and medial prefrontal cortices, and widespread, though lighter, projections emanated from other prefrontal areas as well. The frontal polar area 10, in particular, consistently issued projections to the AM nucleus. These conclusions are supported by a comparison of results obtained from two

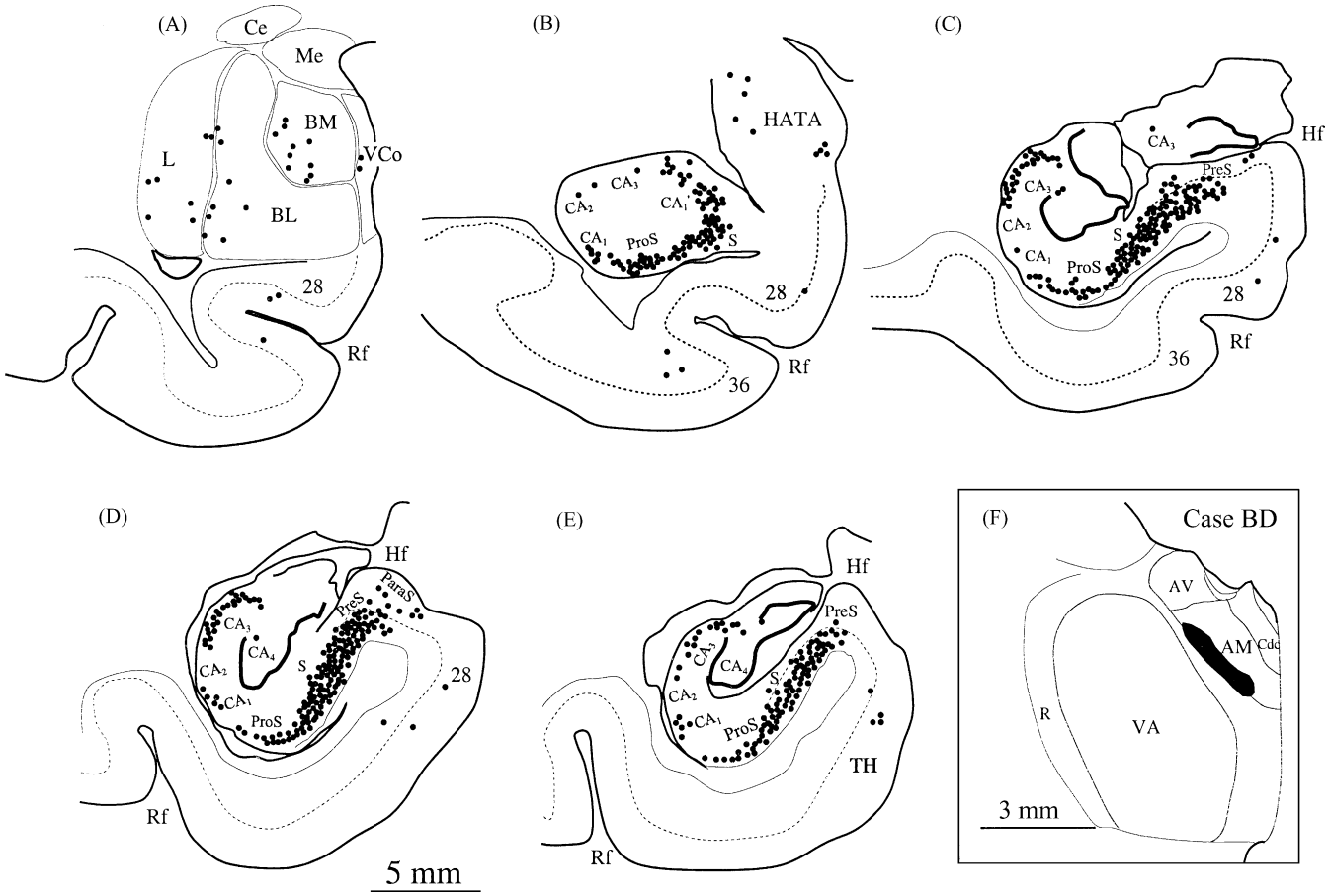


Fig. 7. Projection neurons in temporal structures directed to the ventral part of the anterior medial nucleus. (A) Projection neurons in the lateral (L), basal (BL, BM) and ventral cortical (VCo) nuclei of the amygdala. (B–E) Projection neurons in rostral to caudal coronal sections of the hippocampal formation and adjacent cortices. (F) The injection site of fluoro-ruby was in the ventral part of AM (black area, case BD). Dotted line in A–E represents the bottom of layer 4.

complementary experimental approaches, described in the previous and this study, and summarized in Fig. 12.

4.1. Circuits for emotions linking the AM nucleus with the amygdala

A key structure in the circuit for emotions is the amygdala, which was not initially included in Papez's circuit, but was later implicated in emotions based on the behavior of monkeys after temporal lobectomy (Klüver and Bucy, 1939). The amygdala was subsequently included in the visceral brain (for a review see MacLean, 1949) and its role in emotion has been confirmed in recent studies in humans and animals (for reviews see Adolphs et al., 1995; LeDoux, 2000). Our results provided evidence of projections from the basal nuclei of the amygdala to the AM nucleus. Further, the amygdala strongly innervates orbitofrontal and medial prefrontal cortices in primates (Potter and Nauta, 1979; Porrino et al., 1981; Amaral and Price, 1984; Barbas and De Olmos, 1990; Morecraft et al., 1992; Carmichael and Price, 1995), which are preferentially connected with the AM nucleus, suggesting a close-knit circuit.

4.2. Circuits for distinct aspects of memory linking the AM nucleus with the hippocampal formation, the mammillary body and prefrontal cortices

Another key connection of the AM nucleus is with the hippocampal formation, a structure implicated in long-term memory (for reviews see Alvarez et al., 1994; Kandel, 2001). Our findings indicated strong direct projections from the hippocampal formation to the AM nucleus, originating from the subicular complex, the hippocampus (CA3) and, to a lesser extent, the entorhinal cortex. This finding is consistent with studies in rats (Meibach and Siegel, 1977; Van Groen and Wyss, 1990), cats (Somogyi et al., 1978) and monkeys (DeVito, 1980; Aggleton et al., 1986). We previously noted that caudal medial prefrontal cortices, including areas 25 and the posterior part of area 32 receive robust projections from the hippocampal formation, the entorhinal cortex and perirhinal areas 35 and 36 (Barbas and Blatt, 1995; Barbas et al., 1999). Caudal orbitofrontal cortices also receive projections, albeit not as dense, from the above perirhinal and hippocampal structures (Barbas, 1993; Barbas and Blatt, 1995). Direct projections from the hippocampal

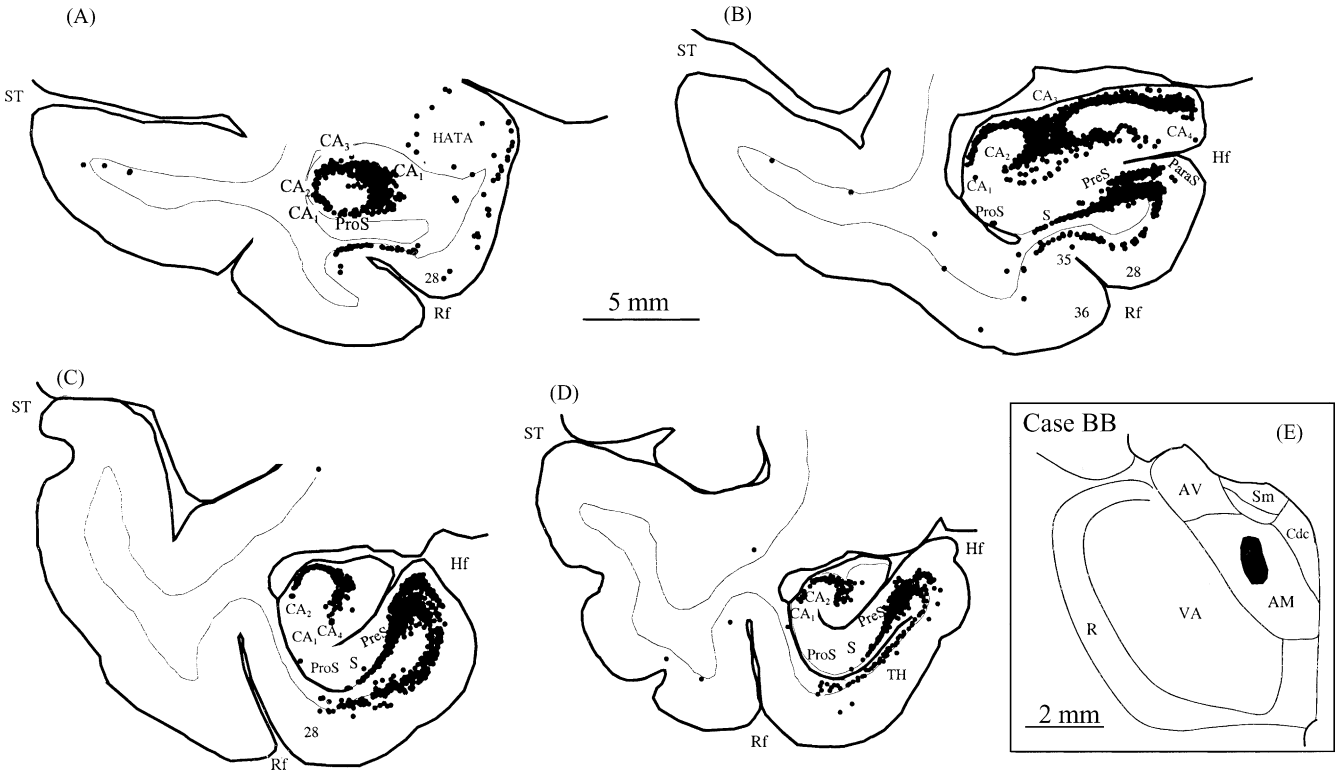


Fig. 8. Projection neurons in temporal structures directed to the central part of the anterior medial nucleus. (A–D) Projection neurons in rostral (A) to caudal (D) coronal sections through the hippocampal formation and adjacent cortices. (E) The injection site of diamidino yellow was in the central part of AM (black area, case BB).

formation to the above prefrontal areas emanate from the subicular complex and CA1. The hippocampal projection to the anterior medial nucleus emanated from the subicular complex as well, but also robustly from CA3. This evidence suggests that a large part of the hippocampal formation projects, either directly, or indirectly through the AM nucleus, to caudal medial and orbitofrontal cortices.

In addition, we noted strong projections to AM from the medial mammillary body, which receives projections from the hippocampal formation as well (for reviews see Saper, 1990; Saper, 2000). This connection may be considered an indirect pathway from the hippocampal formation to the AM nucleus. Previous studies showed that axons or projection neurons from the medial mammillary body terminate in the AM nucleus in rats, cats and monkeys (Cruce, 1975; Somogyi et al., 1978; Veazey et al., 1982; Steriade et al., 1984). Our quantitative analysis showed that a large proportion of all neurons in the medial mammillary body issued projections to the AM nucleus, and in two cases these constituted the majority. Moreover, subpopulations of projection neurons in the medial mammillary body directed to the AM nucleus were positive for parvalbumin and calbindin and may represent, respectively, “specific” and “diffuse” subcortical pathways to the thalamus (for reviews see Jones, 1998a,b, 2001). Interdigitated neurochemically distinct groups of neurons from the medial mammillary body may convey distinct aspects of mnemonic information to the AM nucleus.

Behavioral studies have implicated the AM nucleus in a mnemonic circuit for episodic and spatial memory (Aggleton and Brown, 1999). Previous, though not definitive, evidence was provided when monkeys with lesion of the anterior thalamus, which included the AM nucleus as well as MD, showed impairment in recognition and object–reward associative memory (Aggleton and Mishkin, 1983). Subsequent

Fig. 10. Some projection neurons in the medial mammillary body directed to the anterior medial nucleus were positive for PV or CB. (A) Two populations of neurons (arrows) were positive for PV (red) or CB (green). The mammillothalamic tract is to the left. (B) Population of double-labeled projection neurons (yellow nuclei, arrows) which are positive for PV (red) and single-labeled projection neurons (seen as blue with UV filter). (C) CB positive (red, arrows) projection neurons (yellow nuclei, arrowheads) and single-labeled projection neurons (blue, small arrows). (D) Population of double-labeled projection neurons (yellow nuclei, arrows) which are positive for CB (red) in a larger field of single-labeled neurons (blue). In B–D the nuclei of projection neurons were labeled after injection of diamidino yellow in AM and appear blue when single-labeled (under UV filter), or ivory-yellow when they are also positive for CB or PV (red). All frames are from case BB. Scale bar: 30 μm. Medial is to the right.

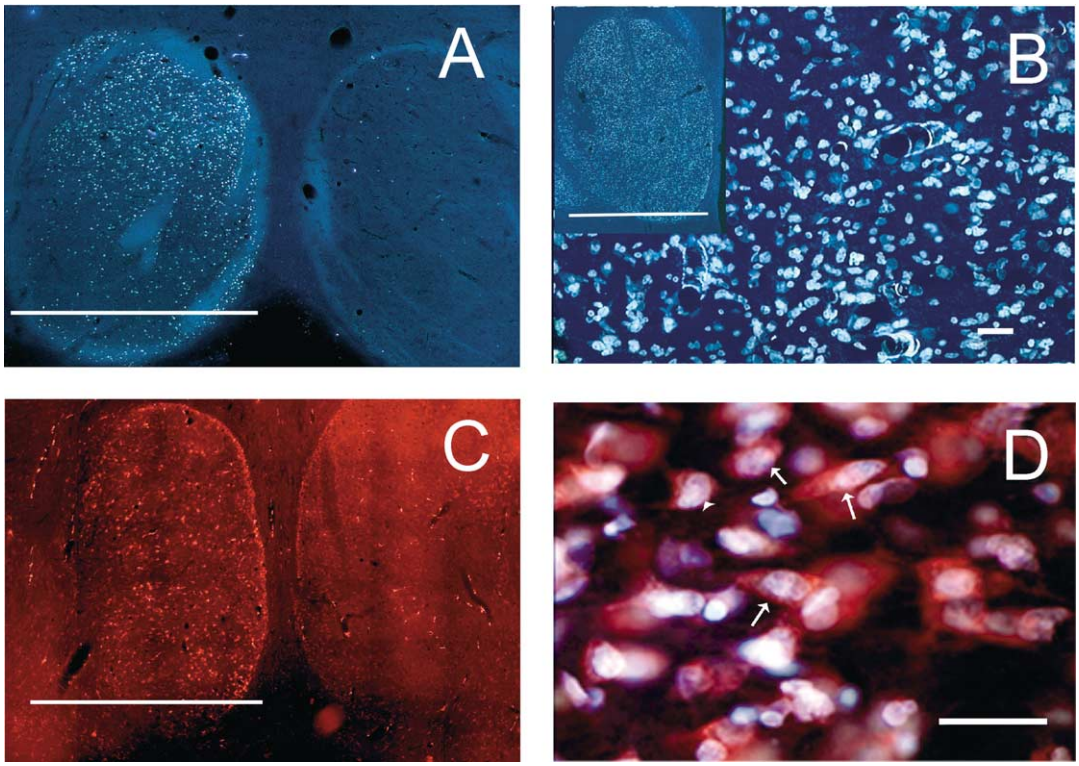
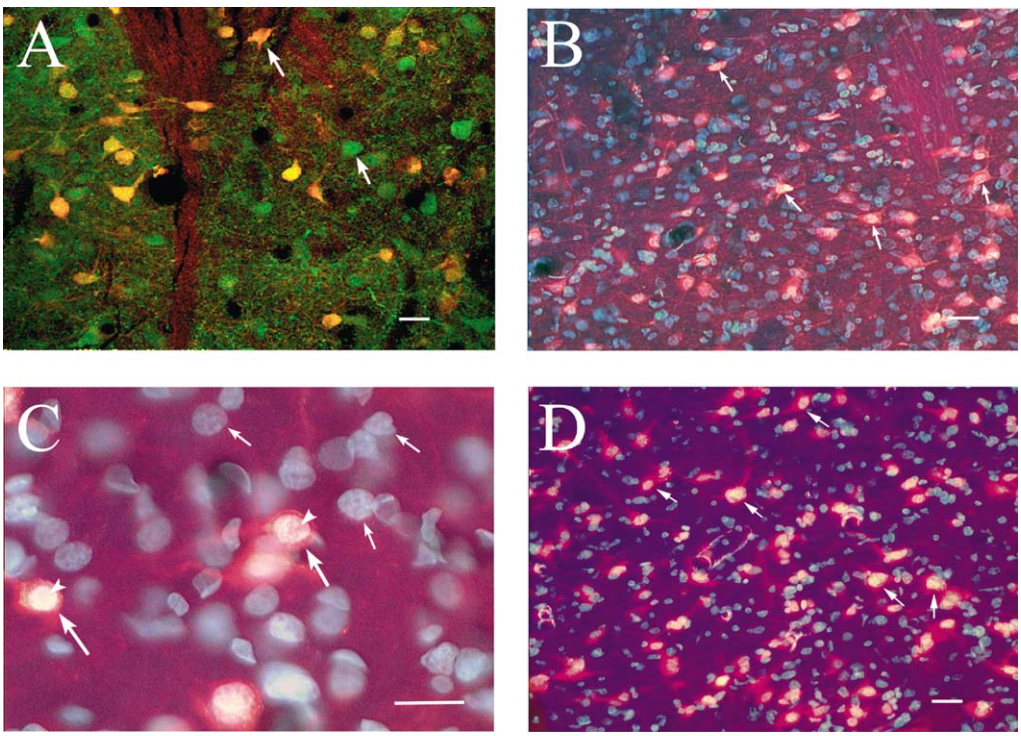


Fig. 9. Projection neurons in the ipsilateral medial mammillary body directed to the anterior medial nucleus. (A) Labeled neurons in the left mammillary body after injection of diamidino yellow at the posterior part of the AM nucleus (case AZ). (B) Labeled neurons in the left mammillary body after injection of diamidino yellow in the central part of the AM nucleus (inset) and under higher magnification (case BB). (C) Labeled neurons in the left mammillary body after injection of fluoro-ruby at the ventral rim of the AM nucleus (case BD). (D) The majority of fluorescent Nissl stained neurons in the mammillary body (red, arrows) are projection neurons labeled after injection of diamidino yellow in AM (their nuclei are labeled with diamidino yellow, arrowhead, and appear blue with filter; case BB). Scale bar: 1 mm in A, B (inset) and C; 30 μm in B and D.



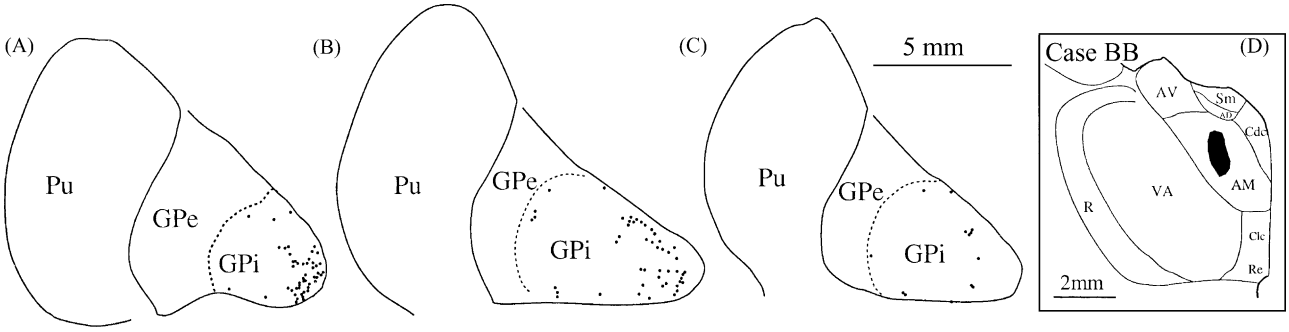


Fig. 11. Projection neurons in the globus pallidus directed to the central part of the anterior medial nucleus. (A–C) Distribution of projection neurons in a series of coronal sections in rostral (A) to caudal (C) sections through the internal segment of the globus pallidus (GPi). The injection site of diamidino yellow was in the central part of AM (D, black area, case BB).

studies in rhesus monkeys showed that lesion of the anterior thalamic nuclei, as well as its sources of projection, the mammillary body and the hippocampus, led to increased errors in remembering object locations superimposed on complex background scenes. Moreover, the memory deficits were more severe than in cases with lesion of one of the cortical outputs of the AM nucleus, the cingulate cortex (Gaffan and Harrison, 1989; Gaffan, 1993, 2002; Parker and Gaffan, 1997a,b; Gaffan et al., 2001). In humans, infarction of the AM nucleus dramatically impaired episodic and recognition memory resulting in anterograde amnesia (Parkin et al., 1994; Ghika-Schmid and Bogousslavsky, 2000; Nolan et al.,

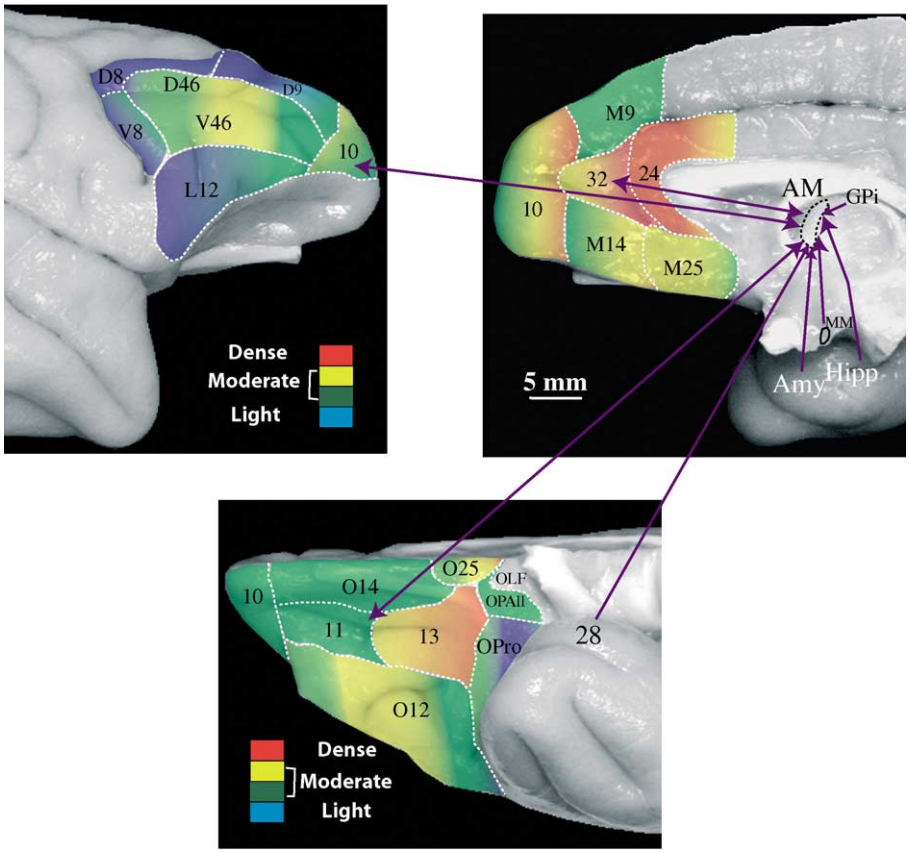


Fig. 12. Summary of the connections of the anterior medial nucleus with prefrontal and temporal structures, the hypothalamic mammillary body and the internal segment of the globus pallidus. The bi-directional arrows for connections of prefrontal cortices with AM summarize findings obtained from this and the preceding study, and are shown on the lateral (top, left), medial (top, right) and basal surfaces (bottom) of the cerebral hemisphere. The projections from temporal cortex, the amygdala, the hippocampus, the hypothalamic mammillary body, and the basal ganglia to AM are according to the findings from this study. Density variations of prefrontal projections to AM are depicted in pseudocolor denoting dense (red) to light (blue) projections, as reconstructed from serial coronal sections (this paper).

2001; Kishiyama et al., 2001; for a review see Gaffan and Gaffan, 1991). Lesions of the AM nucleus, used in treating neurotic patients, caused disorientation in time and place (Spiegel and Wycis, 1962). Finally, structural changes or neuronal loss in the thalamic AM nucleus and the mammillary body have been noted in human diseases affecting memory loss, such as Alzheimer's disease, and Korsakoff's syndrome (Victor et al., 1971; Mair et al., 1979; Braak and Braak, 1991; Kopelman, 1995; Braak et al., 1996).

The involvement of the AM in memory was also noted in non-primate species. Rats and mice were impaired in spatial and non-spatial memory tasks, including non-matching-to-position, radial maze, water maze, or T-maze after lesion of the AM nucleus, its input from the hippocampus, or the fornix (Sutherland and Rodriguez, 1989; Aggleton et al., 1991; Warburton et al., 1997, 2000; Mair et al., 1998; Warburton and Aggleton, 1999; Celerier et al., 2000; for review see Aggleton and Brown, 1999). These deficits may be due to compromise of a network which appears to be critical for spatial recognition memory, including the hippocampus, the mammillary bodies, the anterior nuclei and the medial prefrontal cortex (Sutherland and Rodriguez, 1989; Aggleton and Sahgal, 1993; Byatt and Dalrymple-Alford, 1996; Warburton et al., 1997, 2000; Steckler et al., 1998; Warburton and Aggleton, 1999; Vertes et al., 2001). In addition, the frontal polar cortex, along with the hippocampus, the perirhinal cortex and the lateral prefrontal cortex, appear to be part of a circuit of active memory retrieval (Parkin et al., 1994; Markowitsch et al., 1997; McDermott et al., 1999; Nolan et al., 2001). The circuit linking the AM nucleus with the hippocampal formation, the frontal pole and area 46 may support these functions, and may provide an interface between working memory and long-term memory systems in primates.

4.3. Linkage of the AM nucleus with the basal ganglia

The results showed that the internal segment of the globus pallidus projected to the AM nucleus, a pathway also demonstrated in rats and fascicularis monkeys (Groenewegen, 1988; Parent et al., 2001). Classically, the basal ganglia was thought to receive input from all cortical areas but issue feedback only to premotor and prefrontal areas indirectly through the thalamic ventral anterior (VA) and ventral lateral (VL) nuclei, as well as MD and intralaminar nuclei (Rosvold, 1972; Alexander et al., 1986; Groenewegen et al., 1990; Joel and Weiner, 1994; Haber and McFarland, 2001). This circuit appears to be involved in motor movement and procedural/implicit memory (e.g. Alexander et al., 1986), as well as cognitive and emotional functions, such as attention, memory, reward associations and motivated behavior (Middleton and Strick, 1994; Mitchell et al., 1999; Schultz et al., 2000; Hollerman et al., 2000). Lesion of the striatum in primates elicits changes in emotional behavior, such as lack of expression, display of dominance, motivation and curiosity (Mettler, 1945; MacLean, 1972). In humans, loss of striatopallidal neurons possibly contributes to neuropsychiatric disorders, including obsessive compulsive disorder and Tourette's syndrome (for review see Mitchell et al., 1999). Our findings extend the classic basal ganglia circuit and give additional support for a role of the basal ganglia in cognitive and emotional functions by indicating its direct connection with the AM nucleus in the rhesus monkey.

4.4. The laminar organization of corticothalamic feedback projections

Our results showed that most prefrontal projection neurons to the AM originated from layer VI (about 70–90%), and the rest were found in layer V, consistent with other corticothalamic projections (see reviews Jones, 1985; Steriade et al., 1997). However, we noted significant regional differences in the pattern, whereby dorsal area 9 and medial prefrontal areas included a higher proportion of projection neurons in layer V (about 30%) than lateral and orbital areas (5–20%, OPro was an exception). Layer V pyramidal neurons have basal dendrites running horizontally in the layer, and their apical dendrites ascend to layer I. In contrast, layer VI neurons are generally small with branches in layer IV (for a review see Steriade et al., 1997). There is evidence that layer V neurons terminate as large, grape-like, round and discontinuous terminals (R-type) onto the proximal dendrites of thalamic neurons, whereas layer VI neurons form small plate-like, elongated (E)-type continuous terminals on the distal dendrites of thalamic neurons (Rouiller and Welker, 1991; Ojima, 1994; Rockland, 1996; for review see Rouiller and Welker, 2000). This may explain our findings of punctuated terminal patches in AM originating in axons from medial prefrontal areas, noted in the previous study, which included a higher proportion of their projection neurons in layer V, as seen in this study.

The difference in the laminar distribution of thalamic projection neurons in dorsomedial compared with lateral and orbitofrontal areas suggests functional divergence in the two efferent systems. Thalamic relay neurons that receive projections from cortical layer V project to layer I of the cortex (for reviews see Jones, 1985; Crick and Koch, 1998). In contrast, relay neurons in the thalamus that receive projections from cortical layer VI project to cortical layer IV. By synapsing on the proximal dendrites of thalamic relay neurons, R-type axonal terminations from layer V may influence the excitability of thalamic neurons to a greater extent than terminations by layer VI axons, which target distal dendrites. In addition, layer V projection neurons may not synapse with inhibitory reticular nucleus, as layer VI neurons do (for review see Steriade et al., 1997). Because layer V neurons have faster kinetics, when activated they may trigger strong oscillations by recruiting several cortical areas through widespread projections to layer I and to the thalamus (Bajo et al., 1995; for review see Rouiller and Welker, 2000). There is evidence that abnormality in this circuit may trigger epileptiform

activity (Telfeian and Connors, 1998; see also Crick and Koch, 1998).

In summary, the thalamic AM nucleus appears to be a key interface for systems associated with emotion and distinct types of memory, as well as with the basal ganglia perhaps for central executive functions in emotional situations (Fig. 12). This interconnected system involves the dominant corticothalamic projection from layer VI, as well as substantial projections from layer V. The laminar organization of the prefrontal feedback projections to the AM nucleus, particularly the substantial projections from layer V, may regulate the activity of thalamic neurons to meet emotional and cognitive challenges through extensive recruitment of cortical and thalamic structures.

Acknowledgements

We thank Ms. Karen Trait for technical assistance, Dr. Ron Killiany for MR imaging, and Dr. Sehgal for veterinary care. This work was supported by grants from NIH (NIMH and NINDS).

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