Pergamon

PII: S0306-4522(00)00585-6

www.elsevier.com/locate/neuroscience

*Neuroscience* Vol. 103, No. 3, pp. 593–614, 2001

0306-4522/01 $20.00+0.00

# NEURAL INTERACTION BETWEEN THE BASAL FOREBRAIN AND FUNCTIONALLY DISTINCT PREFRONTAL CORTICES IN THE RHESUS MONKEY

H. T. GHASHGHAEI and H. BARBAS*

Department of Health Sciences, Boston University, Boston, Massachusetts, USA

**Abstract**—The prefrontal cortex in rhesus monkeys is a heterogeneous region by structure, connections and function. Caudal medial and orbitofrontal cortices receive input from cortical and subcortical structures associated with emotions, autonomic function and long-term memory, while lateral prefrontal cortices are linked with structures associated with working memory. With the aid of neural tracers we investigated whether functionally distinct orbitofrontal, medial and lateral prefrontal cortices have specific or common connections with an ascending modulatory system, the basal forebrain. Ascending projections originated in the diagonal band and the basalis nuclei of the basal forebrain in regions demarcated by choline acetyltransferase. Although the origin of projections from the basal forebrain to lateral, medial and orbitofrontal cortices partially overlapped, projections showed a general topography. The posterior part of the nucleus basalis projected preferentially to lateral prefrontal areas while its rostrally adjacent sectors projected to medial and orbitofrontal cortices. The diagonal band nuclei projected to orbitofrontal and medial prefrontal areas. Cortical and subcortical structures that are interconnected appear to have a similar pattern of connections with the basal forebrain. In comparison to the ascending projections, the descending projections were specific, originating mostly in the posterior (limbic) component of medial and orbitofrontal cortices and terminating in the diagonal band nuclei and in the anterior part of the nucleus basalis. In addition, prefrontal limbic areas projected to two other systems of the basal forebrain, the ventral pallidum and the extended amygdala, delineated with the striatal-related markers dopamine, adenosine 3′:5′-monophosphate regulated phosphoprotein of $M_r$ 32 kDa, and the related phosphoprotein Inhibitor-1. These basal forebrain systems project to autonomic nuclei in the hypothalamus and brainstem.

We interpret these results to indicate that lateral prefrontal areas, which have a role in working memory, receive input from, but do not issue feedback projections to the basal forebrain. In contrast, orbitofrontal and medial prefrontal areas, which have a role in emotions and long-term memory, have robust bidirectional connections with the basal forebrain. Moreover, orbitofrontal and medial prefrontal cortices target the ventral pallidum and the extended amygdala, through which high-order association areas may activate motor autonomic structures for the expression of emotions. © 2001 IBRO. Published by Elsevier Science Ltd. All rights reserved.

*Key words:* emotions, working memory, long-term memory, connections, ventral pallidum, extended amygdala.

The prefrontal cortex in primates is a large and heterogeneous region that is connected widely with cortical and subcortical structures. The lateral, medial and orbitofrontal sectors of the prefrontal cortex have diverse connections that probably underlie their functional specialization (for reviews see Refs 10, 11 and 12). For example, the hippocampus and amygdala in the temporal lobe, which are associated with long-term memory and emotional memory,[22,133] target robustly caudal medial and orbitofrontal cortices.[4,15,16,27,85,95] However, lateral prefrontal cortices, which are associated with working memory (for reviews see Refs 38, 39, 49 and 94), have few, if any, connections with the hippocampus and the amygdala, but are connected with parietal cortices associated with cognitive functions (for reviews see Refs 7 and 12).

The question arises of whether the specificity of connections of functionally distinct prefrontal cortices extends to the ascending modulatory systems. These systems include the basal forebrain, the locus coeruleus, the substantia nigra/ventral tegmental area and the raphe nuclei (for reviews see Refs 5, 36, 74, 117 and 118). In this study we focused on the organization of connections between prefrontal cortices and one component of the modulatory systems, the basal forebrain, which is

*Corresponding author. Present address: Department of Health Sciences, Boston University, 635 Commonwealth Ave., Room 431, Boston, MA 02215, USA. Tel.: +1-617-353-5036; fax: +1-617-353-7567.

*E-mail address:* barbas@bu.edu (H. Barbas).

*Abbreviations:* ABC, avidin–biotin-peroxidase complex; AChE, acetylcholinesterase; ChAT, choline acetyltransferase; DAB, 3,3′-diaminobenzidine; DARPP-32, adenosine 3′5-monophosphate regulated phosphoprotein of $M_r$ 32 kDa; DMSO, dimethyl sulphoxide; HRP–WGA, horseradish peroxidase conjugated wheat germ agglutinin; mS, medial septum; nBMa, anterior subdivision of nucleus basalis of Meynart (consisting of nBMam and nBMal); nBMal, anterolateral subdivision of nucleus basalis of Meynart; nBMam, anteromedial subdivision of nucleus basalis of Meynart; nBMi, intermediate subdivision of nucleus basalis of Meynart; nBMp, posterior subdivision of nucleus basalis; nHL, horizontal limb of the diagonal band nuclei; nVL, vertical limb of the diagonal band nuclei; O12, orbital area 12; OPAll, orbital periallocortex (agranular cortex); OPro, orbital proisocortex (dysgranular cortex); PBS, phosphate-buffered saline.

thought to carry out arousal and attentional functions through its connections with the cortex (for reviews see Refs 21, 42, 51, 110 and 132). The basal forebrain has received special attention because of its susceptibility in neurodegenerative and neuropsychiatric diseases (for reviews see Refs 57, 58, 74, 97 and 109).

Previous studies have shown that the basal forebrain is connected with cortical and subcortical structures, with some degree of topographic organization[62,79,93,106,107,131,132] (for review see Ref. 132). For example, the diagonal band nuclei of the basal forebrain project primarily to the hippocampus and olfactory bulb, while the nucleus basalis of Meynert projects to the amygdala and the entire cortex.[79] Among prefrontal cortices the orbitofrontal and medial prefrontal cortices appear to have strong anatomical interactions with the basal forebrain in monkeys[61,64,78–80,106] and rats.[24,44,81,107,132] In monkeys, connections of the prefrontal cortex with the basal forebrain were studied along with the connections of the basal forebrain with the entire cortex.[78,79,83,106]

Since the publication of the above studies, a considerable amount of evidence has been amassed on the functional heterogeneity of both the prefrontal cortex and the basal forebrain. A wealth of information now supports the view that lateral, medial and orbitofrontal cortices have different connections and distinct functions in cognition, memory and emotion (e.g. see Refs 7, 10–12, 37, 39, 49 and 75). Similarly, it is now established that the basal forebrain is composed of several systems[2,58,132] and includes cholinergic as well as non-cholinergic neurons.[79,107,132] The cholinergic component of the basal forebrain has been well characterized.[79] Another component includes the ventral pallidum and occupies roughly the top half of the basal forebrain. In addition, embedded within the classically conceptualized basal forebrain region, there is yet another system, composed of punctuated groups of neurons extending from the central and medial nuclei of the amygdala to the bed nucleus of the stria terminalis, forming the extended amygdala (for reviews see Refs 31, 32 and 58).

In view of new evidence, it has become necessary to re-evaluate the interactions of the basal forebrain with functionally distinct prefrontal cortices. In this study we have focused on this issue and have extended analyses to prefrontal areas not previously explored, including the caudal orbitofrontal region (areas OPro and OPAll) and medial prefrontal area 32. These areas are part of the limbic component of the prefrontal cortex and have been implicated in emotional processes on the basis of their connections and physiological attributes (for reviews see Refs 11 and 12). We addressed the following questions: do prefrontal cortices with distinct functions in memory have a distinct pattern of connections with the basal forebrain, or are the projections from the basal forebrain to the prefrontal cortex diffuse? In light of evidence that there are distinct systems within the basal forebrain, does the pattern of connections between the prefrontal cortices and the basal forebrain include the extended amygdala and the ventral pallidum?

## EXPERIMENTAL PROCEDURES

### *Surgical procedures*

Experiments were conducted on 23 rhesus monkeys (*Macaca mulatta*), obtained through the Washington and New England Regional Primate Research Centers. Experiments were conducted according to the NIH Guide for the Care and Use of Laboratory Animals (NIH publication 86–23, revised 1987), and all efforts were made to minimize animal suffering and to reduce their numbers. The monkeys were anesthetized with ketamine hydrochloride (10 mg/kg, intramuscularly) followed by sodium pentobarbital administered intravenously through a femoral catheter until a surgical level of anesthesia was accomplished (cumulative dose ~30 mg/kg). Surgery was done under aseptic conditions. A craniotomy was made, the dura was cut and the cortex exposed.

In 14 of the animals a solution containing 8% horseradish peroxidase conjugated to wheat germ agglutinin (HRP–WGA; Sigma, St. Louis, MO) was injected, in four animals the fluorescent dye Fast Blue (Sigma, St. Louis, MO) was injected, and finally Fluororuby (dextran tetramethylrhodamine, mol. wt 3000, Molecular Probes, Eugene, OR), was injected in one animal. $^{3}$H-Labeled amino acids were injected in four animals. In all cases the injections were made using a Hamilton syringe (5 μl) mounted on a microdrive with the needle being lowered to a selected cortical site under microscopic inspection. Over a 30-min period, small amounts of the injectate (0.05–0.1 μl, 8% HRP–WGA; 0.4 μl, 3% Fast Blue; 0.8 μl, 10% Fluororuby; 0.4–1.0 μl, [$^{3}$H]leucine and [$^{3}$H]proline, specific activity 40–80 μCi) were delivered 1.5 mm below the pial surface at each of two adjacent sites separated by 1–2 mm.

### *Perfusion and tissue processing*

The animals injected with $^{3}$H-labeled amino acids were anesthetized and perfused with 10% formalin after 10 days of survival. The brain was removed from the skull, photographed,

*Abbreviations used in the figures*

| | |
|---|---|
| A | arcuate sulcus |
| AC | anterior commissure |
| Amy | amygdala |
| CAUD | caudate nucleus |
| CC | corpus callosum |
| Cg | cingulate sulcus |
| EA | extended amygdala |
| fb | Fast Blue |
| Fx | fornix |
| GP | globus pallidus |
| Hypo | hypothalamus |
| IC | internal capsule |
| LF | lateral fissure |
| LO | lateral orbital sulcus |
| MO | medial orbital sulcus |
| MPAll | medial periallocortex (agranular cortex) |
| OC | optic chiasm |
| OLF | olfactory area |
| OT | optic tract |
| P | principalis sulcus |
| PUT | putamen |
| RO | rostral sulcus |
| S | septum |
| ST | superior temporal sulcus |
| THAL | thalamus |
| V | ventricle |
| VP | ventral pallidum |
| V46 | ventral area 46 |
| WM | white matter |

embedded in paraffin, cut in 10 μm coronal sections, and mounted on glass slides. The processing of the sections was according to the autoradiographic method of Cowan *et al.*[29] Sections were counterstained with Thionin and coverslipped.

Following a survival period of 40–48 h animals injected with HRP–WGA were given a lethal dose of anesthetic (sodium pentobarbital) and perfused through the heart with saline followed by 2 liters of fixative (1.25% glutaraldehyde, 1% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4). The fixative was followed by perfusion with 2 liters of cold (4°C) phosphate buffer (0.1 M, pH 7.4). The brain was then removed from the skull, photographed and placed in glycerol phosphate buffer (10% glycerol, 2% dimethyl sulfoxide (DMSO) in 0.1 M phosphate buffer, pH 7.4) for one day and then in 20% glycerol phosphate buffer for two additional days.

The survival period for animals with fluorescent dye injections was 10–14 days. The animals were then anesthetized and perfused with 4% or 6% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4). The brain was removed and photographed as described above and postfixed in a solution of 4% or 6% paraformaldehyde with glycerol and 2% DMSO, as described previously.[8]

In HRP–WGA and fluorescent dye experiments brains were frozen in −75°C isopentane and cut on a freezing microtome in the coronal plane at 40 μm in 10 series. In the HRP–WGA experiments, one series was treated to visualize HRP.[73] The tissue was mounted, dried and counterstained with Neutral Red. In experiments with fluorescent dye injections, two series of sections were mounted for mapping labeled neurons. One series was coverslipped with Fluoromount and stored in light-tight boxes with Drierite at 4°C. The second series was plotted to visualize and localize retrogradely labeled neurons under a microscope equipped with an epi-fluorescence attachment. After plotting, sections were counterstained with Thionin and returned to the microscope to place architectonic borders. In all experiments one series of sections was processed for acetylcholinesterase (AChE) to determine the specific laminar termination of fibers in prefrontal cortices, which presumably arise from the basal forebrain.

### *Histochemical and immunocytochemical procedures used to delineate architectonic borders*

To aid in delineating architectonic borders, adjacent series of sections were stained for Nissl, myelin, choline acetyl transferase (ChAT), AChE, dopamine and adenosine 3′:5′-monophosphate regulated phosphoprotein of $M_r$ 32 kDa (DARPP-32), or Inhibitor-1.[43,46,86,90,91,124] AChE and ChAT can be used to stain cholinergic neurons in the basal forebrain; however, while AChE is an excellent marker for cholinergic neurons it is not a sufficient marker, while ChAT appears to be specific.[79,111] Tissue sections treated for ChAT and/or AChE were used to delineate the cholinergic sectors of the basal forebrain. Tissue treated for DARPP-32 and Inhibitor-1 was used to distinguish the ventral pallidum, which contained positive fibers, from the nucleus basalis, which did not. This tissue also highlighted clusters of neurons belonging to the extended amygdala which are positive for DARPP-32 and Inhibitor-1.

We used standard immunohistochemical procedures for ChAT (rat monoclonal, DiaSorin, INCSTAR, Stillwater, MN), DARPP-32 (mouse monoclonal, a gift from Dr Paul Greengard) and Inhibitor-1 (rabbit polyclonal, immunopurified, gift from Dr Angus Nairn). In brief, the sections were rinsed in 0.1 M phosphate-buffered saline (PBS) several times, blocked with 10% horse serum and 0.3% Triton-X in 0.1 M PBS for 1 h, and placed overnight in the primary antibodies in 0.1 M PBS with 1% horse serum and 0.3% Triton-X (1:200 for ChAT; 1:30,000 for DARPP-32; 1:250 for Inhibitor-1). The sections were then washed in 0.1 M PBS and placed in solution of secondary antibodies in 0.1 M PBS and 1% horse serum (horse anti-rat IgG for ChAT; horse anti-mouse IgG for DARPP-32; horse anti-rabbit IgG for Inhibitor-1; 1:200) for 2 h. The sections were then washed and placed in a solution of avidin and biotin (ABC elite kit, PK 6100, Vector Labs, Burlingame, CA, 1:200 in 0.1 M PBS) for 2 h. The sections were then washed in 0.1 M PBS and treated with 3,3′-diaminobenzidine (DAB; Plus Kit, Zymed, San Francisco, CA) to visualize ChAT, DARPP-32 and Inhibitor-1 positive neurons and fibers.

### *Data analysis*

*Mapping projection neurons.* Sections ipsilateral to the injection site were viewed under bright-field or fluorescence illumination to map retrogradely labeled neurons within the basal forebrain. Mapping of retrogradely labeled neurons was conducted using a microscope–computer interface and software developed in our laboratory. This system allows tracing borders and mapping and counting labeled neurons. The maps were transferred onto paper using a digital plotter (Hewlett Packard, 7475A) which was electronically coupled to the stage of the microscope and a PC.

*Mapping anterograde label.* We used phase-contrast and dark-field illumination to outline and map anterograde label in the basal forebrain in cases with injections of $^3$H-labeled amino acids or HRP–WGA. We used two methods to evaluate anterograde label. First, we rated anterograde label qualitatively by visual inspection as light, moderate or dense. Second, we measured the regional density of anterograde label in the basal forebrain using an image analysis system (MetaMorph, Universal Imaging, West Chester, PA). The system uses a CCD camera mounted on the microscope to capture images directly from brain sections. Measurements of the density of anterograde label were made at ×100 magnification under dark-field illumination, using a fiber optic illuminator which ensures even lighting at low and high magnification (Optical Analysis Corporation, Nashua, NH). Background measurements were taken from adjacent areas with no anterograde label within the basal forebrain. The background measurements were subtracted from the density scores to determine labeling above background level. Density measures were taken from five to six sites within an area, avoiding artifact and retrogradely labeled neurons. After subtraction of background the range of density scores was divided into three. Density scores falling in the bottom third were considered light, in the middle third moderate, and in the top third dense. The rating scores were converted to a scale of (−) to (+++), corresponding to no label (−), light label (+), moderate label (++), and dense label (+++) (Table 3). The final rating scores highly correlated with the independent qualitative analyses (Pearson $r = 0.92$, $P < 0.001$).

*Reconstruction of injection sites, projection zones and photography.* The cortical regions containing the injection sites were reconstructed serially by using the sulci as landmarks, as described previously.[6] Projection zones were shown on representative coronal sections of the basal forebrain. The photomicrographs in Figs 1 and 3 were captured directly from histological brain slides using a CCD camera and the Neurolucida Virtual Slice software (MicroBrightField, Colchester, VT) and were imported into Adobe Photoshop (Adobe Systems, San Jose, CA) for assembly, labeling and adjustment of overall brightness, but were not retouched.

## RESULTS

### *Delineation of basal forebrain nuclei*

The basal forebrain includes the nucleus basalis of Meynert, the nuclei of the diagonal band, consisting of the nucleus of the vertical limb (nVL; Fig. 1A), and the nucleus of the horizontal limb (nHL; Fig. 1B), and the septal nuclei. We delineated the above nuclear groups with the aid of AChE, ChAT and Nissl stained sections. Detailed analyses of the architecture of the above basal forebrain structures in macaque monkeys have been

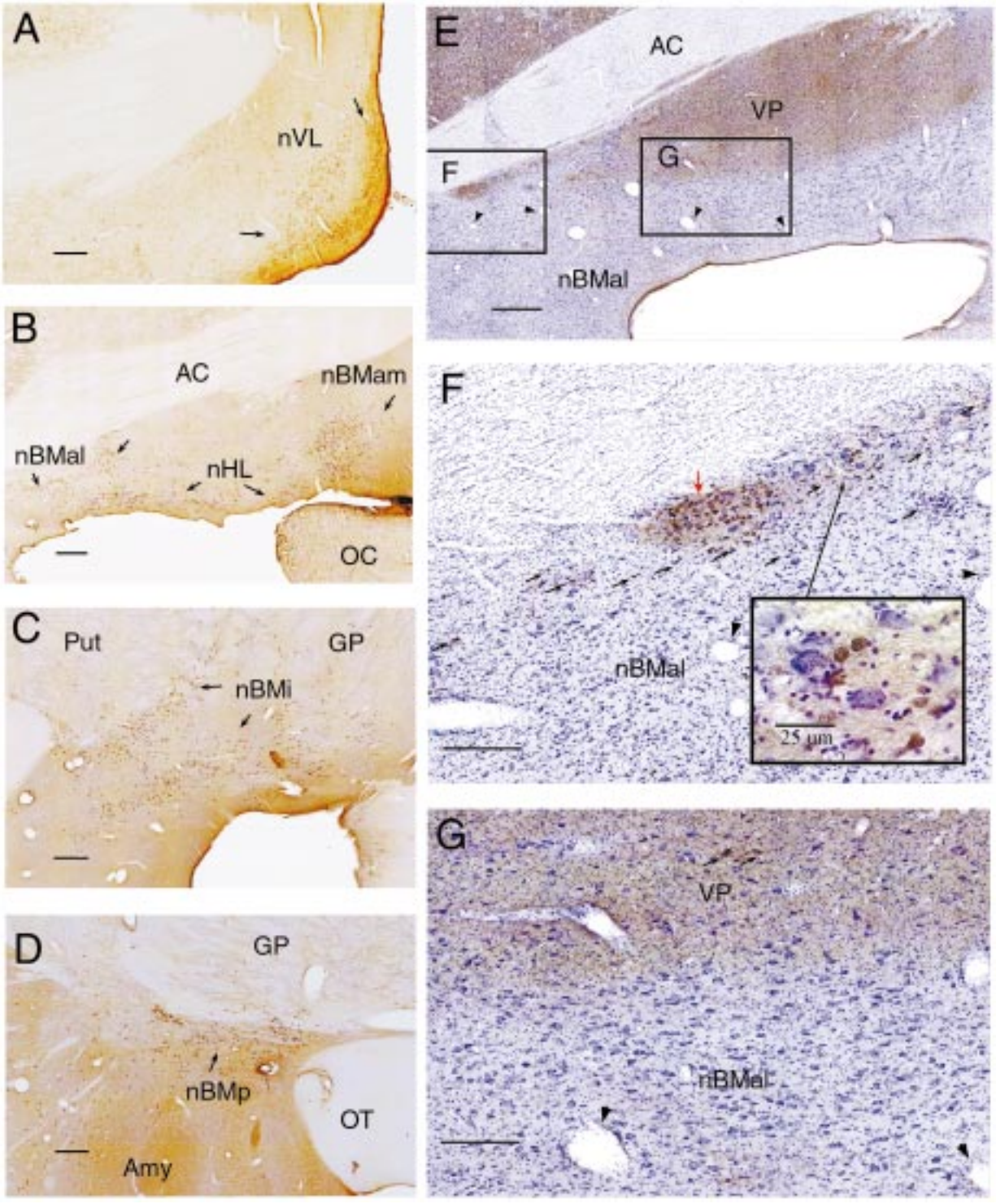


Fig. 1. Architecture of the basal forebrain. (A–D): Bright-field photomicrographs of coronal sections through rostral (A) to caudal (D) extent of the cholinergic basal forebrain showing the different sectors of the nucleus basalis in tissue treated for ChAT. (A) The vertical limb of the diagonal band nuclei (nVL; Ch2). (B) Anterior divisions of the nucleus basalis (nBMam, nBMal; Ch4), and the horizontal limb of the diagonal band (nHL; Ch3). (C) Intermediate division of the nucleus basalis (nBMi; Ch4). (D) Posterior extent of the nucleus basalis (nBMp; Ch4). (E, F) Distinction of the nucleus basalis, the ventral pallidum, and the extended amygdala. (E) Photomicrograph of tissue treated for the phosphoprotein DARPP-32 and counterstained with Nissl in the nBMa region, showing the border between the ventral pallidum containing DARPP-32 immunoreactive neuropil (brown region) and the negative nBM stained for Nissl (blue neurons below the brown region). (F) Small neurons of the extended amygdala are positive for DARPP-32 (dark brown cells, small black arrows) and are distinguishable from the magnocellular neurons in nBMal stained only with Nissl (blue cells). Areas in F, G are shown below at higher magnification. Inset in F is a higher magnification of DARPP-32 positive neurons in the region of the extended amygdala, indicated by the long black arrow. The red arrow shows a ventral extension of the putamen which includes neurons of the putamen as well as neurons of the extended amygdala. Scale bars = 1 mm (A–E), 250 μm (F–G).

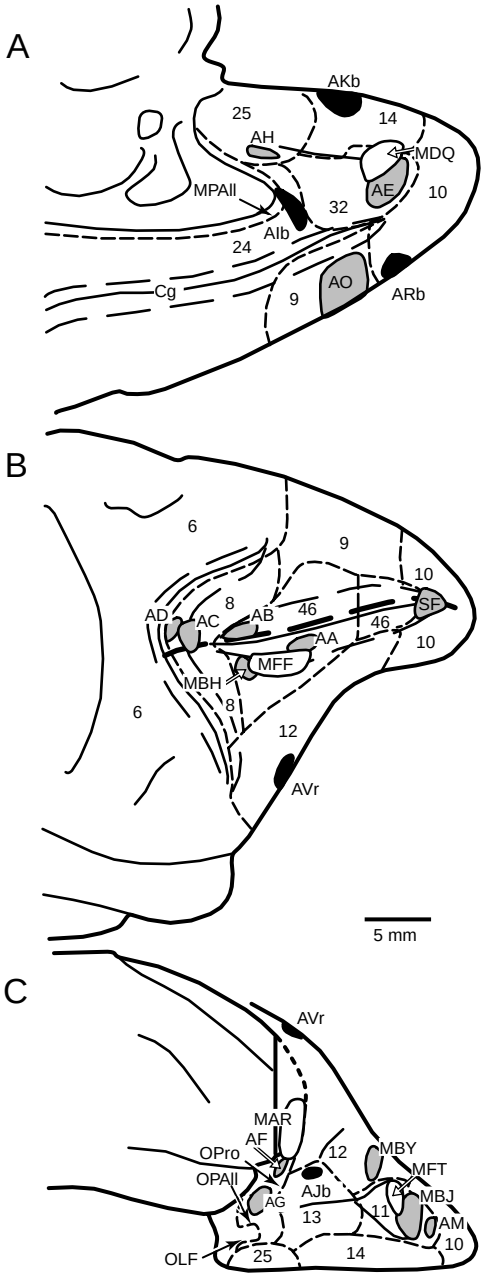


Fig. 2. Composite of injection sites shown on the medial (A) lateral (B) and orbital (C) surfaces of the frontal lobe. The injection sites are superimposed on an architectonic map of the prefrontal cortex (Barbas and Pandya, 1989).[20a] Dotted lines demarcate architectonic areas indicated by numbers. MPAll, OPAll, OPro and OLF refer to architectonic areas. All other letter combinations refer to cases. The injection site patterns refer to the type of tracer used: black area, fluorescent dyes; black outline, [$^3$H]amino acids; gray, HRP–WGA.

published in previous studies[79] and will not be described here.

The nucleus of the vertical limb (nVL; Fig. 1A) and the medial septum (mS; dorsal to nVL, not shown) are located rostromedially within the basal forebrain and correspond to the Ch1 and Ch2 groups of Mesulam *et al.*[79] The nucleus of the horizontal limb (nHL) is located posterior to the nVL and extends along the ventral edge of the forebrain (Ch3 group of Mesulam

Table 1. Injection sites, tracer types and analyses conducted in prefrontal cases

| Area injected | Case | Tracer type |
|---|---|---|
| Orbitofrontal | | |
| OPro/OPAll | AG | HRP–WGA*† |
| OPro | AF | HRP–WGA*† |
| OPro | MAR | [$^3$H]Amino acids† |
| 13 | AJb | Fast Blue* |
| O12 | MBY | HRP–WGA*† |
| 11 | MFT | [$^3$H]Amino acids† |
| 11 | AM | HRP–WGA* |
| 11 | MBJ | HRP–WGA* |
| Medial | | |
| 25 | AH | HRP–WGA†‡ |
| 32/24 | AIb | Fast Blue* |
| 32 | AE | HRP–WGA*† |
| 32 | MDQ | [$^3$H]Amino acids† |
| 14 | AKb | Fast Blue* |
| M9 | AO | HRP–WGA*† |
| M10 | ARb | Fast Blue* |
| Lateral | | |
| 8 | AD | HRP–WGA* |
| 8 | AC | HRP–WGA*† |
| D46 | AB | HRP–WGA† |
| V46 | MBH | HRP–WGA*† |
| V46 | MFF | [$^3$H]Amino acids† |
| V46 | AA | HRP–WGA*† |
| L12/O12 | AVr | Fluororuby* |
| D10/R46 | SF | HRP–WGA*† |

*Quantitative analysis of projection neurons in the basal forebrain directed to prefrontal cortices,
†Analysis of anterograde label in the basal forebrain,
‡Qualitative analysis only; confirmatory cases.

*et al.*;[79] Fig. 1B). The nucleus basalis of Meynert (Ch4 group[79]) which is situated caudal to the nVL and dorsal to the nHL, is divided into anatomically distinct subdivisions: an anterior (nBMa) portion with medial (nBMam) and lateral parts (nBMal; Fig. 1B), an intermediate region (nBMi; Fig. 1C), and a posterior group (nBMp; Fig. 1D), all of which are segregated solely by topography.

In addition, our descriptions extend into the nearby striatal-related part of the basal forebrain delineated with the aid of DARPP-32, a phosphoprotein first described in the neostriatum and its efferent projections.[90,124] The striatal-related parts of the basal forebrain include the ventral pallidum and the extended amygdala. Fibers positive for DARPP-32 labeled the upper part of the basal forebrain, which includes the ventral pallidum, and delineated it from the bottom half of the basal forebrain where neither fibers nor the magnocellular neurons of the nucleus basalis were positive for DARPP-32 (Fig. 1E, G). The extended amygdala is composed of punctuated groups of neurons extending from the central and medial nuclei of the amygdala to the bed nucleus of the stria terminalis (Fig. 1F, G). In contrast to other neurons of the basal forebrain, cell clusters of the extended amygdala were positive for DARPP-32 (Fig. 1F, G, small black arrows), akin to the positive neurons of the central nucleus of the amygdala described previously.[18] The DARPP-32-positive neurons in the basal forebrain were morphologically distinct from the islands of neurons situated below the anterior commissure that belong to

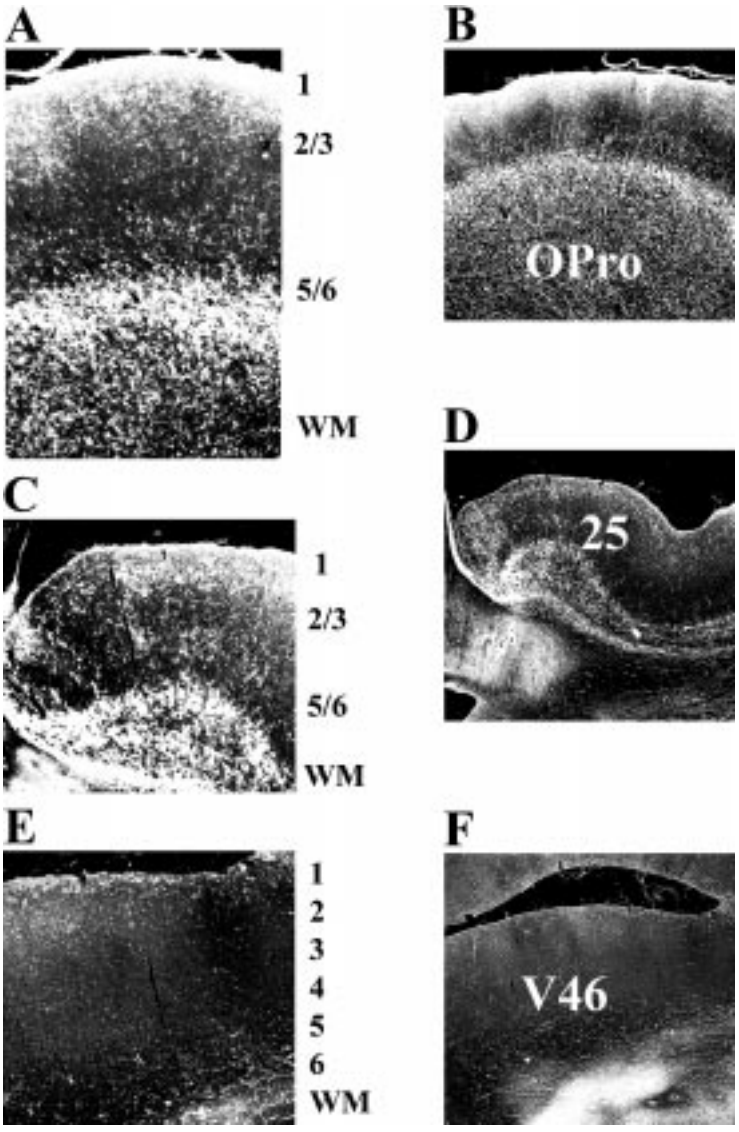


Fig. 3. The distribution of acetylcholinesterase fibers in the prefrontal cortex. Dark-field images of coronal sections showing AChE-positive terminals and fibers (white) within orbitofrontal (A, B), medial (C, D), and lateral (E, F) prefrontal cortices. The numbers to the right of A, C and E correspond to the cortical layers.

the ventral putamen.[91] A similar pattern was noted by labeling with Inhibitor-1.

Our analyses of striatal structures were restricted to regions that share territory with the basal forebrain and do not include the nucleus accumbens or more dorsally situated striatal parts described in previous studies.[34,48,129]

*Injection sites*

A composite diagram of the location of tracer injections within prefrontal cortices is shown in Fig. 2. We injected three different types of tracers: a bidirectional tracer (HRP–WGA), retrograde tracers (Fast Blue and Fluororuby) and anterograde tracers ([$^3$H]amino acids). The cases, the types of dyes injected, the corresponding injection sites within prefrontal areas and the type of analyses conducted are shown in Table 1. Injections of tracers in eight cases were in orbitofrontal areas (Fig. 2C), in seven cases in medial areas (Fig. 2A) and in eight cases in lateral areas (Fig. 2B). Details for the majority of the injection sites here were described previously in studies investigating the cortical, thalamic, amygdaloid, hippocampal and hypothalamic connections of prefrontal cortices.[8,9,15–17,19,33,99,100] Some of the data on the connections of these prefrontal areas with the basal forebrain have appeared as an abstract.[47]

*Termination of basal forebrain fibers within prefrontal cortices*

The distribution of AChE-positive terminals in a series of coronal sections through the prefrontal cortices is shown in Fig. 3. AChE-positive fibers were most densely distributed in posterior orbital (Fig. 3A, B) and medial (Fig. 3C, D) prefrontal regions and were found predominantly in layer 1 and the superficial aspect of the

Table 2. Distribution of labeled neurons in basal forebrain nuclei projecting to prefrontal cortices

| | | Basal forebrain projection zone | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Area injected | Case | mS/nVL | nHL | nBMam | nBMal | nBMi | nBMp | Total *n* |
| Orbitofrontal | | | | | | | | |
| OPro/OPAll | AG | – | 24 | 3 | 25 | 35 | 13 | 116 |
| OPro | AF* | – | 11 | 3 | 26 | 48 | 12 | 118 |
| 13 | AJb | 9 | 11 | 14 | 28 | 37 | 2 | 130 |
| O12 | MBY* | – | 20 | 27 | 2 | 24 | 27 | 45 |
| 11 | MBJ* | 2 | 28 | 17 | 17 | 25 | 11 | 125 |
| 11 | AM | 9 | 44 | 20 | - | 20 | 7 | 65 |
| Medial | | | | | | | | |
| 32/24 | AIb* | 34 | 14 | 25 | 21 | 3 | 3 | 191 |
| 32 | AE* | 27 | 2 | 28 | 28 | 11 | 3 | 777 |
| 14 | AKb* | 16 | 22 | 7 | 24 | 31 | – | 55 |
| M9 | AO | 14 | 8 | 19 | 12 | 42 | 5 | 74 |
| M10 | ARb | 10 | 10 | 23 | 7 | 33 | 17 | 30 |
| Lateral | | | | | | | | |
| 8 | AD | 7 | 19 | 12 | 12 | 17 | 33 | 108 |
| 8 | AC | – | – | 12 | 6 | 6 | 76 | 17 |
| V46 | MBH | 12 | 4 | 25 | 18 | 16 | 25 | 51 |
| V46 | AA | 5 | 27 | 17 | 2 | 27 | 22 | 60 |
| L12/O12 | AVr | 7 | – | – | 21 | 47 | 27 | 15 |
| D10/R46 | SF | 6 | – | 15 | 10 | 38 | 31 | 70 |

Data in columns below area or nucleus designations are expressed in percentages. The last column (total *n*) shows the total number of labeled neurons in the basal forebrain in each case; –, areas with no evidence of labeled neurons.
*Cases with possible labeled neurons in the ventral pallidum or extended amygdala.

deep layers. Labeling was also noted in lateral prefrontal cortices but was substantially less dense than in medial and orbitofrontal areas and was seen mostly in layer 1 and sparsely in layer 6 (Fig. 3E, F). This pattern confirms previous findings in the cortex using AChE and ChAT staining procedures.[20,23,66,72,76,79,80,82,122]

### *Retrogradely labeled neurons in the basal forebrain*

The distribution of retrogradely labeled neurons in the different nuclei of the basal forebrain after injection of retrograde tracers in prefrontal cortices is shown in Table 2. About two-thirds of the labeled neurons in the basal forebrain were found in and around the nucleus basalis of Meynert (nBM) in all cases. The remaining labeled neurons were located in the diagonal band nuclei and medial septal region. Overall, the majority of the labeled neurons appeared magnocellular in size, although a few small labeled neurons were scattered among the magnocellular neurons and were noted after caudal medial and orbitofrontal injections. Moreover, a few of the labeled neurons were found in the territory of the extended amygdala and ventral pallidum in medial and orbitofrontal cases, although they were magnocellular neurons, suggesting that they belonged to the nucleus basalis.

### *Projections to orbitofrontal cortices*

In case AG, with an HRP–WGA injection in orbital agranular area OPAll and dysgranular area Opro (Figs 2C, 4E), the highest proportion of labeled neurons was observed within the nBMi region, posterior to the anterior commissure and optic chiasm, accounting for 35% of all labeled neurons in this case (Fig. 4C). Similarly, in case AF, with an injection in neighboring orbitofrontal area OPro (Fig. 5E), labeled neurons in nBMi accounted for 48% of all labeled neurons (Fig. 5B, C). At the level of the rostral aspect of the anterior commissure, in each case, a quarter of the labeled neurons were found within the nBMal (Figs 4A, B, 5A). Ventral to the nBMa, labeled neurons were found within nHL on the ventral edge of the forebrain (case AG, 24%; case AF, 11%; Figs 4B, 5A). Labeled neurons were also observed within the caudal extent of the nucleus basalis, in nBMp, just lateral to the optic tract (13% in case AG and 12% in case AF; Figs 4D, 5D). In both cases, the nBMam contained only 3% of all labeled neurons. There was no evidence of labeled neurons within nVL in either case.

As in the above cases, in case AJb, with an injection of Fast Blue in adjacent area 13 (Fig. 2C), labeled neurons were seen in nBMi (37%) and nBMal (28%; data not shown). The nBMam, at the level of the anterior commissure, contained some labeled neurons (14%; Table 2), as did the mS/nVL region (9%) and the nHL (11%). Only a few labeled neurons were observed within the nBMp.

More rostrally within the orbitofrontal region, areas O12 and 11 differed from caudal orbitofrontal areas as recipient of a more robust projection from nBMam (Table 2). In case MBY, with an injection in the rostral part of area 12 (Fig. 6E), labeled neurons were seen in nBMam (27%; Fig. 6A, B), nBMi (24%; Fig. 6C), and lateral to the optic tract within nBMp (27%; Fig. 6D), and in nHL (20%; Fig. 6A). There were only a few labeled neurons in nBMal (Fig. 6A, B) and none could be seen in the nVL (not shown).

Cases with injections in orbitofrontal area 11 were distinguished from the rest of the orbitofrontal cases by a higher proportion of labeled neurons in nHL (28% in

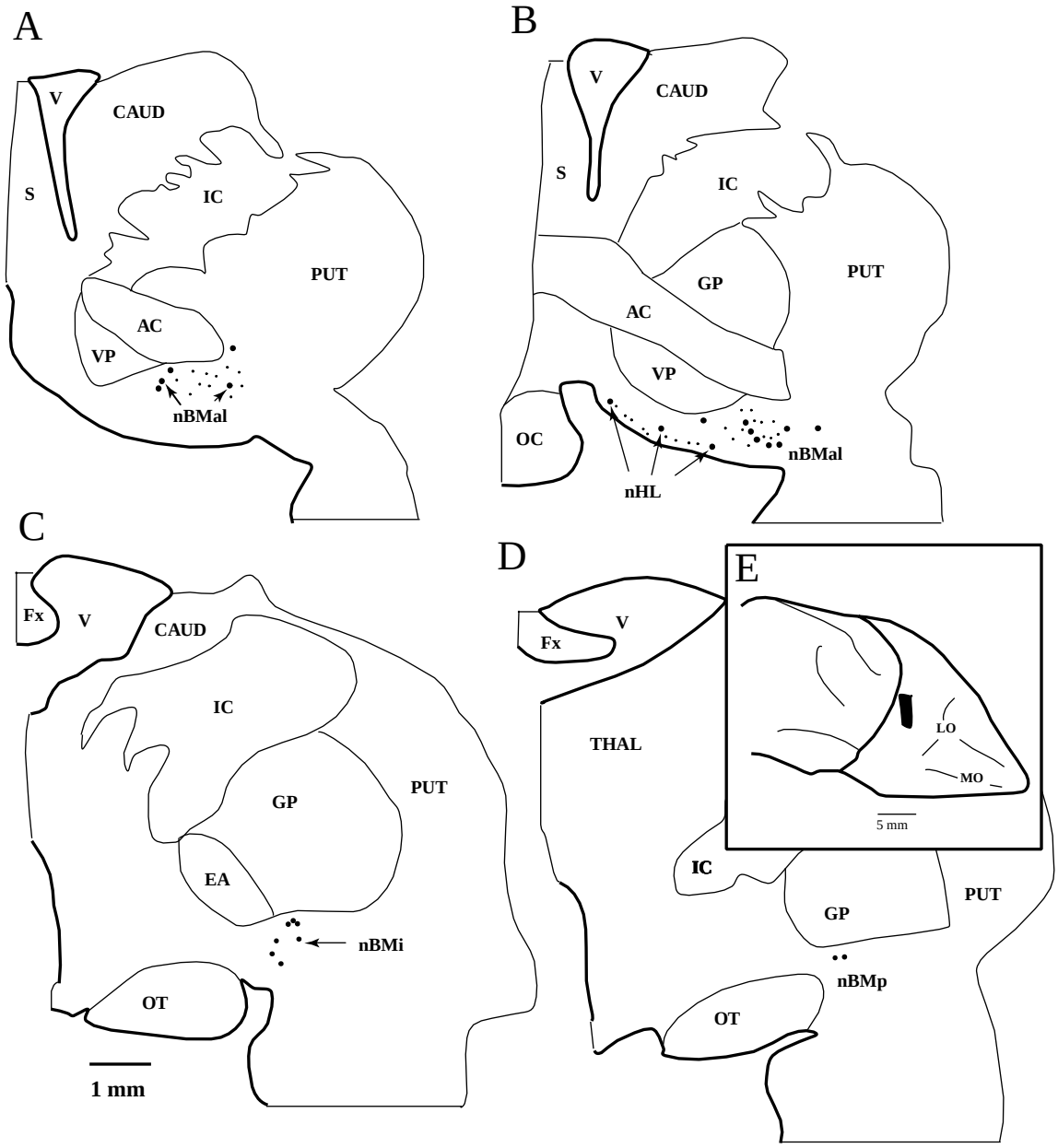


Fig. 4. Bidirectional connections between the basal forebrain and orbitofrontal area OPro/OPAll. Case AG: Distribution of labeled neurons (large dots) and anterograde label (small dots) in a series of coronal sections in rostral to caudal (A–D) basal forebrain nuclei after injection of HRP–WGA in orbitofrontal areas OPro and OPAll (E, black area).

case MBJ and 44% in case AM; Figs 7A, 8A, B). Similar to other orbitofrontal cases, the nBMi included a significant portion of all labeled neurons in both cases (25% in case MBJ and 20% in case AM; Figs 7C, 8D). In case MBJ, a cluster of labeled neurons was observed within the central part of nBMa, extending into both nBMam and nBMal (17% each; Fig. 7A, B). In case AM there were labeled neurons within nBMam (20%) but none was seen in nBMal (Fig. 8B, C). More caudally, immediately lateral to the optic tract, nBMp contained a cluster of labeled neurons in both cases (11% in case MBJ and 7% in case AM; Fig. 7D). Ventral to the septal region, only a few labeled neurons were found within nVL (2% in case MBJ and 9% in case AM; not shown).

*Projections to medial cortices*

Medial prefrontal cortices differed from the orbitofrontal by their higher proportion of labeled neurons in the mS/nVL (Table 2). In cases AIb and AE with injections in area 32 (Figs 9E, 10E) about a third to a quarter of the labeled neurons were seen in the mS/nVL (Figs 9A, 10A) and in nBMam and nBMal (Figs 9B, 10B; Table 2). In case AE it was difficult to delineate the basal forebrain subgroups because of the extensive retrograde labeling. Nuclear boundaries in case AE were drawn on the basis of adjacent series stained for AChE or Thionin.

The injection in case AIb was posterior to that in case

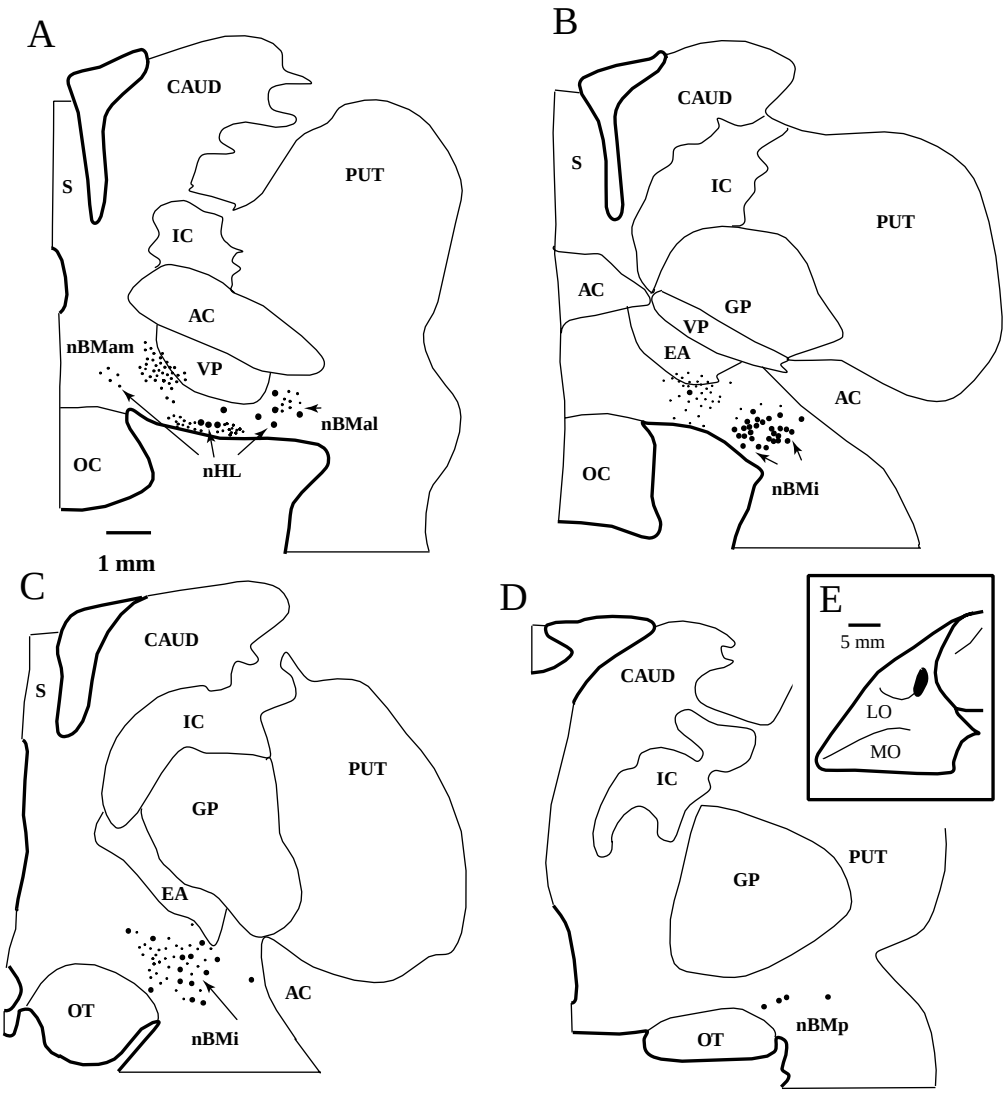


Fig. 5. Bidirectional connections between the basal forebrain and orbitofrontal area OPro. Case AF: Distribution of labeled neurons (large dots) and anterograde label (small dots) in a series of coronal sections in rostral to caudal (A–D) basal forebrain nuclei after injection of HRP–WGA in orbitofrontal area OPro (E, black area).

AE and it spread into area 24. These cases differed somewhat with more labeled neurons seen in the nHL in case AIb (14%; Fig. 9B, C) than in case AE (2%; Fig. 10B, C), and by more labeled neurons in the nBMi in case AE (11%; Fig. 10C, D) compared to case AIb (3%; Fig. 9C). In both cases, only a few labeled neurons were found within the nBMp (3%; Fig. 9D). In both cases a few labeled neurons were noted in the territory of the extended amygdala and ventral pallidum (Figs 9C, D, 10B–D).

In case AKb the injection of Fast Blue was in area 14 (Fig. 11E), ventral to the above injections. This case differed from the above by more extensive retrograde labeling in nBMi (31%; Fig. 11C, D), but like the above, it had labeled neurons in nBMal (24%; Fig. 11B), nHL (22%; Fig. 11B), the nVL (16%; Fig. 11A) and in nBMam (7%; Fig. 11B). There was no evidence of labeled neurons in the nBMp.

In cases AO and ARb, injection of HRP–WGA was in medial area 9 and the mediodorsal aspect of area 10 (M10), respectively (Fig. 2A). These cases differed from cases with injections in area 32 or 24 by having a large proportion of labeled neurons in nBMi (Table 2: 42% in case AO and 33% in case ARb; not shown), and a smaller proportion in nBMal (12% in case AO and 7% in case ARb; Table 2) and in the nVL (14% in case AO and 10% in case ARb). Like cases AIb and AE with injections in areas 24 and 32, however, nBMam had a substantial portion of labeled neurons in both cases (19% in case AO and 23% in case ARb). The nHL included some labeled neurons in both cases (8% in case AO and 10% in case ARb). In case ARb there were more labeled neurons within nBMp (17%) than in case AO (5%).

### *Projections to lateral cortices*

Lateral areas differed from the rest by their robust projections from nBMp, ranging from about a quarter

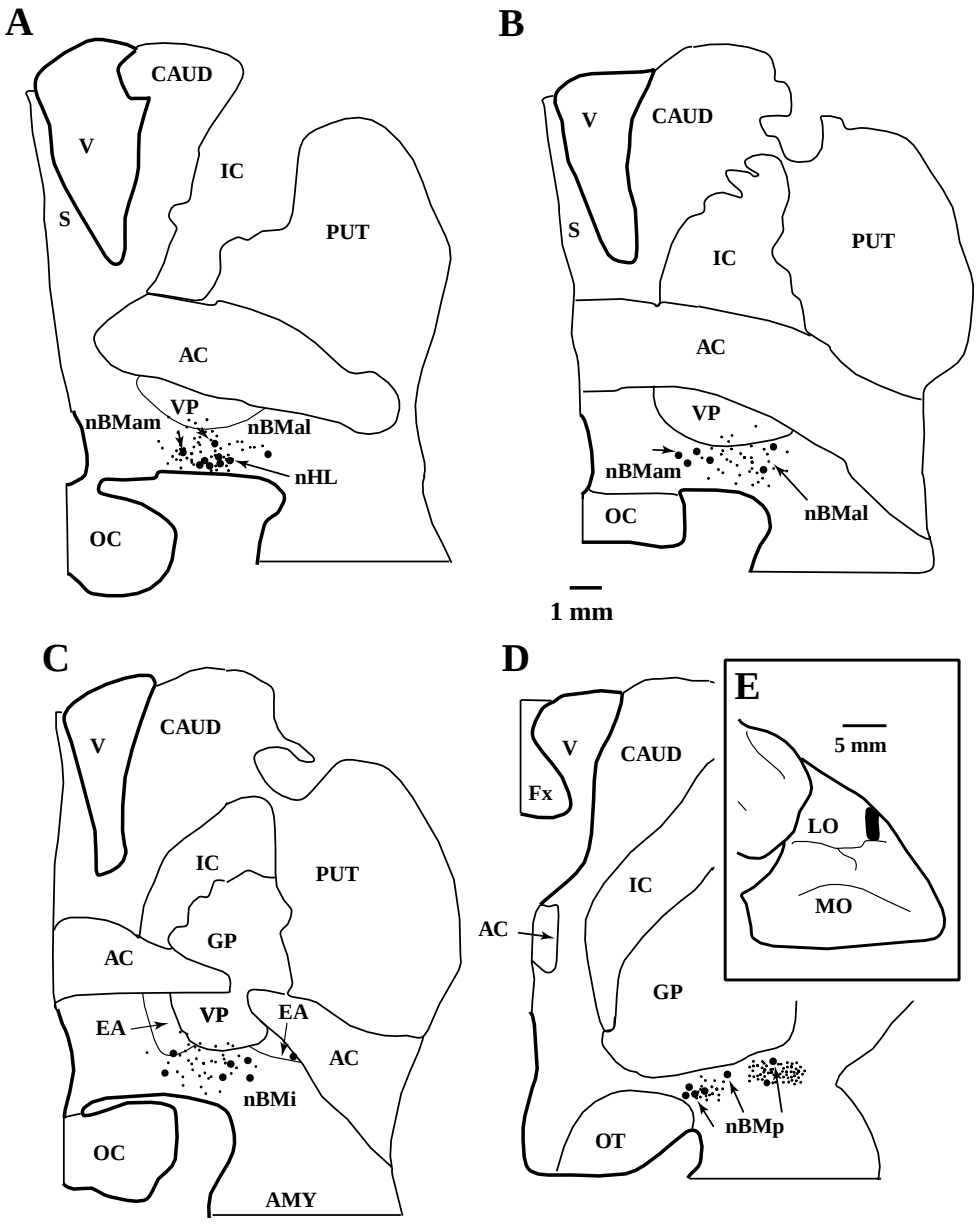


Fig. 6. Bidirectional connections between the basal forebrain and orbitofrontal area 12. Case MBY: Distribution of labeled neurons (large dots) and anterograde label (small dots) in a series of coronal sections in rostral to caudal (A–D) basal forebrain nuclei after injection of HRP–WGA in orbitofrontal area 12 (E, black area).

to a majority of all labeled neurons. In cases AD and AC, injections of HRP–WGA were in area 8. In case AD, labeled neurons were found in nBMp (33%; Fig. 12C, D), nBMi (17%; Fig. 12B) and the nHL (19%; Fig. 12A). A cluster of labeled neurons was seen in the center of the nBMa region, ventral to the anterior commissure and ventral pallidum, within nBMam (12%) and nBMal (12%; not shown). Only a few labeled neurons were found in nVL (Fig. 12A). As in case AD, in case AC most of the labeled neurons were found in nBMp (76%, Table 2; not shown), and a few were seen in nBMam (12%). Only a few labeled neurons were seen in nBMi and nBMal (6% each). There was no evidence of labeled neurons in the diagonal band nuclei or in the medial septum.

In cases with more rostral injections in lateral prefrontal areas there were fewer, but still a substantial proportion of labeled neurons in nBMp. In cases MBH and AA injections of HRP–WGA were in ventral area 46 (Figs 2B, 13E). In case MBH, labeled neurons were seen in nBMam (25%; Fig. 13B), nBMp (25%; Fig. 13D) and nBMal (18%; Fig. 13B). Labeled neurons were also noted in nBMi (16%; Fig. 13C) and nVL (12%; Fig. 13A). Only a few labeled neurons were seen within the nHL (Fig. 13B). In case AA, where the injection was in a more rostral part of ventral area 46 than in case MBH (Fig. 2B), the most extensive labeling was observed in nHL (27%), nBMi (27%), nBMp (22%) and nBMam (17%). Only a few labeled neurons were found in nVL or nBMal.

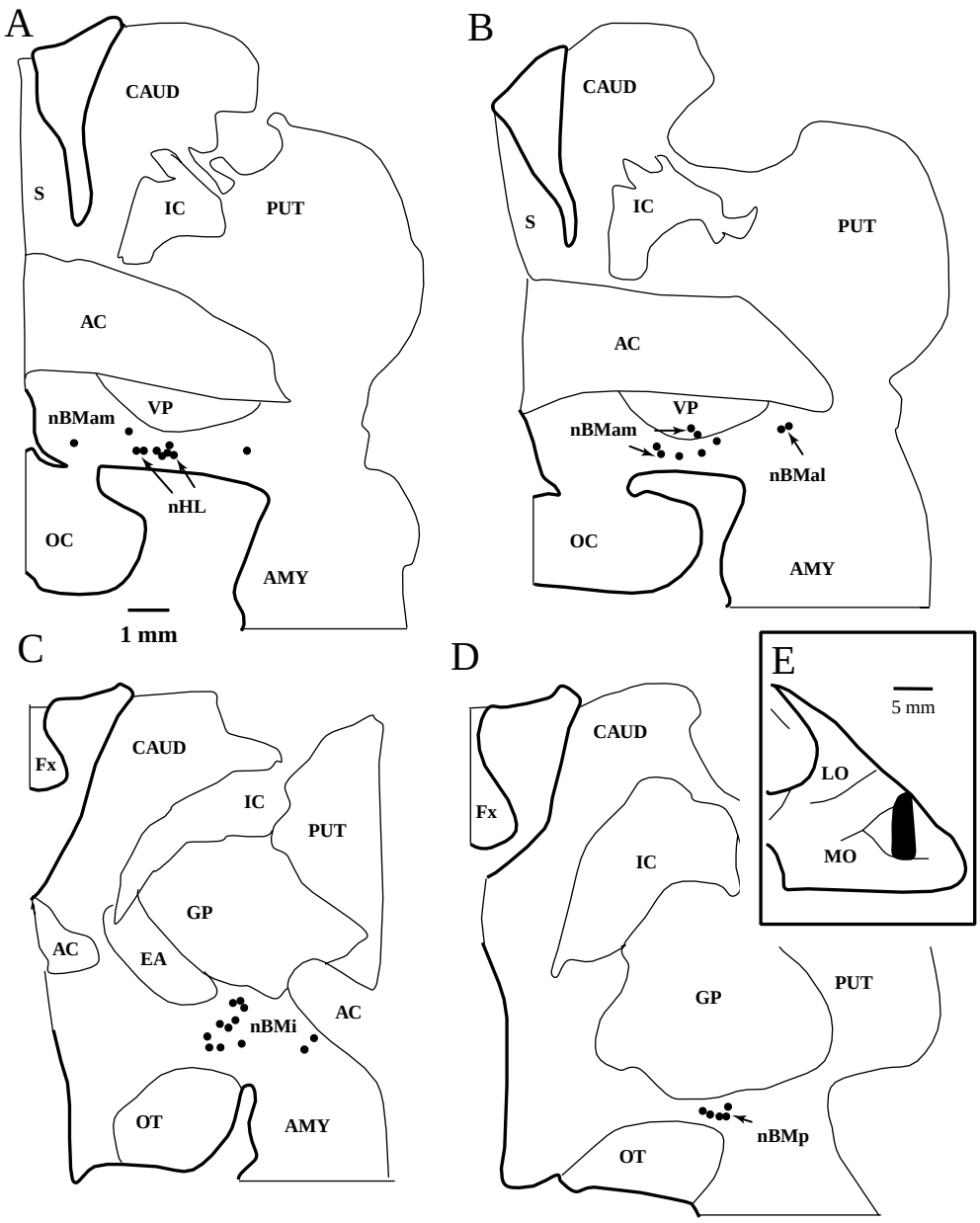


Fig. 7. Ascending projections from the basal forebrain to orbitofrontal area 11. Case MBJ: Distribution of labeled neurons (large dots) in a series of coronal sections in rostral to caudal (A–D) basal forebrain nuclei after injection of HRP–WGA in orbitofrontal area 11 (E, black area).

In case AVr with an injection of Fluororuby in lateral and orbital area 12, the highest proportion of labeled neurons was noted in nBMi, similar to case SF which had an injection of HRP–WGA in rostral area 46 and dorsal 10 (47% in case AVr and 38% in case SF; Table 2; not shown). These cases resembled other lateral cases by a substantial projection from the nBMp (27% in case AVr and 31% in case SF). In both cases, labeled neurons were also found within nBMal (21% in case AVr and 10% in case SF). The nBMam included labeled neurons in case SF (15%), but not in case AVr (Table 2). There were only a few labeled neurons within nVL in both cases (7% in case AVr and 6% in case SF), and there was no evidence of labeled neurons in nHL.

*Topography of basal forebrain neurons projecting to lateral, medial and orbitofrontal regions*

The above analysis indicated some differences in the topography of projection neurons directed to distinct prefrontal cortices. We next investigated whether there were overall differences in the topography of basal forebrain projections to lateral, medial and orbitofrontal cortices. The most notable difference was in the preferential distribution of projection neurons within nBMp after injections in lateral prefrontal cortices (average 36%) compared with orbitofrontal cases (12%) or medial cases (6%; Fig. 14). In a rostrocaudal direction within the nucleus basalis, there appears to be a crude topographic

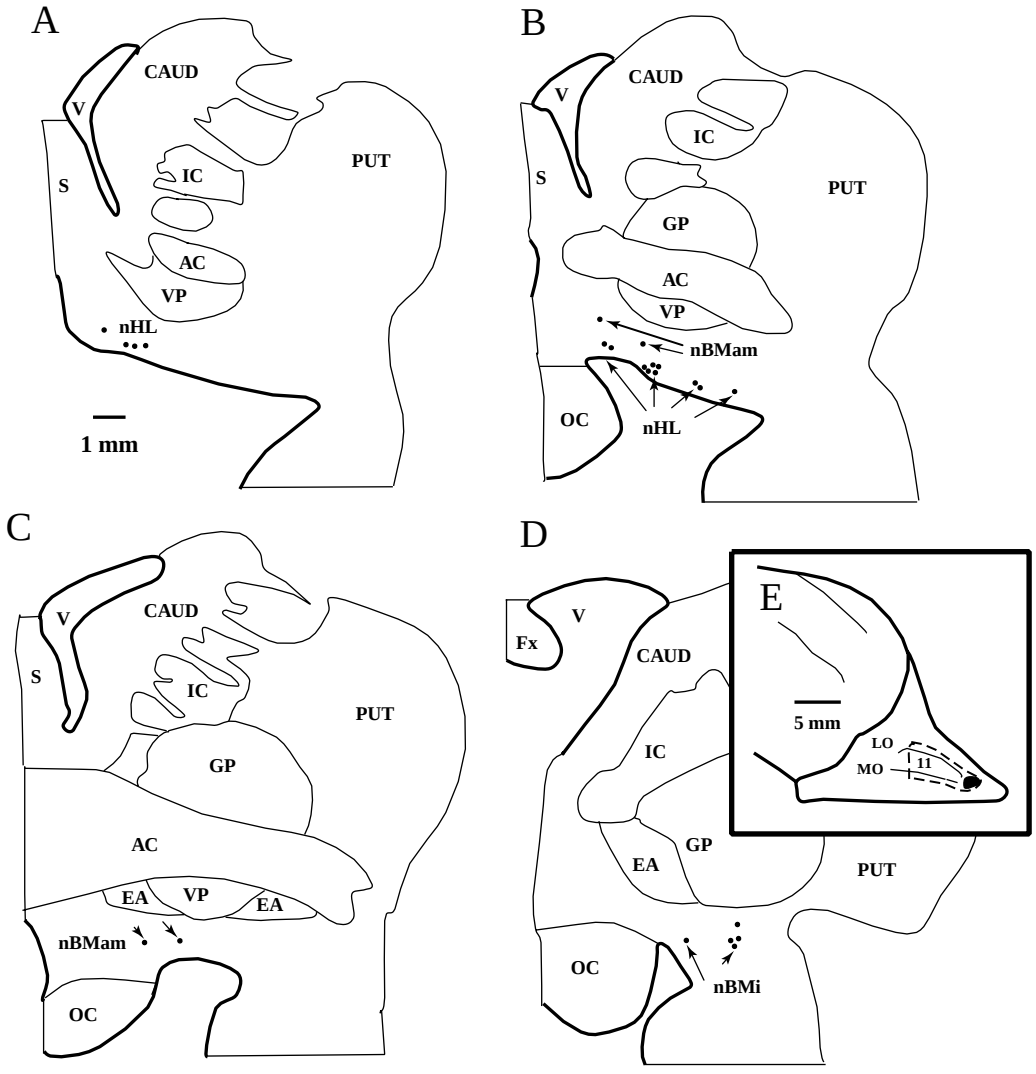


Fig. 8. Ascending projections from the basal forebrain to orbitofrontal area 11. Case AM: Distribution of labeled neurons (large dots) in a series of coronal sections in rostral to caudal (A–D) basal forebrain nuclei after injection of HRP–WGA in orbitofrontal area 11 (E, black area).

organization in the projection neurons, whereby nBMa (medial and lateral) sends more robust projections to medial, nBMi to orbitofrontal and nBMp to lateral prefrontal cortices.

There were differences in the projections from the mS and the diagonal band nuclei to different prefrontal cortices (Fig. 14). The mS and the nVL were grouped together because of their proximity. There was a significant difference in the percentage of labeled neurons within mS/nVL directed to prefrontal sectors ($F = 9.64$, $P < 0.05$), so that medial prefrontal injections labeled a significantly higher percentage of neurons than lateral ($t = 2.57$, $P < 0.05$) or orbital injections ($t = 3.3$, $P < 0.05$).

*Topography of descending projections from prefrontal cortices to the basal forebrain*

We next investigated whether the connections between the basal forebrain and the prefrontal cortices were reciprocal. Anterograde label was evident in the basal forebrain after injections of HRP or $^3$H-labeled amino acids only in some of the cases studied (Table 3). The highest density of anterograde label was observed after injections in orbitofrontal cortices (cases AF, MAR, MBY and MFT), and was seen in the nBMal in all cases, and in most cases in the nBMam, nHL and nBMi as well (Figs 4–6). In orbitofrontal cases AF, MAR and MBY anterograde label was also seen in the ventral pallidum and the extended amygdala (Figs 5B, 6B, C). In addition, there was a dense cluster of anterograde label in nBMp after injection of HRP–WGA in orbital area 12 in case MBY (Fig. 6D). Other orbitofrontal cases had little or no anterograde label in nBMp.

Among medial cortices, injection of HRP in area 32 (case AE) resulted in significant anterograde label in all basal forebrain regions (Fig. 10). Anterograde label in the ventral pallidum and extended amygdala was diffusely distributed, and was less dense than in the bottom half of the basal forebrain (Fig. 10B, C). Dense anterograde

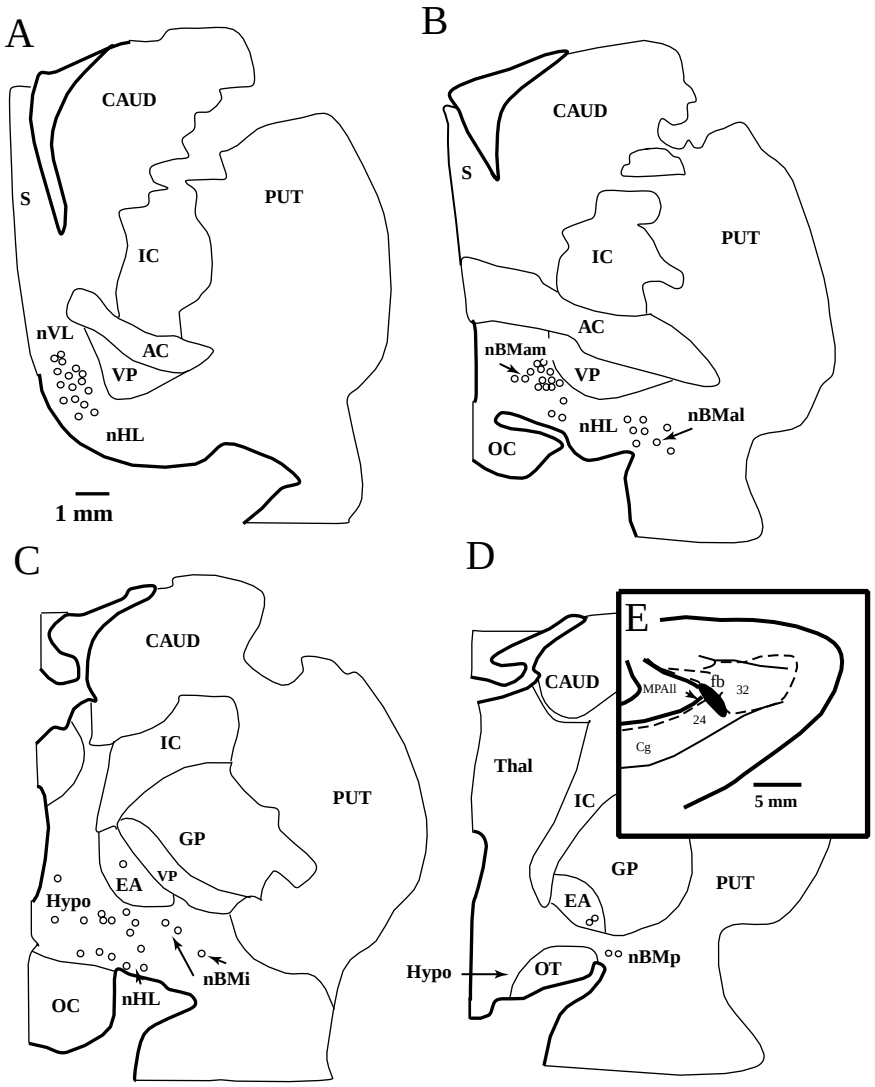


Fig. 9. Ascending projections from the basal forebrain to medial areas 32/24. Case AIb: Distribution of labeled neurons (open circles) in a series of coronal sections in rostral to caudal (A–D) basal forebrain nuclei after injection of Fast Blue in medial prefrontal areas 32/24 (E, black area).

label, in case AE, was found mostly within nVL, nHL and nBMam (Fig. 10A–C). In case AH, with an injection of HRP in area 25, light anterograde label was seen in the mS/nVL and in nHL of the diagonal band (not shown). In case MDQ with an injection of [$^3$H]amino acids in rostral area 32, there was no labeling in any of the basal forebrain nuclei except for sparse label in the mS. In case AO with an injection in area 9, or in cases with lateral prefrontal injections, there was no evidence of significant, if any, anterograde label within the basal forebrain (Table 3).

### *Relationship of injection size with retrograde and anterograde label*

There was no significant correlation between the size of the reconstructed injection site and the total number of labeled neurons in the basal forebrain (Spearman rank order correlation, $r_s = 0.073$; $P > 0.05$) or between the injection size and the intensity of anterograde label ($r_s = -0.38$; $P > 0.05$). These results suggest that the prevalence of ascending and descending connections between the basal forebrain and prefrontal cortices depends on the location of the injection but not its size.

## DISCUSSION

### *Basal forebrain innervation of the cortex*

The results suggest considerable overlap in the origin of projections to orbitofrontal, medial and lateral prefrontal cortices, confirming and extending previous findings in rats[44,53,54,68,71,120] and primates.[79,84,93,106,115] Nevertheless, there were some notable biases in these connections as well. For example, the nBMp targeted mostly lateral prefrontal cortices, whereas the nVL projected preferentially to medial prefrontal, and the nHL to orbitofrontal cortices.

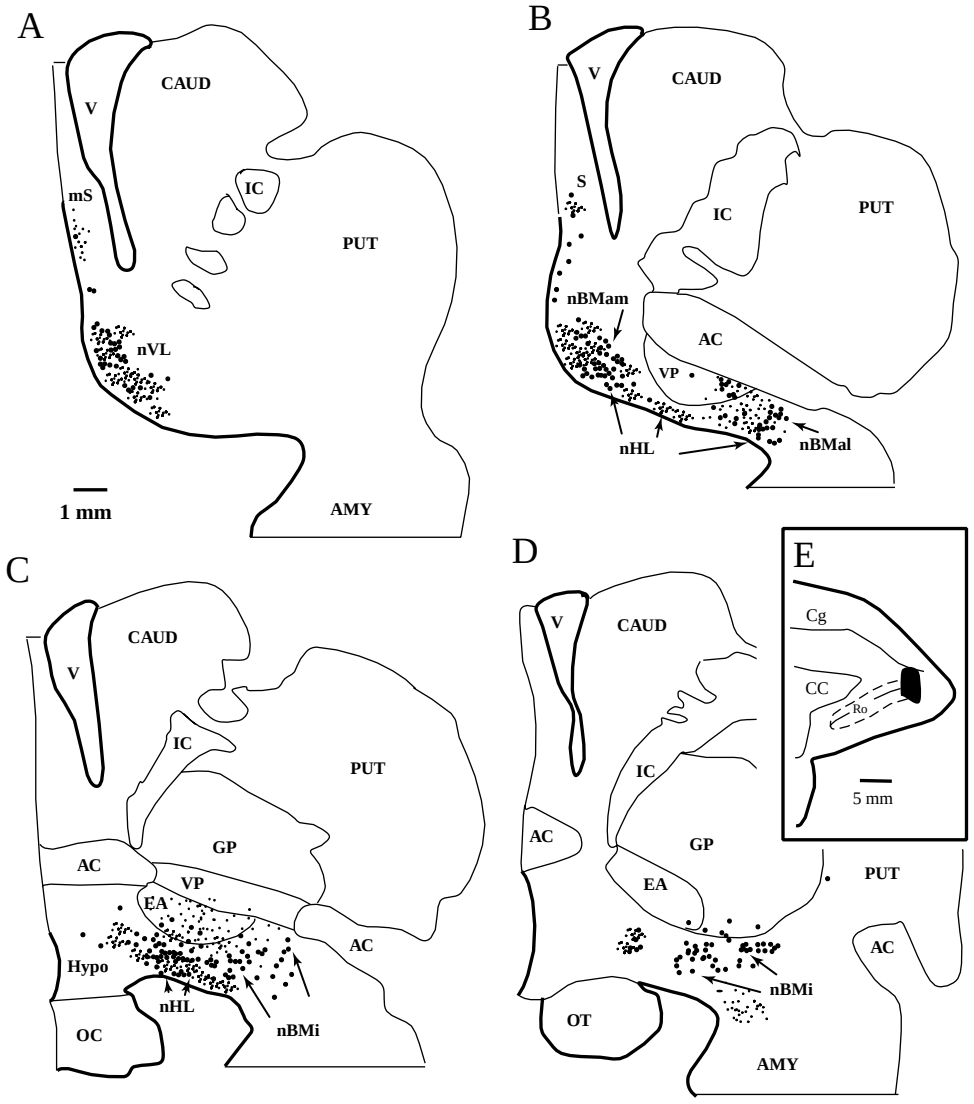


Fig. 10. Bidirectional connections between the basal forebrain and medial area 32. Case AE: Distribution of labeled neurons (large dots) and anterograde label (small dots) in a series of coronal sections in rostral to caudal (A–D) basal forebrain nuclei, including the territory of VP and EA, after injection of HRP–WGA in medial area 32 (E, black area).

The basal forebrain is made up of cholinergic as well as non-cholinergic neurons whose proportion varies within subsectors of the basal forebrain.[79,107,132] There is no general agreement on the proportion of cholinergic to non-cholinergic neurons that project to the cortex[52,80,107] (for review see Ref. 132), and our data did not specifically address this question. However, the majority of the projection neurons in this study had the morphology of the magnocellular cholinergic neurons of the nucleus basalis of Meynert, while a few were small, suggesting that cholinergic as well as non-cholinergic neurons project to prefrontal cortices.

Previous findings indicated that the termination of basal forebrain projections in the cortex differs regionally in density and laminar distribution.[23,66,77,122] Using the cholinergic degradative enzyme AChE, we noted that positive fibers were more densely distributed in medial and orbitofrontal limbic cortices than in lateral prefrontal cortices. These results are consistent with previous findings indicating that the density of ChAT-positive fibers is higher in agranular and dysgranular (limbic) cortices than in granular (eulaminate) areas,[122] a pattern also seen using AChE in different cortical areas.[20,84] In our own material the most dense concentration of AChE-positive fibers in medial and orbitofrontal cortices was noted in layer 1 and the superficial part of layer 5, with a gradual decrease in density in layer 6. In contrast, in lateral prefrontal areas, layer 1 had AChE-positive fibers, but layer 6 included only a sparse distribution of fibers. These findings suggest that projections from the basal forebrain may influence to a greater extent neurons in different layers in medial and orbitofrontal than in lateral prefrontal cortices.

### *Common origin of basal forebrain projections to interconnected neural structures*

The posterior part of the nucleus basalis targeted

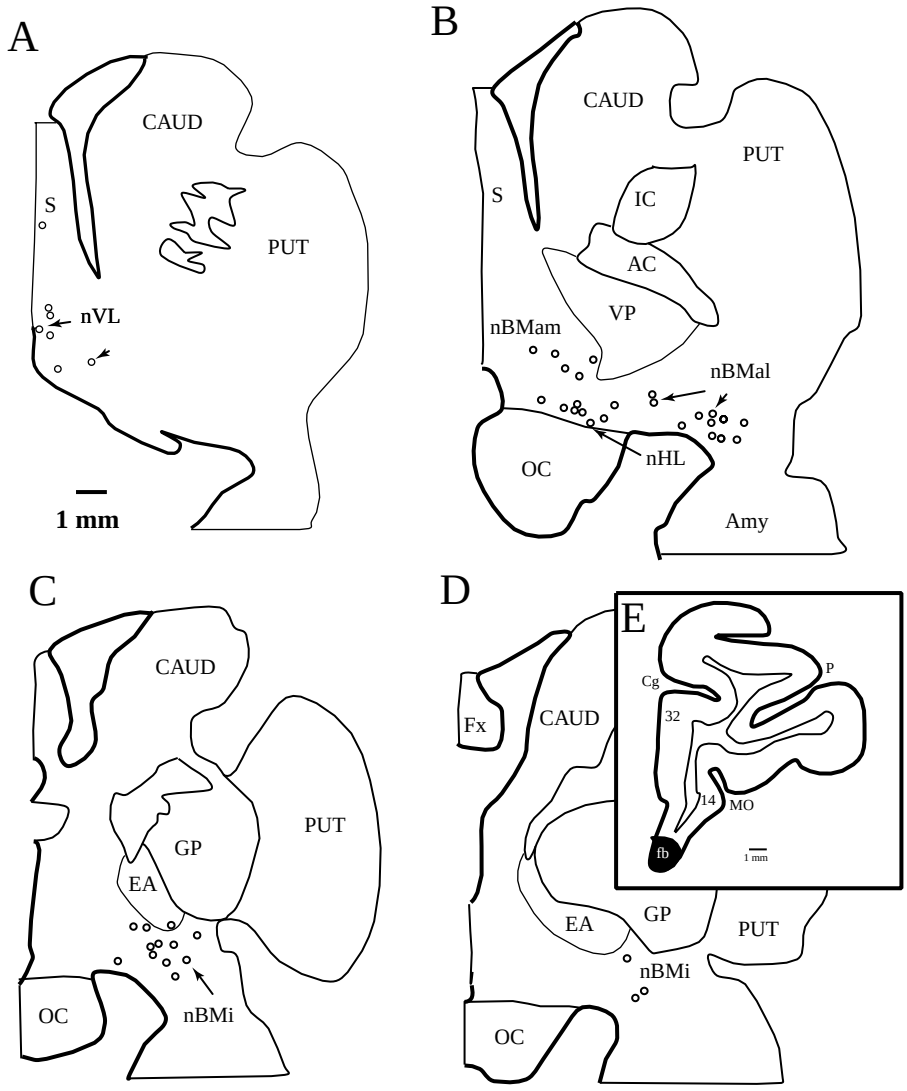


Fig. 11. Ascending projections from the basal forebrain to prefrontal area 14. Case AKb: Distribution of labeled neurons (open circles) in a series of coronal sections in rostral to caudal (A–D) basal forebrain nuclei after injection of the fluorescent dye Fast Blue in prefrontal area 14 (E, black area).

preferentially lateral prefrontal cortices, a pattern noted for auditory association and temporal polar areas as well.[79] In this context, it may be significant that the lateral prefrontal areas 8, 46, 12 and 10 studied here have robust connections with visual and auditory association cortices (for reviews see Refs 7, 10 and 92). This evidence suggests that through a set of common connections, the nBMp may preferentially activate prefrontal, auditory and visual cortices, which are linked through corticocortical connections. Such activation may facilitate the recruitment of signals necessary for cognitive processes that rely on lateral prefrontal cortices (for reviews see Refs 39, 49 and 94).

Similarly, the preferential connection of the diagonal band nuclei mS/nVL with medial prefrontal areas is consistent with other common connections of these structures. For example, medial prefrontal cortices are connected preferentially with the hippocampal formation, parahippocampal, rhinal and perirhinal areas, and midline thalamic nuclei (for reviews see Refs 11 and 14), as is the case with the mS/nVL complex.[44,54,60,71,79,106] In addition, the nHL projected to orbitofrontal cortices, as shown here, and to the olfactory bulb,[79,105] matching an equally robust projection from olfactory areas to the posterior orbitofrontal cortices.[8,25,85,96] Finally, there is evidence that the projections from the basal forebrain to the amygdala originate predominantly from the lateral part of the nBMa (nBMal),[79] which also projects to medial and orbitofrontal cortices. Like the medial and orbitofrontal cortices, among the nuclei of the amygdala the basolateral has a pronounced cholinergic innervation,[3] and issues robust projections to orbitofrontal and medial prefrontal cortices.[1,4,16,27,85,95]

Thus, although the projections of the basal forebrain to different prefrontal regions overlap, a set of basal forebrain nuclei issues projections to interconnected cortices forming a more elaborate but unique network. This evidence suggests that within a seemingly diffuse system,

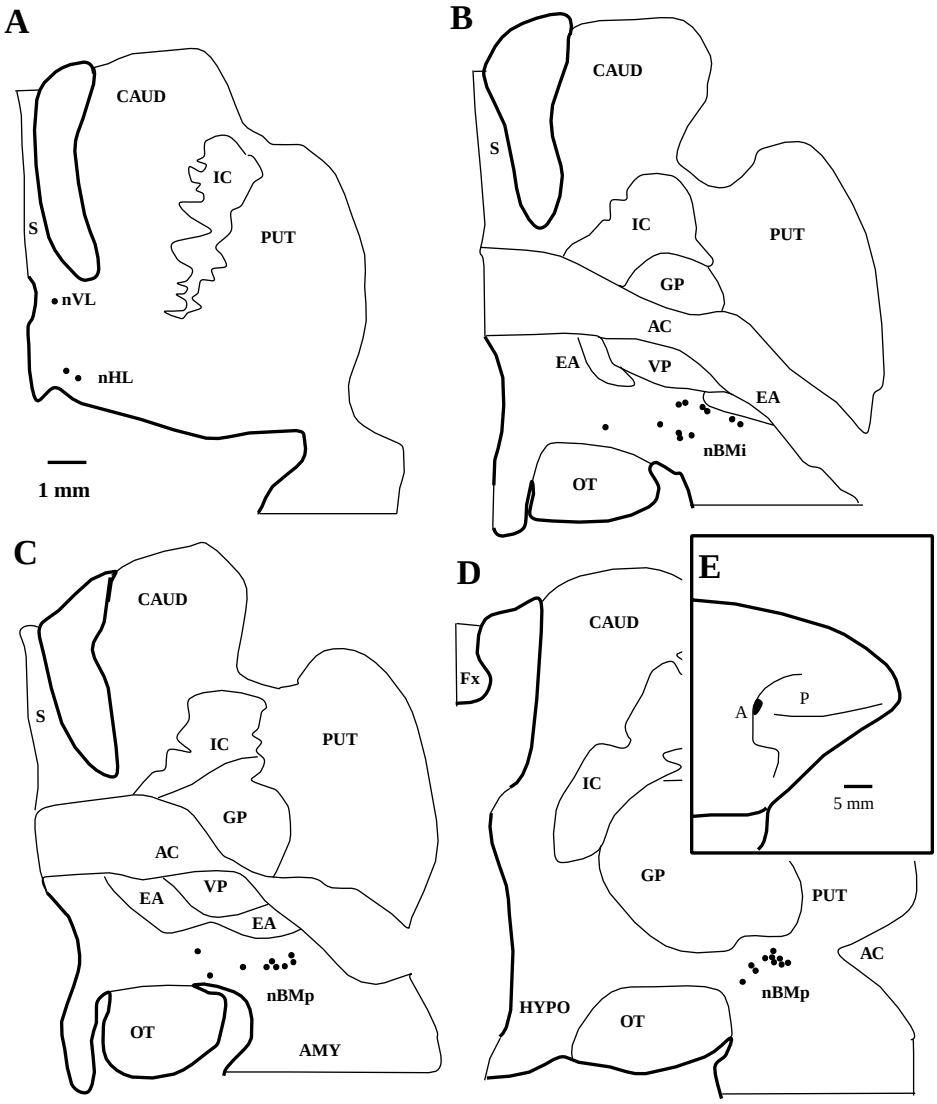


Fig. 12. Ascending projections from the basal forebrain to lateral area 8. Case AD: Distribution of labeled neurons (large dots) in a series of coronal sections in rostral to caudal (A–D) basal forebrain nuclei after injection of HRP–WGA in lateral area 8 (E, black area).

a certain degree of specificity may be afforded by concomitant activation by the basal forebrain of interconnected neural structures. This pattern of innervation suggests that the arousal and attentional functions of the basal forebrain[102,110,123] may be effected through activation of circuits that recruit functionally distinct prefrontal cortices and the areas with which they are connected.[54,93,132]

*Selective output from medial and orbitofrontal cortices to the basal forebrain, ventral pallidum and the extended amygdala*

Critical to the analysis of prefrontal descending projections was deciphering different systems within the basal forebrain in tissue treated for the degradative enzyme (AChE) or the biosynthetic enzyme (ChAT) as markers for cholinergic neurons. In addition, the striatal-related phosphoprotein DARPP-32 and the related phosphoprotein Inhibitor-1 aided demarcation of another basal forebrain system, the ventral pallidum, where fibers were positive for DARPP-32.[91] This marker also delineated the system of the extended amygdala, highlighted by punctuated groups of neurons through the basal forebrain that were positive for DARPP-32 and Inhibitor-1. Using these neurochemical stains we noted that, in addition to the general topography of projections, there was another distinction in the neural association of different regions of the prefrontal cortex with the basal forebrain. Medial and orbitofrontal cortices issued descending projections to the basal forebrain that terminated rostrally within the nBMa, nHL and parts of nVL. These results confirm previous findings in monkeys[78] and rats.[45] Further, our results extend previous findings by showing that in addition to cortical terminations in the nucleus basalis, projections from medial areas 32 and 25 terminated in the diagonal band nuclei as well. Moreover, we noted descending projections from some

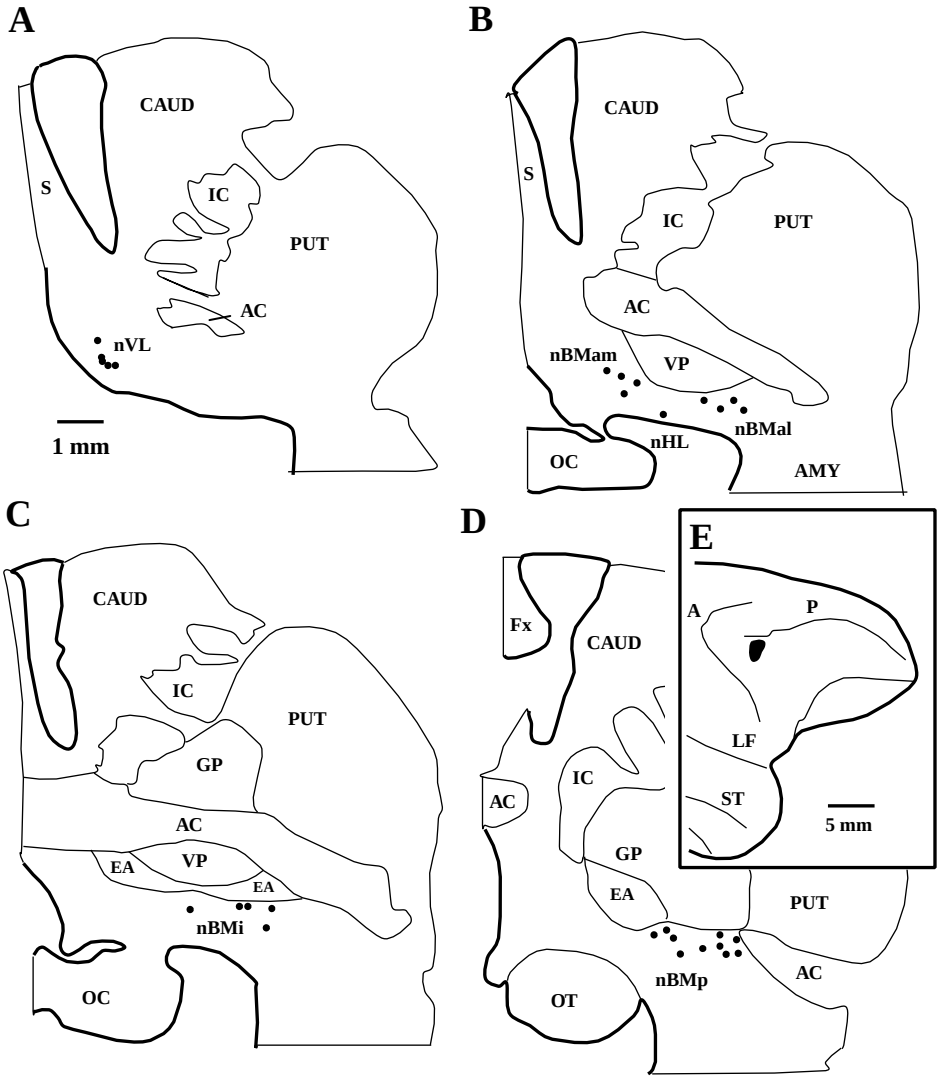


Fig. 13. Ascending projections from the basal forebrain to lateral area V46. Case MBH: Distribution of labeled neurons (large dots) in a series of coronal sections in rostral to caudal (A–D) basal forebrain nuclei after injection of HRP–WGA in lateral area V46 (E, black area).

orbitofrontal, and particularly from medial area 32, to the extended amygdala and the ventral pallidum, as has been noted in several other species.[28,63,69,104,128] In marked contrast, lateral prefrontal cortices do not appear to issue descending projections to the basal forebrain.

*Functional implications*

The present findings indicated a general but consistent topography, whereby granular types of cortices on the lateral surface of the frontal lobe appear to receive their basal forebrain input from posterior parts, while the agranular and dysgranular limbic cortices on the medial and orbitofrontal surfaces are interconnected with its more rostral sectors. In the case of the lateral prefrontal cortices this linkage was unidirectional, since they did not issue feedback projections to the basal forebrain. This evidence suggests that the projections from the basal forebrain to lateral prefrontal cortices constitute an open-loop system, which may be related to "on-line" processing in cognitive tasks. Activation of a network consisting of lateral prefrontal cortices and their interconnected visual, auditory and other sensory cortices by the basal forebrain may be associated with recruitment and retrieval of perceptual information in working memory functions.[39,49,75]

In the case of orbitofrontal and medial prefrontal cortices, the connections with the basal forebrain were bidirectional, although ultrastructural analysis indicated that prefrontal axons synapse with interneurons of the basal forebrain, but provided no evidence of synapses with cholinergic neurons, at least in rats.[131] However, orbitofrontal and medial prefrontal cortices and the basal forebrain have common connections with several diencephalic structures and the amygdala,[30,55,60,79,89,101,106,119,130] suggesting that they are part of a more elaborate network. From a functional perspective the basal forebrain has been associated with processing novelty and reinforcement of sensory stimuli,[125,126] functions that have been attributed to orbitofrontal

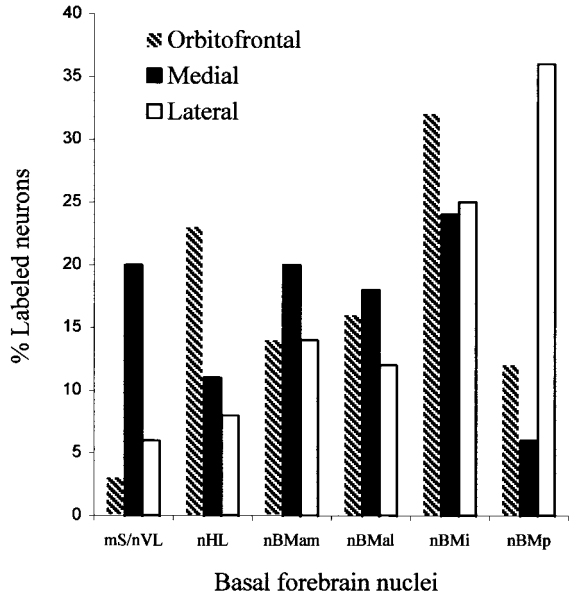


Fig. 14. Histogram showing the proportion of projection neurons in each of the basal forebrain nuclei directed to areas within orbitofrontal, medial and lateral prefrontal regions ($n = 19$). The six bars designating projections to each prefrontal region add up to 100%.

cortices and the amygdala as well. Specifically, neurons in the orbitofrontal cortex and the amygdala fire selectively in response to cues based on their associative significance,[67,103,112–114,121] and their activity is correlated and adjusted as the salience of cues in a behavioral task changes.[41,112,114] As recipient of input from all sensory modalities and the amygdala,[4,8,16,26,27,85,95] the orbitofrontal cortex may be capable of encoding cross-modal associations in reward-associated tasks involving the gustatory, olfactory and somatosensory modalities.[67] Like the orbitofrontal cortex, the amygdala has multimodal connections (for review see Ref. 10), and is implicated in cross-modal associations.[40] Information about reward associations may be transmitted to the basal forebrain by the descending projections from medial and orbitofrontal cortices as well as temporal cortices and the amygdala.[78] Feedback projections may be necessary to focus attention on the task at hand and adjust performance.[35]

In addition to strong interconnections with the amygdala, the caudal orbitofrontal and medial limbic prefrontal cortices and the basal forebrain have common connections with the hippocampal formation, the rhinal and entorhinal cortices, and the mediodorsal nucleus of the thalamus[19,33,44,50,54,56,60,71,79,88,116] associated with long-term memory (for reviews see Refs 2, 11, 14, 31, 70 and 133). The bidirectional connections between the limbic prefrontal areas and the basal forebrain may influence this extended network in the process of monitoring the motivational significance of associated events and their encoding into long-term memory.

Finally, our data provided evidence that descending projections from restricted medial and orbitofrontal areas terminated within the territory of the extended amygdala and the ventral pallidum, a venue to the output of the amygdala through hypothalamic and brainstem autonomic structures (for reviews see Refs 2, 58, 59 and 98). This pathway is positioned in parallel to a direct projection from medial prefrontal and orbitofrontal cortices to hypothalamic and brainstem autonomic centers,[65,87,89,99,108,127] and represents yet another set of common connections for these structures. Recent evidence indicates that direct cortical projections from orbitofrontal and medial prefrontal cortices are positioned in series with hypothalamic projections to brainstem[89,99] as well as spinal autonomic centers,[101] a final common pathway that innervates peripheral autonomic

Table 3. Distribution of anterograde label in the basal forebrain originating from prefrontal cortices

| | | Basal forebrain termination zone | | | | | |
|---|---|---|---|---|---|---|---|
| Area injected | Case | mS/nVL | nHL | nBMam | nBMal | nBMi | nBMp |
| Orbitofrontal | | | | | | | |
| OPro/OPAll | AG | – | + | – | + | – | – |
| OPro | AF* | + | +++ | +++ | ++ | + | – |
| OPro | MAR* | – | – | + | ++ | – | + |
| O12 | MBY* | – | ++ | + | + | + | +++ |
| 11 | MFT | – | + | + | ++ | + | + |
| Medial | | | | | | | |
| 25 | AH | + | + | – | – | – | – |
| 32 | AE* | +++ | +++ | +++ | ++ | - | ++ |
| 32 | MDQ | + | – | – | – | – | – |
| M9 | AO | – | – | – | – | – | – |
| Lateral | | | | | | | |
| 8 | AC | + | – | – | – | – | – |
| D46 | AB | + | + | + | – | – | – |
| V46 | MBH | – | – | – | – | – | – |
| V46 | MFF | – | – | – | – | – | – |
| V46 | AA | – | + | – | – | + | – |
| D10/R46 | SF | – | – | – | – | – | – |

+, light anterograde label; ++, moderate anterograde label; +++, dense anterograde label; –, no evidence of anterograde label.
*Cases with possible anterograde label in the ventral pallidum or extended amygdala.

organs. This evidence provides the structural basis of parallel pathways through which high-order association orbitofrontal and medial prefrontal cortices may influence the emotional motor system.[2,59]

In summary, the presented evidence suggests that in contrast with lateral prefrontal cortices, caudal orbitofrontal and medial prefrontal cortices, which make up the limbic component of the prefrontal cortex, have a bidirectional association with the basal forebrain. Moreover, the prefrontal limbic cortices appear to be part of a more extensive network that includes a cluster of diencephalic, temporal structures and the basal forebrain, all of which show particular vulnerability in neurodegenerative and psychiatric diseases.[13,57,58,97,109]

*Acknowledgements*—We thank Drs Angus Nairn, Hugh Hemmings and Paul Greengard for providing the Inhibitor-1 and DARPP-32 antibodies. This work was supported by grants from NIH (NINDS and NIMH).

## REFERENCES


1. Aggleton J. P., Burton M. J. and Passingham R. E. (1980) Cortical and subcortical afferents to the amygdala of the rhesus monkey (*Macaca mulatta*). *Brain Res.* **190,** 347–368.
2. Alheid G. F. and Heimer L. (1996) Theories of basal forebrain organization and the "emotional motor system". *Prog. Brain Res.* **107,** 461–484.
3. Amaral D. G. and Bassett J. L. (1989) Cholinergic innervation of the monkey amygdala: An immunohistochemical analysis with antisera to choline acetyltransferase. *J. comp. Neurol.* **281,** 337–361.
4. Amaral D. G. and Price J. L. (1984) Amygdalo-cortical projections in the monkey (*Macaca fascicularis*). *J. comp. Neurol.* **230,** 465–496.
5. Aston-Jones G., Rajkowski J., Kubiak P., Valentino R. J. and Shipley M. T. (1996) Role of the locus coeruleus in emotional activation. *Prog. Brain Res.* **107,** 379–402.
6. Barbas H. (1988) Anatomic organization of basoventral and mediodorsal visual recipient prefrontal regions in the rhesus monkey. *J. comp. Neurol.* **276,** 313–342.
7. Barbas H. (1992) Architecture and cortical connections of the prefrontal cortex in the rhesus monkey. In *Advances in Neurology* (eds Chauvel P., Delgado-Escueta A. V., Halgren E. and Bancaud J.), Vol. 57. Raven, New York.
8. Barbas H. (1993) Organization of cortical afferent input to orbitofrontal areas in the rhesus monkey. *Neuroscience* **56,** 841–864.
9. Barbas H. (1995) Pattern in the cortical distribution of prefrontally directed neurons with divergent axons in the rhesus monkey. *Cereb. Cortex* **5,** 158–165.
10. Barbas H. (1995) Anatomic basis of cognitive–emotional interactions in the primate prefrontal cortex. *Neurosci. Behav. Rev.* **19,** 499–510.
11. Barbas H. (1997) Two prefrontal limbic systems: their common and unique features. In *The Association Cortex: Structure and Function* (eds Sakata H., Mikami A. and Fuster J. M.). Harwood Academic Publications, Amsterdam.
12. Barbas H. (2000) Complementary role of prefrontal cortical regions in cognition, memory and emotion in primates. *Adv. Neurol.* **84,** 87–110.
13. Barbas H. (2000) Neuroanatomic basis for reorganization of function after prefrontal damage in primates. In *Cerebral Reorganization of Function after Brain Damage* (eds Levin H. S. and Grafman J.). Oxford University Press, New York.
14. Barbas H. (2000) Connections underlying the synthesis of cognition, memory, and emotion in primate prefrontal cortices. *Brain Res. Bull.* **52,** 319–330.
15. Barbas H. and Blatt G. J. (1995) Topographically specific hippocampal projections target functionally distinct prefrontal areas in the rhesus monkey. *Hippocampus* **5,** 511–533.
16. Barbas H. and De Olmos J. (1990) Projections from the amygdala to basoventral and mediodorsal prefrontal regions in the rhesus monkey. *J. comp. Neurol.* **301,** 1–23.
17. Barbas H., Ghashghaei H., Dombrowski S. M. and Rempel-Clower N. L. (1999) Medial prefrontal cortices are unified by common connections with superior temporal cortices and distinguished by input from memory-related areas in the rhesus monkey. *J. comp. Neurol.* **410,** 343–367.
18. Barbas H., Gustafson E. L. and Greengard P. (1993) Comparison of the immunocytochemical localization of DARPP-32 and I-1 in the amygdala and hippocampus of the rhesus monkey. *J. comp. Neurol.* **334,** 1–18.
19. Barbas H., Henion T. H. and Dermon C. R. (1991) Diverse thalamic projections to the prefrontal cortex in the rhesus monkey. *J. comp. Neurol.* **313,** 65–94.
20. Barbas H. and Pandya D. N. (1983) Distribution of acetylcholinesterase in the medial and ventral prefrontal cortex of the rhesus monkey. *Neurosci. Abstr.* **9,** 877.

20a. Barbas H. and Pandya D. N. (1989) Architecture and intrinsic connections of the prefrontal cortex in the rhesus monkey. *J. comp. Neurol.* **286,** 353–375.

21. Baxter M. G. and Chiba A. A. (1999) Cognitive functions of the basal forebrain. *Curr. Opin. Neurobiol.* **9,** 178–183.
22. Cahill L. (1999) A neurobiological perspective on emotionally influenced, long-term memory. *Semin. Clin. Neuropsychiat.* **4,** 266–273.
23. Campbell M. J., Lewis D. A., Foote S. L. and Morrison J. H. (1987) Distribution of choline acetyltransferase-, serotonin-, dopamine-B-hydroxylase-, tyrosine hydroxylase-immunoreactive fibers in monkey primary auditory cortex. *J. comp. Neurol.* **261,** 209–220.
24. Carlsen J., Zaborszky L. and Heimer L. (1985) Cholinergic projections from the basal forebrain to the basolateral amygdaloid complex: a combined retrograde fluorescent and immunohistochemical study. *J. comp. Neurol.* **234,** 155–167.
25. Carmichael S. T., Clugnet M.-C. and Price J. L. (1994) Central olfactory connections in the macaque monkey. *J. comp. Neurol.* **346,** 403–434.
26. Carmichael S. T. and Price J. L. (1995) Sensory and premotor connections of the orbital and medial prefrontal cortex of macaque monkeys. *J. comp. Neurol.* **363,** 642–664.
27. Carmichael S. T. and Price J. L. (1995) Limbic connections of the orbital and medial prefrontal cortex in macaque monkeys. *J. comp. Neurol.* **363,** 615–641.
28. Cassell M. D. and Wright D. J. (1986) Topography of projections from the medial prefrontal cortex to the amygdala in the rat. *Brain Res. Bull.* **17,** 321–333.
29. Cowan W. M., Gottlieb D. I., Hendrickson A. E., Price J. L. and Woolsey T. A. (1972) The autoradiographic demonstration of axonal connections in the central nervous system. *Brain Res.* **37,** 21–51.
30. Cullinan W. E. and Zaborszky L. (1991) Organization of ascending hypothalamic projections to the rostral forebrain with special reference to the innervation of cholinergic projection neurons. *J comp. Neurol* **306,** 631–667.
31. De Olmos J. S. (1990) Amygdala. In *The Human Nervous System* (ed. Paxinos G.). Academic, San Diego.
32. De Olmos J. S. and Heimer L. (1999) The concepts of the ventral striatopallidal system and extended amygdala. *Ann. N.Y. Acad. Sci.* **877,** 1–32.
33. Dermon C. R. and Barbas H. (1994) Contralateral thalamic projections predominantly reach transitional cortices in the rhesus monkey. *J. comp. Neurol.* **344,** 508–531.

34. Eblen F. and Graybiel A. M. (1995) Highly restricted origin of prefrontal cortical inputs to striosomes in the macaque monkey. *J. Neurosci.* **15,** 5999–6013.
35. Elliott R., Frith C. D. and Dolan R. J. (1997) Differential neural response to positive and negative feedback in planning and guessing tasks. *Neuropsychologia* **35,** 1395–1404.
36. Foote S. L. and Morrison J. H. (1987) Extrathalamic modulation of cortical function. *A. Rev. Neurosci.* **10,** 67–95.
37. Frith C. and Dolan R. (1996) The role of the prefrontal cortex in higher cognitive functions. *Cog. Brain Res.* **5,** 175–181.
38. Funahashi S. and Kubota K. (1994) Working memory and prefrontal cortex. *Neurosci. Res.* **21,** 1–11.
39. Fuster J. M. (1989) *The Prefrontal Cortex*, 2nd edn. Raven, New York.
40. Gaffan D. and Murray E. A. (1990) Amygdalar interaction with the mediodorsal nucleus of the thalamus and the ventromedial prefrontal cortex in stimulus reward associative learning in the monkey. *J. Neurosci.* **10,** 3479–3493.
41. Gallagher M., McMahan R. W. and Schoenbaum G. (1999) Orbitofrontal cortex and representation of incentive value in associative learning. *J. Neurosci.* **19,** 6610–6614.
42. Gallagher M. and Schoenbaum G. (1999) Functions of the amygdala and related forebrain areas in attention and cognition. *Ann. N.Y. Acad. Sci.* **877,** 397–411.
43. Gallyas F. (1979) Silver staining of myelin by means of physical development. *Neurol. Res.* **1,** 203–209.
44. Gaykema R. P., Luiten P. G., Nyakas C. and Traber J. (1990) Cortical projection patterns of the medial septum-diagonal band complex. *J. comp. Neurol.* **293,** 103–124.
45. Gaykema R. P., Vanweeghel R., Hersh L. B. and Luiten P. G. M. (1991) Prefrontal cortical projections to the cholinergic neurons in the basal forebrain. *J. comp. Neurol.* **303,** 563–583.
46. Geneser-Jensen F. A. and Blackstad T. W. (1971) Distribution of acetyl cholinesterase in the hippocampal region of the guinea pig. *Z. Zellforsch. mikrosk. Anat.* **114,** 460–481.
47. Ghashghaei H. and Barbas H. (1997) Topographic organization of projections from the basal forebrain to functionally distinct prefrontal cortices in the rhesus monkey. *Soc. Neurosci. Abstr.* **23,** 901.
48. Goldman P. S. and Nauta W. J. (1977) An intricately patterned prefronto-caudate projection in the rhesus monkey. *J. comp. Neurol.* **72,** 369–386.
49. Goldman-Rakic P. S. (1988) Topography of cognition: parallel distributed networks in primate association cortex. *A. Rev. Neurosci.* **11,** 137–156.
50. Goldman-Rakic P. S. and Porrino L. J. (1985) The primate mediodorsal (MD) nucleus and its projection to the frontal lobe. *J. comp. Neurol.* **242,** 535–560.
51. Gray T. S. (1999) Functional and anatomical relationships among the amygdala, basal forebrain, ventral striatum, and cortex. An integrative discussion. *Ann. N.Y. Acad. Sci.* **877,** 439–444.
52. Gritti I., Mainville L., Mancia M. and Jones B. E. (1997) GABAergic and other noncholinergic basal forebrain neurons, together with cholinergic neurons, project to the mesocortex and isocortex in the rat. *J. comp. Neurol.* **383,** 163–177.
53. Grove E. A. (1988) Neural associations of the substantia innominata in the rat: afferent connections. *J. comp. Neurol.* **277,** 315–346.
54. Grove E. A. (1988) Efferent connections of the substantia innominata in the rat. *J. comp. Neurol.* **277,** 347–364.
55. Haber S. N., Lynd-Balta E. and Mitchell S. J. (1993) The organization of the descending ventral pallidal projections in the monkey. *J. comp. Neurol.* **329,** 111–128.
56. Heckers S., Ohtake T., Wiley R. G., Lappi D. A., Geula C. and Mesulam M.-M. (1994) Complete and selective cholinergic denervation of rat neocortex and hippocampus but not amygdala by an immunotoxin against the p75 NGF receptor. *J. Neurosci.* **14,** 1271–1289.
57. Heimer L. (2000) Basal forebrain in the context of schizophrenia. *Brain Res. Brain Res. Rev.* **31,** 205–235.
58. Heimer L., Harlan R. E., Alheid G. F., Garcia M. M. and De Olmos J. (1997) Substantia innominata: a notion which impedes clinical-anatomical correlations in neuropsychiatric disorders. *Neuroscience* **76,** 957–1006.
59. Holstege G. (1991) Descending motor pathways and the spinal motor system: limbic and non-limbic components. *Prog. Brain Res.* **87,** 307–421.
60. Hreib K. K., Rosene D. L. and Moss M. B. (1988) Basal forebrain efferents to the medial dorsal thalamic nucleus in the rhesus monkey. *J. comp. Neurol.* **277,** 365–390.
61. Irle E. and Markowitsch H. J. (1986) Afferent connections of the substantia innominata/basal nucleus of Meynert in carnivores and primates. *J. Hirnforsch.* **27,** 343–367.
62. Jones E. G., Burton H., Saper C. B. and Swanson L. W. (1976) Midbrain, diencephalic and cortical relationships of the basal nucleus in primates. *J. comp. Neurol.* **167,** 385–420.
63. Kapp B. S., Schwaber J. S. and Driscoll P. A. (1985) Frontal cortex projections to the amygdaloid central nucleus in the rabbit. *Neuroscience* **15,** 327–346.
64. Kievit J. and Kuypers H. G. J. M. (1975) Basal forebrain and hypothalamic connections to frontal and parietal cortex in the rhesus monkey. *Science* **187,** 660–662.
65. Leichnetz G. R. and Astruc J. (1976) The efferent projections of the medial prefrontal cortex in the squirrel monkey (*Saimiri sciureus*). *Brain Res.* **109,** 455–472.
66. Lewis D. A. (1991) Distribution of choline acetyltransferase-immunoreactive axons in monkey frontal cortex. *Neuroscience* **40,** 363–374.
67. Lipton P. A., Alvarez P. and Eichenbaum H. (1999) Crossmodal associative memory representations in rodent orbitofrontal cortex. *Neuron* **2,** 349–359.
68. Luiten P. G., Spencer D. G. Jr, Traber J. and Gaykema R. P. (1985) The pattern of cortical projections from the intermediate parts of the magnocellular nucleus basalis in the rat demonstrated by tracing with *Phaseolus vulgaris*-leucoagglutinin. *Neurosci. Lett.* **57,** 137–142.
69. McDonald A. J., Shammah-Lagnado S. J., Shi C. and Davis M. (1999) Cortical afferents to the extended amygdala. *Ann. N.Y. Acad. Sci.* **877,** 309–338.
70. McGaugh J. L., Cahill L. and Roozendaal B. (1996) Involvement of the amygdala in memory storage: interaction with other brain systems. *Proc. natn. Acad. Sci. USA* **93,** 13,508–13,514.
71. McKinney M., Coyle J. T. and Hedreen J. C. (1983) Topographic analysis of the innervation of the rat neocortex and hippocampus by the basal forebrain cholinergic system. *J. comp. Neurol.* **217,** 103–121.
72. Mesulam M.-M. and Geula C. (1988) Nucleus basalis (Ch4) and cortical cholinergic innervation in the human brain: observations based on the distribution of acetylcholinesterase and choline acetylcholinesterase. *J. comp. Neurol.* **275,** 216–240.
73. Mesulam M.-M., Hegarty E., Barbas H., Carson K. A., Gower E. C., Knapp A. G., Moss M. B. and Mufson E. J. (1980) Additional factors influencing sensitivity in the tetramethyl benzidine method for horseradish peroxidase neurohistochemistry. *J. Histochem. Cytochem.* **28,** 1255–1259.
74. Mesulam M.-M. (1995) The cholinergic contribution to neuromodulation in the cerebral cortex. *Semin. Neurosci.* **7,** 297–307.
75. Mesulam M. M. (1998) From sensation to cognition. *Brain* **121,** 1013–1052.

76. Mesulam M. M., Geula C., Cosgrove R., Mash D. and Brimijoin S. (1991) Immunocytochemical demonstration of axonal and perikaryal acetylcholinesterase in human cerebral cortex. *Brain Res.* **539,** 233–238.
77. Mesulam M. M., Hersh L. B., Mash D. C. and Geula C. (1992) Differential cholinergic innervation within functional subdivisions of the human cerebral cortex: a choline acetyltransferase study. *J. comp. Neurol.* **318,** 316–328.
78. Mesulam M. M. and Mufson E. J. (1984) Neural inputs into the nucleus basalis of the substantia innominata (Ch4) in the rhesus monkey. *Brain* **107,** 253–274.
79. Mesulam M. M., Mufson E. J., Levey A. I. and Wainer B. H. (1983) Cholinergic innervation of cortex by the basal forebrain: cytochemistry and cortical connections of the septal area, diagonal band nuclei, nuclei, nucleus basalis (substantia innominata), and hypothalamus in the rhesus monkey. *J. comp. Neurol.* **214,** 170–197.
80. Mesulam M. M., Mufson E. J. and Wainer B. H. (1986) Three-dimensional representation and cortical projection topography of the nucleus basalis (Ch4) in the macaque: concurrent demonstration of choline acetyltransferase and retrograde transport with a stabilized tetramethylbenzidine method for horsera. *Brain Res.* **367,** 301–308.
81. Mesulam M. M., Mufson E. J., Wainer B. H. and Levey I. A. (1983) Central cholingeric pathways in the rat: an overview based on an alternative nomenclature (Ch1–Ch6). *Neuroscience* **10,** 1185–1201.
82. Mesulam M. M., Rosen A. D. and Mufson E. J. (1984) Regional variations in cortical cholinergic innervation: chemoarchitectonics of acetylcholinesterase-containing fibers in the macaque brain. *Brain Res.* **311,** 245–258.
83. Mesulam M. M. and Van Hoesen G. W. (1976) Acetylcholinesterase-rich projections from the basal forebrain of the rhesus monkey to neocortex. *Brain Res.* **109,** 152–157.
84. Mesulam M. M., Volicer L., Marquis J. K., Mufson E. J. and Green R. C. (1986) Systematic regional differences in cholinergic innervation of the primate cerebral cortex: distribution of enzyme activities and some behavioral implications. *Ann. Neurol.* **281,** 611–633.
85. Morecraft R. J., Geula C. and Mesulam M.-M. (1992) Cytoarchitecture and neural afferents of orbitofrontal cortex in the brain of the monkey. *J. comp. Neurol.* **323,** 341–358.
86. Nairn A. C., Hemmings H. C. Jr, Walaas S. I. and Greengard P. (1988) DARPP-32 and phosphatase inhibitor-1, two structurally related inhibitors of protein phosphatase-1, are both present in striatonigral neurons. *J. Neurochem.* **50,** 257–262.
87. Neafsey E. J. (1990) Prefrontal cortical control of the autonomic nervous system: anatomical and physiological observations. *Prog. Brain Res.* **85,** 147–166.
88. Ohtake T., Heckers S., Wiley R. G., Lappi D. A., Mesulam M. M. and Geula C. (1997) Retrograde degeneration and colchicine protection of basal forebrain cholinergic neurons following hippocampal injections of an immunotoxin against the P75 nerve growth factor receptor. *Neuroscience* **78,** 123–133.
89. Ongur D., An X. and Price J. L. (1998) Prefrontal cortical projections to the hypothalamus in macaque monkeys. *J. comp. Neurol.* **401,** 480–505.
90. Ouimet C. C. and Greengard P. (1990) Distribution of DARPP-32 in the basal ganglia: an electron microscopic study. *J. Neurocytol.* **19,** 39–52.
91. Ouimet C. C., Lamantia A. S., Goldman-Rakic P., Rakic P. and Greengard P. (1992) Immunocytochemical localization of DARPP-32, a dopamine and cyclic-AMP-regulated phosphoprotein, in the primate brain. *J. comp. Neurol.* **323,** 209–218.
92. Pandya D. N., Seltzer B. and Barbas H. (1988) Input-output organization of the primate cerebral cortex. In *Comparative Primate Biology: Neurosciences* (eds Steklis H. D. and Erwin J.), Vol. 4. Alan R. Liss, New York.
93. Pearson R. C., Gatter K. C., Brodal P. and Powell T. P. (1983) The projection of the basal nucleus of Meynert upon the neocortex in the monkey. *Brain Res.* **259,** 132–136.
94. Petrides M. (1989) Frontal lobes and memory. In *Handbook of Neuropsychology* (eds Boller F. and Grafman J.), Vol. 3. Elsevier Science Publishers B.V. (Biomedical Division), New York.
95. Porrino L. J., Crane A. M. and Goldman-Rakic P. S. (1981) Direct and indirect pathways from the amygdala to the frontal lobe in rhesus monkeys. *J. comp. Neurol.* **198,** 121–136.
96. Potter H. and Nauta W. J. H. (1979) A note on the problem of olfactory associations of the orbitofrontal cortex in the monkey. *Neuroscience* **4,** 361–367.
97. Price D. L., Martin L. J., Sisodia S. S., Wagster M. V., Koo E. H., Walker L. C., Koliatsos V. E. and Cork L. C. (1991) Aged non-human primates: an animal model of age-associated neurodegenerative disease. *Brain Path.* **1,** 287–296.
98. Price J. L., Russchen F. T. and Amaral D. G. (1987) The limbic region. II. The amygdaloid complex. In *Handbook of Chemical Neuroanatomy Integrated Systems of the CNS, Part I* (eds Björklund A., Hökfelt T. and Swanson L. W.), Vol. 5. Elsevier, Amsterdam.
99. Rempel-Clower N. and Barbas H. (1998) Topographic organization of connections between the hypothalamus and prefrontal cortex in the rhesus monkey. *J. comp. Neurol.* **398,** 393–419.
100. Rempel-Clower N. L. and Barbas H. (2000) The laminar pattern of connections between prefrontal and anterior temporal cortices in the rhesus monkey is related to cortical structure and function. *Cereb. Cortex* **10,** 851–865.
101. Rempel-Clower N. L., Ghashghaei H. and Barbas H. (1999) Serial pathways from orbital and medial prefrontal cortices reach hypothalamic, brainstem, and spinal autonomic centers in the rhesus monkey. *Soc. Neurosci. Abstr.* **25,** 362.
102. Robbins T. W., Granon S., Muir J. L., Durantou F., Harrison A. and Everitt B. J. (1998) Neural systems underlying arousal and attention. Implications for drug abuse. *Ann. N. Y. Acad. Sci.* **846,** 222–237.
103. Rolls E. T., Critchley H. D., Browning A. S., Hernadi I. and Lenard L. (1999) Responses to the sensory properties of fat of neurons in the primate orbitofrontal cortex. *Neurosci. Lett.* **2,** 117–120.
104. Room P., Russchen F. T., Groenewegen H. J. and Lohman A. H. (1985) Efferent connections of the prelimbic (area 32) and the infralimbic (area 25) cortices: an anterograde tracing study in the cat. *J. comp. Neurol.* **242,** 40–55.
105. Rosene D. L., Heimer L. and Van Hoesen G. W. (1978) Centrifugal efferents to the olfactory bulb in the rhesus monkey. *Soc. Neurosci. Abstr.* **4,** 91.
106. Russchen F. T., Amaral D. G. and Price J. L. (1985) The afferent connections of the substantia innominata in the monkey, *Macaca fascicularis*. *J. comp. Neurol.* **242,** 1–27.
107. Rye D. B., Wainer B. H., Mesulam M. M., Mufson E. J. and Saper C. B. (1984) Cortical projections arising from the basal forebrain: a study of cholinergic and noncholinergic components employing combined retrograde tracing and immunohistochemical localization of choline acetyltransferase. *Neuroscience* **13,** 627–643.
108. Saper C. B., Loewy A. D., Swanson L. W. and Cowan W. M. (1976) Direct hypothalamo-autonomic connections. *Brain Res.* **117,** 305–312.
109. Sarter M. and Bruno J. P. (1999) Abnormal regulation of corticopedal cholinergic neurons and impaired information processing in neuropsychiatric disorders. *Trends Neurosci.* **22,** 67–74.
110. Sarter M. and Bruno J. P. (2000) Cortical cholinergic inputs mediating arousal, attentional processing and dreaming: differential afferent regulation of the basal forebrain by telencephalic and brainstem afferents. *Neuroscience* **95,** 933–952.
111. Satoh K., Armstrong D. M. and Fibiger H. C. (1983) A comparison of the distribution of central cholinergic neurons as demonstrated by acetycholinesterase pharmacohistochemistry and choline acetyltransferase immunohistochemistry. *Brain Res. Bull.* **11,** 693–720.

112. Schoenbaum G., Chiba A. A. and Gallagher M. (1998) Orbitofrontal cortex and basolateral amygdala encode expected outcomes during learning. *Nature Neurosci.* **1,** 155–159.
113. Schoenbaum G., Chiba A. A. and Gallagher M. (1999) Neural encoding in orbitofrontal cortex and basolateral amygdala during olfactory discrimination learning. *J. Neurosci.* **19,** 1876–1884.
114. Schoenbaum G., Chiba A. A. and Gallagher M. (2000) Changes in functional connectivity in orbitofrontal cortex and basolateral amygdala during learning and reversal training. *J. Neurosci.* **20,** 5179–5189.
115. Selden N. R., Gitelman D. R., Salamon-Murayama N., Parrish T. B. and Mesulam M. M. (1998) Trajectories of cholinergic pathways within the cerebral hemispheres of the human brain. *Brain* **121,** 2249–2257.
116. Siwek D. F. and Pandya D. N. (1991) Prefrontal projections to the mediodorsal nucleus of the thalamus in the rhesus monkey. *J. comp. Neurol.* **312,** 509–524.
117. Steriade M. (1995) Neuromodulatory systems of thalamus and neocortex. *Semin. Neurosci.* **7,** 361–370.
118. Steriade M. (1996) Arousal: revisiting the reticular activating system. *Science* **272,** 225–226.
119. Steriade M., Parent A., Pare D. and Smith Y. (1987) Cholinergic and non-cholinergic neurons of cat basal forebrain project to reticular and mediodorsal thalamic nuclei. *Brain Res.* **408,** 372–376.
120. Stewart D. J., MacFabe D. F. and Leung L. W. S. (1985) Topographical projection of cholinergic neurons in the basal forebrain to the cingulate cortex in the rat. *Brain Res.* **358,** 404–407.
121. Tremblay L. and Schultz W. (1999) Relative reward preference in primate orbitofrontal cortex. *Nature* **398,** 704–708.
122. Voytko M. L., Kitt C. A. and Price D. L. (1992) Cholinergic immunoreactive fibers in monkey anterior temporal cortex. *Cereb. Cortex* **2,** 48–55.
123. Voytko M. L., Olton D. S., Richardson R. T., Gorman L. K., Tobin J. R. and Price D. L. (1994) Basal forebrain lesions in monkeys disrupt attention but not learning and memory. *J. Neurosci.* **14,** 167–186.
124. Walaas S. I. and Ouimet C. C. (1989) The ventral striatopallidal complex: an immunocytochemical analysis of medium-sized striatal neurons and striatopallidal fibers in the basal forebrain of the rat. *Neuroscience* **28,** 663–672.
125. Wilson F. A. and Rolls E. T. (1990) Neuronal responses related to the novelty and familiarity of visual stimuli in the substantia innominata, diagonal band of broca and periventricular region of the primate basal forebrain. *Expl Brain Res.* **80,** 104–120.
126. Wilson F. A. and Rolls E. T. (1990) Neuronal responses related to reinforcement in the primate basal forebrain. *Brain Res.* **509,** 213–231.
127. Wouterlood F. G., Steinbusch H. W. M., Luiten P. G. M. and Bol J. G. J. M. (1987) Projection from the prefrontal cortex to histaminergic cell groups in the posterior hypothalamic region of the rat. Anterograde tracing with *Phaseolus vulgaris* leucoagglutinin combined with immunocytochemistry of histidine decarboxylase. *Brain Res.* **406,** 330–336.
128. Yasui Y., Breder C. D., Saper C. B. and Cechetto D. F. (1991) Autonomic responses and efferent pathways from the insular cortex in the rat. *J. comp. Neurol.* **303,** 355–374.
129. Yeterian E. H. and Pandya D. N. (1991) Prefrontostriatal connections in relation to cortical architectonic organization in rhesus monkeys. *J. comp. Neurol.* **312,** 43–67.
130. Zaborszky L. and Cullinan W. E. (1989) Hypothalamic axons terminate on forebrain cholinergic neurons: an ultrastructural double-labeling study using PHA-L tracing and ChAT immunocytochemistry. *Brain Res.* **479,** 177–184.
131. Zaborszky L., Gaykema R. P., Swanson D. J. and Cullinan W. E. (1997) Cortical input to the basal forebrain. *Neuroscience* **79,** 1051–1078.
132. Zaborszky L., Pang K., Somogyi J., Nadasdy Z. and Kallo I. (1999) The basal forebrain corticopetal system revisited. *Ann. N Y. Acad. Sci.* **877,** 339–367.
133. Zola-Morgan S. and Squire L. R. (1993) Neuroanatomy of memory. *A. Rev. Neurosci.* **16,** 547–563.

(*Accepted* 15 *December* 2000)