Basal Amygdaloid Complex Afferents to the Rat Nucleus Accumbens Are Compartmentally Organized

Christopher I. Wright, Arno V. J. Beijer, and Henk J. Groenewegen

Graduate School Neurosciences Amsterdam, Research Institute Neurosciences, Department of Anatomy and Embryology, Vrije Universiteit, Amsterdam, The Netherlands

The basal amygdaloid complex (BAC) topographically projects to the nucleus accumbens (Acb) in patchy, inhomogeneous patterns. These termination patterns may be related to the histological features of the Acb that define the shell, core, and adjacent ventral caudate-putamen (CPv), and the ventral striatal compartments providing output to different autonomic, motor, and endocrine targets. Knowledge of the relationships of BAC afferents with these compartments is essential for understanding the activities of amygdalostratial circuits. Therefore, anterograde tracing experiments were performed, combined with calbindin-D_{28K} (CaB) immunohistochemistry or Nissl staining.

The results demonstrated that the caudal parvocellular basal amygdala (Bpc) projected primarily to cell clusters in the dorsal shell of the medial Acb, and to patches in the core/CPv. Fibers from the caudal accessory basal nucleus (AB) selectively reached CaB-immunoreactive cell clusters in the ventral shell,

avoiding the core/CPv. The rostral AB projected to the same ventral shell compartments as the caudal AB; in addition, dense terminations were found in the matrix of the core/CPv, avoiding the patches. Caudal magnocellular basal amygdala (Bmg) fibers reached ventral parts of the shell, including the CaB-immunoreactive cell clusters. The caudal Bmg projected strongly to the patches of the core/CPv, evading the matrix. Finally, the rostral Bmg densely innervated the moderately CaB-immunoreactive lateral shell and the patches of the core/CPv, largely avoiding the matrix. These results indicate the specific compartmental relationships of the patchy BAC terminations and suggest that BAC subregions differentially influence particular ventral striatal outputs.

Key words: amygdala; anterograde tracing; basal ganglia; calbindin; immunohistochemistry; nucleus accumbens; ventral striatum

The nucleus accumbens (Acb) and the basal amygdala have been linked as components of a brain network subserving stimulus-reward/punishment-related processes (Cador et al., 1989; Everitt et al., 1989, 1991) and the motor/effector activities of basic biological drives (Mogenson et al., 1980). This is consistent with the anatomical findings that Acb outputs, directly and via the ventral pallidum (VP), reach structures important for coordinating complex motor and autonomic responses, such as the lateral hypothalamus, ventral tegmental area (VTA), substantia nigra (SN), midbrain extrapyramidal area (MEA), and periaqueductal gray (PAG) (Nauta et al., 1978; Heimer et al., 1991; Berendse et al., 1992a; Groenewegen et al., in press). Via the basal amygdala, the Acb receives highly processed viscerosensory and auditory information (Turner and Herkenham, 1991; LeDoux, 1992). These projections are topographically arranged such that the caudal basal amygdaloid nucleus projects to the medial Acb, whereas rostral parts send fibers laterally (Kelley et al., 1982; MacDonald, 1991a,b). However, the termination patterns are patchy, and it is

unclear whether this heterogeneity is related to the different histochemical and cellular features of the Acb.

The Acb has two major subregions—the shell and the core—defined by a variety of histochemical, cellular, hodological, and electrophysiological criteria (Záborszky et al., 1985; Voorn et al., 1989; Zahm and Heimer, 1990; Heimer et al., 1991; Pennartz et al., 1992; Zahm and Brog, 1992; Jongen-Rêlo et al., 1993, 1994). Based on similar criteria, these two subregions are further compartmentalized. For example, the shell has different longitudinally oriented zones (Wright and Groenewegen, 1995), distinguished by different calbindin-D_{28K} (CaB) immunoreactivities (Voorn et al., 1989; Jongen-Rêlo et al., 1994), cellular densities (Herkenham et al., 1984), and projections to either the VTA or MEA/PAG (Berendse et al., 1992a). The core and adjacent ventral caudate-putamen (CPv) contain patches with low CaB immunoreactivity surrounded by a CaB-rich matrix containing cells projecting to the dopaminergic and nondopaminergic SN, respectively (Berendse et al., 1992a). This pattern of organization is like that of the dorsal striatum, in which striatal compartmentation was first described (Graybiel and Ragsdale, 1978; Gerfen et al., 1987).

Basal amygdaloid projections to the dorsal striatum demonstrate specific compartmental relationships. For example, in cats the basal amygdala projects preferentially to patches (Ragsdale and Graybiel, 1988), whereas in rats amygdaloid projections principally reach the matrix (Kita and Kitai, 1990). Likewise, the heterogeneous patterns of the caudal basal amygdaloid terminations are related to the Acb compartments, because the (parvocellular) basal amygdala gives selective input to the cell cluster zone of the caudomedial Acb and to patches in the medial

Received June 9, 1995; revised Oct. 25, 1995; accepted Dec. 12, 1995.

This work was supported by a Human Frontier Science Program long-term fellowship (C.I.W.) and a Harvard Medical School Moseley Fellowship (C.I.W.). The critical comments of Dr. A. H. M. Lohman on this manuscript are much appreciated. We thank Dirk de Jong for his expert photographic skills, Yvonne Galis-de Graaf, Jolinda Kos, and Joan Hage for their technical and secretarial assistance. We are also indebted to Dr. Jan G. Wolters and Allert J. Jonker for generating some of the experimental material used in this study.

Correspondence should be addressed to Dr. Henk J. Groenewegen, Department of Anatomy and Embryology, Faculty of Medicine, Vrije Universiteit, van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands.

Copyright © 1996 Society for Neuroscience 0270-6474/96/161877-17\$05.00/0

core/CPv (Wright and Groenewegen, 1995). However, the basal amygdaloid complex (BAC; i.e., the basal and accessory basal nuclei) projects to a large extent of the ventral striatum (McDonald, 1991b; Brog et al., 1993). From the latter studies, it remains unclear whether specific compartments are involved.

Because the Acb compartments reflect specific output pathways, probably with different functional (motor and autonomic) affiliations, knowledge of the precise organization of amygdaloid afferents is important for understanding the activities of amygdalostratial circuits. We therefore examined the specific immunohistochemical and cytoarchitectural relationships of the BAC afferents in the Acb, using simultaneous visualization of anterogradely labeled fibers and CaB immunoreactivity or Nissl substance.

Abbreviations

<i>AB</i>	accessory basal amygdaloid nucleus
<i>ABmg</i>	magnocellular accessory basal amygdaloid nucleus
<i>ABpc</i>	parvocellular accessory basal amygdaloid nucleus
<i>ac</i>	anterior commissure
<i>Acb</i>	nucleus accumbens
<i>AOP</i>	posterior anterior olfactory nucleus
<i>BAC</i>	basal amygdaloid complex BLvbasolateral amygdala
<i>Bmg</i>	magnocellular basal amygdaloid nucleus
<i>Bpc</i>	parvocellular basal amygdaloid nucleus
<i>BNST</i>	bed nucleus of the stria terminalis
<i>C</i>	core of the nucleus accumbens
<i>Ce</i>	central amygdaloid nucleus
<i>PAG</i>	periaqueductal gray
<i>CPv</i>	ventral caudate-putamen
<i>En</i>	endopiriform nucleus
<i>GP</i>	globus pallidus
<i>I</i>	intercalated amygdaloid nucleus
<i>iCjm</i>	insula Calleja major
<i>La</i>	lateral amygdaloid nucleus
<i>LH</i>	lateral hypothalamus
<i>MEA</i>	midbrain extrapyramidal area
<i>OT</i>	olfactory tubercle
<i>PAG</i>	periaqueductal gray
<i>Sh</i>	shell of the nucleus accumbens
<i>SNC</i>	substantia nigra pars compacta
<i>SNr</i>	substantia nigra pars reticulata
<i>VTa</i>	ventral tegmental area
<i>VP</i>	ventral pallidum

MATERIALS AND METHODS

Animal surgery and anterograde tracer injections. Sixty-five adult female Wistar rats (Harlan CPB, Zeist, The Netherlands) weighing between 180 and 230 gm were used. Rats were deeply anesthetized using a 4:3 mixture of Aescoket (1% ketaminum HCl; Aesculaap, Bostel, The Netherlands) and Rompun (2% xylathine HCl; Bayer, Belgium), using one intraperitoneal and two intramuscular injections. Each injection was 0.05 ml/100 gm of body weight of the Aescoket–Rompun mixture. Anesthetized rats were mounted in a stereotaxic apparatus, the brain was exposed through small burr holes, and injections of the anterograde tracers were placed at coordinates derived from the atlas of Paxinos and Watson (1986).

Phaseolus vulgaris-leucoagglutinin (PHA-L; Vector Laboratories, Burlingame, CA) (Gerfen and Sawchenko, 1984) and biotinylated dextran amine (BDA; Molecular Probes, Eugene, OR) (Veenman et al., 1992) were used as tracers. Injections were achieved iontophoretically using glass micropipettes as described previously (Wright and Groenewegen, 1995). Because PHA-L and BDA are visualized via different histochemical procedures that do not interfere with each other, in all rats both tracers were injected in the right side of the brain, each at different coordinates. Postinjection survival time was 7–14 d. Before perfusion (300 ml of heparinized 0.9% NaCl solution) and brain fixation [400 ml of 4% paraformaldehyde, in 0.1 M phosphate buffer (PB), pH 7.4], animals were

deeply anesthetized with Nembutal (sodiumpentobarbital 1 ml/kg; Sanofi, Maassluis, The Netherlands). After removal, brains were post-fixed (1–2 hr, same fixative) and soaked overnight in 20% glycerol/2% dimethylsulfoxide (DMSO) in PB at 4°C. Forty- μ m-thin sections were cut with a freezing microtome and collected in 0.05 M Tris-HCl-buffered saline, pH 7.60, 0.5% Triton X-100 (TBS-T) for immunohistochemistry, or in glycerol-DMSO solution for storage at –20°C.

Histochemistry. Histochemistry was performed to visualize each tracer individually. In all rats, series of sections were counterstained with Nissl to determine the location of the injection site and the relationships of fibers to cytoarchitectural features. In a few cases, additional sections were stained for tracer and acetylcholinesterase to confirm injection location.

Tracers were detected as described previously. Briefly, BDA was visualized using the Vectastain ABC kit (Vector Laboratories). A 1:1 mixture of reagents A (avidin) and B (biotinylated horseradish peroxidase) prereacted for 30 min (8 μ l of A and 8 μ l of B per milliliter TBS-T) was incubated with brain tissue for 1.5 hr. All incubations were at room temperature unless otherwise noted. Sections were then rinsed with TBS-T and TBS or with 0.1 M PB, pH 7.4. Avidin–biotin peroxidase activity was detected using TBS containing 0.05% 3'-3' diaminobenzidine (DAB) and 0.0015% H₂O₂ (brown reaction product), or 0.05% DAB plus 0.5% nickel ammonium sulfate (DAB-Ni) and 0.0015% H₂O₂ in PB (blue-black reaction product). After color development, sections were rinsed with TBS, mounted on slides from Tris-HCl (0.05 M, pH 7) containing 0.2% gelatin, and dried. For Nissl stain, the mounted sections were incubated in 0.3% cresyl violet (in H₂O) for 1–3 min. Sections were then dehydrated with an ethanol gradient and coverslipped from xylene using Entellan (Merck, Darmstadt, Germany).

PHA-L was detected using the peroxidase anti-peroxidase (PAP) method of Sternberger (1979). After several rinses with TBS-T, sections were treated overnight with a rabbit anti-PHA-L serum (α PHA-L; diluted 1:2000 in TBS-T; Dako, Glostrup, Denmark) at 4°C. After additional rinses, sections were incubated with a swine anti-rabbit serum (Sw α R; 1:100 in TBS-T; Dako) for 45 min, rinsed again, and finally incubated with rabbit PAP (r-PAP; Dako) diluted 1:800 in TBS-T for 45 min. After the final rinses, peroxidase activity was visualized as above with DAB or DAB-Ni as chromogen. Sections were also Nissl-stained and coverslipped as above.

Acetylcholinesterase enzyme (AChE) histochemistry was performed using either the Tago modification of the Karnovsky–Roots procedure (Tago et al., 1986) or the method of Geneser (1987). In these experiments, anterograde tracer staining was initially performed as outlined above, sections were then mounted and air dried, and AChE activity was detected. Subsequently, sections were dehydrated and coverslipped with Entellan (Merck).

CaB-antegrade tracer double histochemistry. In all cases, a series of sections was stained to detect both CaB and the anterograde tracer. For histochemical detection of BDA-labeled fibers and CaB immunoreactivity (IR), BDA was first detected with the avidin–biotin procedure (as described above) using DAB-Ni as a chromogen (blue-black). Calbindin immunohistochemistry was then performed using the PAP method. Briefly, tissue was incubated overnight at 4°C with a 1:5000 dilution mouse CaB monoclonal antibody (α CaB; Sigma, St. Louis, MO) in TBS-T containing 2–4% bovine serum albumin (Sigma) (TBS-TB). Sections were then rinsed, incubated with a goat anti-mouse antibody (G α M; 1:50 in TBS-TB; Dako) for 1 hr, rinsed again, and incubated with monoclonal mouse PAP (m-PAP) complex (1:100 in TBS-T; Dako). After the m-PAP incubation and thorough rinsing, peroxidase activity was visualized as a brown reaction product using DAB as chromogen. Sections were washed, mounted, and coverslipped as for other experiments.

For visualization of PHA-L-labeled fibers and CaB IR, sections were first incubated with a solution containing the two different primary antibodies (i.e., α PHA-L and α CaB), rinsed, and then incubated with a solution containing the two secondary antibodies (i.e., Sw α R and G α M). Thus, after preincubation with TBS-TB, tissue was incubated overnight at 4°C with the primary antibodies (α PHA-L at 1:2000 and α CaB at 1:5000 in TBS-TB), rinsed thoroughly with TBS-T, and incubated for 1 hr with a mixture of secondary antibodies (Sw α R, 1:100 dilution; G α M, 1:50 dilution) in TBS-TB. After thorough rinsing with TBS-T, the incubation with the appropriate PAP complex (i.e., mouse or rabbit) and the detection of peroxidase were performed separately. Thus, sections were first incubated with r-PAP for 1 hr, and PHA-L IR was visualized with DAB-Ni. Then, after several rinses with TBS-T, tissue was incubated with

Figure 1. Patterns of CaB IR in the Acb. Sections are transversely cut and are from different caudal-to-rostral levels. *A*, At the caudal extreme, where the ventral pallidum (VP) is prominent and the shell of the Acb and the BNST merge, a small group of CaB-positive cells (arrows) is present dorsally which appear to extend rostrally (*B*). Also evident are elements of the moderate CaB-immunoreactivity in the lateral shell (*Sh_l*, positioned ventrolateral to the anterior commissure), present both in cells and neuropil. In the ventral caudate putamen (CPv) and the caudal core (C) of the Acb surrounding the anterior commissure (ac), lightly staining patches (stars) are present within a CaB-rich matrix. *B*, At the level of the iCjm (open star), the shell and the core/CPv regions of the Acb are most prominent. In the medial shell (*Sh_m*), smaller areas with very low CaB IR (arrowheads) and CaB-immunoreactive cells (arrows) intermingle with larger zones having low, homogeneous CaB IR. The latter zones include the so-called "cone-shaped" area and a dorsomedial area directly adjacent to the lateral septum (Wright and Groenewegen, 1995). The light, homogeneous CaB IR zones intermingle and extend into ventral/central parts of the shell at more rostral levels. Ventrally in the shell (*Sh_v*), areas with CaB-immunoreactive cells (arrows) are also present. The moderately CaB-immunoreactive *Sh_l* is prominent at this level. In the core/CPv patch (closed stars) and matrix areas are present. *C*, At this level, the shell is primarily medially and ventrally located with respect to the anterior commissure (ac), which lies laterally against the ventral tip of the external capsule. Medial (*Sh_m*) and *Sh_l* shell areas are characterized by light and moderate CaB neuropil staining, respectively. The medial shell (*Sh_m*) has dorsal and ventral parts based on a border with spots having very low CaB IR (arrowheads) or small groups of CaB-immunoreactive cells (arrows). Patch (closed stars) and matrix areas are visible in the core/CPv. *D*, In the most rostral section of the Acb, a central area with low CaB IR can be distinguished from a partly circumferential CaB-rich area. The core/CPv is not present at this level. Scale bars: *A*, *B*, *C*, 200 μ m; *D*, 300 μ m.

Figure 2. Nissl staining in transverse sections of the Acb. *A*, In a section through the caudal shell at the level of the iCjm (adjacent to that of Fig. 1*B*), cell clusters (arrowheads, arrows) are associated with very low CaB IR (arrowheads) or with CaB-immunoreactive cells (arrows). *B*, In the rostral shell (section is adjacent to that shown in Fig. 1*C*), small cell clusters (arrowheads) are present primarily along the border separating the dorsal and ventral parts of the medial shell and correspond to the spots of very low CaB IR (Fig. 1*C*). Ventrally in both sections *A* and *B*, the OT and the islands of Calleja are visible. Scale bar (shown in *B*), 300 μ m.

m-PAP for 1 hr, and CaB IR was visualized using DAB. Finally, sections were rinsed, mounted, and coverslipped as for other experiments.

In all the above experiments, stained sections were charted using standard light microscopy and a drawing tube. Charting of anterogradely labeled fibers and CaB IR or cytoarchitectural features shown with Nissl stain was similarly performed.

Amygdala nomenclature. In general, we follow the rat amygdala nomenclature of Price et al. (1987). With respect to the basal amygdaloid nucleus, rostral magnocellular (Bmg) and caudal parvocellular (Bpc) portions are recognized (see Fig. 3*A–F*). These correspond to the anterior and posterior basolateral amygdala (Krettek and Price, 1977; McDonald, 1991a; Alheid et al., 1995). We also recognize a rostrocaudally extensive accessory division of the basal amygdala (AB) (see Fig. 3*A–F*). However, we explicitly include in our definition of the AB the large-celled, ventral division of the basolateral amygdala (BLv of McDonald, 1991a; Alheid et al., 1995), and we parcel the remainder of the AB according to the description of Turner and Zimmer (1984) (see also McDonald, 1991a; Alheid et al., 1995). Thus, we distinguish a magnocellular AB (ABmg) that is coincident with the BLv [and with the AB₂ of Turner and Zimmer, (1984)], and a parvocellular AB (ABpc) that includes Turner and Zimmer's (1984) AB rostrally, AB₂ midrostrocaudally, and AB₄ caudally (Turner and Herkenham, 1991).

RESULTS

Immunohistochemical and cellular features of the Acb

As in previous studies (Voorn et al., 1989; Zahm and Brog, 1992; Jongen-Rêlo et al., 1994; Wright and Groenewegen, 1995), CaB immunohistochemistry was used to define the shell, the core, and the adjacent CPv of the Acb. Four different rostrocaudal levels of the Acb immunostained for CaB are illustrated in Figure 1. Olfactory structures [posterior anterior olfactory nucleus (AOP) and olfactory tubercle (OT)], the islands of Calleja, and the cell clusters in the Acb were examined in Nissl-stained sections di-

rectly adjacent to those used to examine CaB IR (Figs. 1*B,C*, 2*A,B*). The drawings in Figures 5, 6, 8, 9, and 11, in which the distribution of amygdalostratial fibers are charted, are based on the CaB IRs and Nissl-stain features described below (see Figs. 1, 2).

At the most caudal level of the Acb, the transition area of the bed nucleus of the stria terminalis (BNST) and the shell of the Acb (Fig. 1*A*) were marked dorsally by CaB-positive cells and ventrally by homogeneous, light CaB IR. At this caudal level, the lateral shell showed moderate CaB IR. In the core/CPv, lightly staining patches were visible (Fig. 1*A*). At the level of the major island of Calleja (iCjm; Figs. 1*B*, 2*A*), the shell was characterized by its general, weak staining intensity for CaB (Voorn et al., 1989; Zahm and Brog, 1992; Jongen-Rêlo et al., 1994; Wright and Groenewegen, 1995). However, both the CaB and Nissl staining showed distinct inhomogeneities in the form of weak homogeneous CaB IR zones, distinct from the cell clusters (Nissl) with very low (or no) CaB IR or CaB-immunoreactive neurons (Wright and Groenewegen, 1995). The moderately CaB-immunoreactive lateral shell and the patches and matrix of the core/CPv were all prominent at this level. More rostrally (Fig. 1*C*), the light, homogeneous CaB IR medial shell is divided into dorsal and ventral parts by a region with CaB-immunoreactive cells and spots having very low or no CaB IR associated with small cell clusters in the Nissl staining (Fig. 2*B*). At the most rostral level (Fig. 1*D*), CaB staining demonstrated a lightly staining central area with a partly circumferential, darkly staining region, which was most evident ventrally overlying the anterior commissure.

General aspects of BAC injections

Anterograde tracers (BDA or PHA-L) were deposited at various rostrocaudal levels throughout the BAC (Figs. 3, 4). The deposits extended ~250–1000 μ m in the rostrocaudal direction and were spherical or ellipsoidal. All injections examined were restricted to subregions of the Bmg (Fig. 4A–D), AB (Fig. 4E–F), or Bpc (Fig. 4G).

Labeled fibers from the BAC coursed toward the Acb by two major pathways. Fibers originating in the rostral two-thirds of the Bmg primarily reached the Acb directly via the external capsule. Fibers stemming from all other regions of the BAC (including the caudal third of the Bmg and all regions of the AB and Bpc) reached the Acb primarily via the stria terminalis and BNST. From the BNST, these fibers directly entered the medial parts of the Acb (including the core/CPv) caudal to the crossing of the anterior commissure, or extended rostrally past the commissural crossing to enter the medial Acb.

As in previous studies, a gross medial-to-lateral topography of basal amygdala afferents to the ventral striatum was evident (Kelley et al., 1982; Russchen and Price, 1984; Kita and Kitai, 1990; McDonald, 1991a,b; Shinonaga et al., 1994). For example, injections in the caudomedial Bpc resulted in dense collections of labeled fibers medially in the Acb, whereas those in the rostral Bmg led to labeling laterally (compare Figs. 5 and 11). Fibers from the AB were densest in the central Acb, avoiding the dorsomedial and ventrolateral extremes (Figs. 6, 8). For the following description of the amygdalostratial projections, the emphasis is on the immunohistochemical and cytoarchitectural relationships of these fibers. In keeping with the known topography of the basal amygdalostratial fibers, we examined these relationships proceeding from medial to lateral in the Acb. Thus, the histochemical relationships of labeled fibers from the Bpc injections (Fig. 5) are described, followed by those from the AB (see Figs. 6, 8) and those from the Bmg (see Figs. 9, 11).

Immunohistochemical and cytoarchitectural relationships of basal parvocellular amygdaloid afferents to the Acb

Caudomedial Bpc injections

The bulk of labeled fibers to the medial Acb arose from caudomedial Bpc injections (93221B, 93260P; Fig. 3F,F'). These fibers passed through medial parts of the dorsomedial BNST/shell transition area, producing a fork-shaped pattern of intense fiber labeling in the dorsomedial part of the caudal shell (Fig. 5A,B). Fibers were associated with areas of very low CaB IR or areas containing CaB-immunoreactive cells in the cell cluster zone (Wright and Groenewegen, 1995), but avoided those areas having low, homogeneous CaB IR (Fig. 5B). In the medial core/CPv, fibers were present in patches and near the shell/core border, as well as medially in the OT. In the more rostral, compared with caudal shell, fewer labeled fibers were observed, located primarily along its medial and lateral borders (Fig. 5C). Fibers were also found along the dorsal/ventral border region of the rostral shell in association with CaB-immunoreactive cells and spots with very low or no CaB IR. The surrounding areas with light, homogeneous CaB IR were avoided. At the most rostral level (Fig. 5D), a bundle of fibers oriented toward the medial prefrontal cortex was present along the medial edge of the Acb. Otherwise, only a small number of fibers were present primarily in areas with dark CaB staining, and in the medial AOP.

Other Bpc injections

In general, injections in the caudolateral (93199B, 93273P, 94140P; Fig. 3F,F'), compared with the caudomedial (93221B, 93260P; Fig. 3F,F'), parts of the Bpc gave rise to fewer labeled fibers in the Acb. These fibers involved the same histochemically defined areas as those from caudomedial Bpc and extended somewhat further ventrally in the caudal shell. Injections primarily involving rostral parts of the Bpc (93280P, 95037B) gave similar results as those located caudolaterally. Lateral injections centered at midrostrocaudal levels of the Bpc (93220P, 93281P; Fig. 3E, E') gave somewhat denser labeling in ventral parts of the caudal shell and in the medial patches of the core than did the caudolateral injections. An injection centered medially at midrostrocaudal levels of the Bpc (93267P; Fig. 3E,E'), compared with those centered caudomedially, resulted in less dense projections to the same histochemically defined areas.

Immunohistochemical and cytoarchitectural relationships of accessory basal amygdaloid afferents to the Acb

Caudal ABpc injections

The majority of labeled fibers from injections in the caudal parts of the ABpc (94036B, 94246P, 95013P, 95014P) extended through more lateral parts of the dorsomedial BNST/shell transition area compared with the fibers of the Bpc (Fig. 6A vs Fig. 5A). In sections at the level of the iCjm (Fig. 6B), patchy collections of labeled fibers were concentrated ventromedially in the shell, where they were associated with regions having very low CaB IR or CaB-immunoreactive cells and cell clusters (see Figs. 6B, 7A,B). The fibers from caudal AB injections, like those from caudomedial Bpc injections, avoided the regions in the medial shell with moderate cell density staining lightly (and homogeneously) for CaB (see Figs. 6B, 7A,B). In the core/CPv, occasional labeled fibers were observed in the matrix along the shell/core border and, rarely, in a patch. In the ventromedial parts of the OT, a substantial concentration of labeled fibers was present (Fig. 6B).

In all parts of the two most rostral, compared with caudal sections through the Acb, the concentration of labeled fibers was greatly reduced. In the section in which the core/CPv was apparent, the few remaining fibers in the Acb were associated with the dorsal/ventral shell border region and with the ventromedial OT (Fig. 6C). In the most rostral section, the few remaining fibers were associated primarily with the OT and ventromedial AOP (Fig. 6D).

Further aspects of caudal ABpc injections

Other injections in the caudal ABpc demonstrated the same immunohistochemical and cytoarchitectural relationships as noted above, and no significant differences in projection patterns were seen when injections at different rostrocaudal positions in the caudal ABpc were compared (Fig. 3E,E',F,F'). However, a medial-to-lateral gradient in the shell was noted when injections involved different extents of the medial or lateral parts of the caudal ABpc. Thus, a medially positioned injection (95013P) gave rise to a greater density of labeled fibers medially in the ventromedial shell along the border with the iCjm and the medial OT, compared with laterally in the ventromedial shell along the border with the core. A laterally positioned injection (95014P) resulted in a greater density of labeled fibers laterally, compared with medially, along the ventromedial shell border. In general, projection patterns in the ventromedial shell were reflective of the extent to which injections (94236B, 94236P, 94246P) involved the medial or lateral parts of the caudal ABpc. The injection (94236P) presented above involved a large extent of the caudal ABpc

Figure 3. Two rows of drawings illustrating, at six rostrocaudal levels, the divisions of the BAC (*A–F*) and the positions of the anterograde tracer injections (*A'–F'*) in transverse sections. The approximate distance of the sections from bregma is noted for each drawing. *A–F*, Magnocellular (*Bmg*) and parvocellular (*Bpc*) basal and accessory basal (*ABmg*, *ABpc*) divisions were recognized based on Nissl staining and (Figure continues)

(Figs. 3*E',F'*, 6), resulting in labeling in medial and lateral parts of the ventromedial shell.

Midrostrocaudal *ABpc* injections

Midrostrocaudal injections in the *ABpc* (90092P, 94250B, 94251P) at a level where the *Bpc* and *Bmg* are adjacent to each other (see Figs. 3*C,D*, 8) resulted in different immunohistochemical and cytoarchitectural relationships than those found after more caudal injections. Labeled fibers from midrostrocaudal *ABpc* injections traverse ventral and ventromedial parts of the BNST/shell transition areas to involve the ventral shell and extensive parts of the caudal core/CPv surrounding the anterior commissure (Fig. 8*A,B*). Ventrally in the caudal shell, fibers were associated with CaB-immunoreactive cells and cell clusters and

the adjacent (ventromedial) parts of the moderately CaB-immunoreactive lateral shell (Fig. 8*B*). The rest of the medial and lateral shell (Fig. 8*B*) was virtually free of fibers. In the core/CPv, fibers were almost entirely restricted to the matrix and were only very rarely found in patches (Figs. 7*C,D*, 8*A,B*). The most medial and lateral parts of the core/CPv had fewer fibers compared with the central parts surrounding the anterior commissure. In the caudal OT, projections were concentrated ventrally.

Rostral sections through the Acb (Fig. 8*C,D*) exhibited far fewer labeled fibers than caudal sections with the exception of olfactory structures. In the rostral parts of the shell, fibers were limited to the dorsal/ventral border region and the lateral shell and avoided areas with light, homogeneous CaB staining. The few

acetylcholinesterase histochemistry. *A'–F'* show the rostrocaudal location and case number of BDA (case number is followed by a *B*) or PHA-L (case number is followed by a *P*) tracer injections (see Fig. 4). Injections from cases used for drawings in Figures 5, 6, 8, 9, and 11 are indicated in *italic bold type*. Scale bar, 1 mm.

fibers in the core/CPv were limited mostly to the matrix adjacent to the anterior commissure. In the most rostral Acb (Fig. 8*D*), occasional fibers present expressed a slight preference for CaB-rich areas, whereas many fibers were concentrated in central parts of the AOP.

Further aspects of ABpc injections

The available injections (90092P, 94250B, 94251P, 94251P; Fig. 3*C',D'*) showed no differences in the topographic patterns of midrostrocaudal ABpc projections to the Acb. It was only found

that injections in rostral parts of the ABpc (94250P, 95001P, 95002B; Fig. 3*A',B'*) gave very few projections to the Acb. These fibers were restricted to the ventral BNST/shell transition area and to ventral parts of the shell more caudal than shown here.

ABmg injections

Injections restricted principally to the ABmg (88480P, 88602P, 94251B, 95075P; Fig. 3*B',C'*) gave rise to labeling in the Acb like that after midrostrocaudal ABpc injections. Thus, fibers in the shell were limited mostly to its caudal and ventral portions and

were associated with CaB-immunoreactive cells. In the core/CPv, fibers were concentrated in the matrix and avoided the weakly CaB-immunoreactive patches. However, the densest labeling in the core/CPv from ABmg injections was found ventrally and laterally to the anterior commissure, whereas fewer projections were observed dorsally and medially.

Immunohistochemical and cytoarchitectural relationships of basal magnocellular amygdaloid afferents to the Acb

Caudal Bmg injections

Injections (95013B, 94237B, 95002P, 95001B) in caudal parts of the Bmg (Fig. 3C',D') gave rise to fibers in the caudal shell with similar immunohistochemical and cytoarchitectural affiliations as those of the midrostrocaudal ABpc, the ABmg, and the caudo-medial Bpc. Thus, many labeled fibers were found medially and ventrally in the shell in association with CaB-immunoreactive and CaB-poor cell cluster regions, whereas very few were present in the areas of the medial shell with low, homogeneous CaB IR or in the moderately CaB-immunoreactive lateral shell (e.g., injection 94237B; Fig. 9A,B). In the caudal core/CPv, dense collections of labeled fibers in the medial patches were present after the caudal Bmg injections (Figs. 9A,B, 10A), in sharp contrast to the strong labeling found in the matrix after midrostrocaudal ABpc and ABmg injections. However, a small number of fibers were seen in the medial parts of the matrix adjacent to its border with the shell. In the caudal OT, fibers were concentrated ventrally and medially.

In sections through the rostral shell (Fig. 9C), labeled fibers from caudal Bmg injections, in contrast to those from other injections, were present along the dorsal/ventral shell border as well as dorsally in the area with low, homogeneous CaB IR. In the core/CPv, fibers occupied the medial-most patches and generally avoided the matrix. The few fibers observed in the most rostral Acb were in the central areas with low, homogeneous CaB IR (Fig. 9D). In rostral olfactory structures, the ventral and medial parts of the OT contained dense fiber plexi, whereas fibers in the AOP were limited to a small dorsal region.

Projections from the rostral Bmg

As noted above, labeled fibers from rostral injections in the Bmg (Fig. 3A') entered the Acb primarily via a direct route through the external capsule. In the caudal sections of the Acb (Fig. 11A), labeled fibers in the shell were limited principally to its moderately CaB-immunoreactive lateral part. At the level of the iCjm, fibers were also associated with CaB-immunoreactive cell clusters in the ventral-most regions of the shell (Fig. 11B). In the core/CPv, fibers were present in all the patches at the level of the anterior commissure and more dorsally (Fig. 11A,B), with the laterally positioned patches having a somewhat denser concentration of labeled fibers than those positioned medially (Fig. 10B–D). In the matrix, small, patchy collections of fibers were restricted to

its lateral part. Fibers in the OT were concentrated laterally and tapered off in density medially.

Rostrally in the Acb (Fig. 11C), labeled fibers were also laterally positioned, involving the dorsal/ventral shell border zone and the regions ventral and lateral to this, including the rostral parts of the lateral shell. In the core/CPv, fibers involved the lateral patches to a greater extent than the medial ones, and very few fibers were present laterally in the matrix. Most rostrally (Fig. 11D), fibers were concentrated ventrally in the area staining darkly for CaB and in the lateral parts of the AOP.

Further aspects of Bmg injections

No major differences in histochemical relationships or projection patterns were observed when injections in different parts of the caudal Bmg were compared with one another (94237B, 95001B, 95002P, 95013B; Fig. 3C',D') or when those in different parts of the rostral Bmg were compared (94008P, 94017B, 94018B, 94070P, 94148B, 94149B, 94151P, 94288B; Fig. 3A'). However, medial (94052P, 94069P, 94139P) and lateral (94229B, 94240P) injections in the midrostrocaudal parts of the Bmg (Fig. 3B,B') gave rise to different projection patterns that involved the immunohistochemical and cytoarchitectural features of the Acb to different extents.

Injections in the medial parts of the midrostrocaudal Bmg (94052P, 94069P, 94139P; Fig. 3B,B'), compared with rostral injections (Fig. 3A,A'), resulted in fewer labeled fibers laterally, but in more fibers ventrally in the caudal shell and ventrolaterally in the rostral shell. Substantial collections of fibers were present in the patches of the core/CPv directly dorsal to the anterior commissure. In the matrix, labeling was restricted to small areas of the lateral core. In contrast to the rostral and caudal Bmg injections, medially positioned midrostrocaudal injections resulted in more fibers in centrally located patches (e.g., above the anterior commissure) than in medially and laterally located patches. Thus, fibers from medial, midrostrocaudal injections resulted in a pattern of labeling intermediate to that observed after rostral and caudal Bmg injections.

The pattern of labeled fibers observed after injections in lateral parts of the midrostrocaudal Bmg (94229B, 94240P; Fig. 3B,B') resembled that found after injections in the rostral parts. Thus, fibers were present in the lateral, moderately CaB-immunoreactive shell, and in the directly adjacent patches and medial matrix. However, compared with the rostral Bmg injections, the lateral midrostrocaudal injections resulted in weaker labeling in the lateral shell, but in stronger labeling in the adjacent matrix.

DISCUSSION

Topographical aspects of BAC afferents to the Acb

The present study confirms the mediolateral organization of the major Acb projections from the Bpc and the rostral Bmg as shown by previous authors (Kelley et al., 1982; Russchen and Price, 1984;

Figure 4. Photomicrographs showing anterograde tracer injections in Nissl-counterstained tissue. *A*, An injection of PHA-L (94070P) in the rostral magnocellular basal amygdala (Bmg), as shown in a transverse section. *B*, A horizontal section showing a BDA injection (94270B) in the rostral Bmg. The medially positioned caudal Bmg (arrowhead) and the adjacent parvocellular basal amygdala (Bpc) are also visible. *C*, Horizontal section demonstrating an injection of BDA (95015B) in the caudal (medial) Bmg. *D*, A caudal (medial) Bmg injection with BDA (94237B) shown in a transverse section. The Bpc and the parvocellular (ABpc) and magnocellular (ABmg) parts of the AB demonstrate no labeled cells. *E*, An injection of BDA in the midrostrocaudal parts of the parvocellular accessory basal region (ABpc) as shown in a transverse section (94250B). Whereas fibers extend into the ABmg, cells labeled for the tracer are concentrated in the ABpc. *F*, A PHA-L injection limited to the caudal ABpc (94236P); the Bpc lies dorsally. *G*, A caudomedial Bpc injection with BDA (93221B). The ventrally located ABpc and the dorsally positioned lateral nucleus (La) demonstrate very few cells labeled for the tracer. Scale bars: *A*, 150 μ m; *B–G*, 200 μ m.

Figure 5. A series of drawings of labeled fiber patterns at four different levels of the Acb after a BDA injection (93221B) in the caudal parvocellular basal amygdala (Bpc). *A–D*, The series begins caudally and ends rostrally and is comparable to the series of photomicrographs in Figure 1*A–D*. At each level, drawings are based on two adjacent sections: one stained for BDA and CaB IR, and the other for BDA and Nissl substance. Fiber relationships with the cytoarchitecturally defined olfactory structures (i.e., OT and anterior part of the posterior olfactory nucleus; AOP) were determined with sections stained for both tracer and Nissl substance. Scale bar, 1 mm.

Figure 6. Patterns of fiber labeling at four different caudal-to-rostral levels of the Acb after a PHA-L injection in the caudal parvocellular accessory basal nucleus (ABpc; 94236P). *A–D*, Drawings are based on adjacent calbindin and Nissl-stained sections as described in the legend of Figure 5. The region indicated with the rectangle in *B* is shown in the photomicrograph of Figure 7*B* (7B). Scale bar, 1 mm.

Figure 7. Cytoarchitectural and immunohistochemical relationships of AB afferents in the Acb. *A*, A Nissl-counterstained section directly adjacent to *B* (arrowheads indicate same blood vessel) where fibers from the ABpc are associated with cell clusters. The surrounding moderately cellular areas are avoided. *B*, Photomicrograph of ABpc fibers in association with CaB-immunoreactive cells in the shell (as indicated in Fig. 6*B*; injection 94236P). In *C* and *D*, relationships of afferents from the midrostrocaudal AB with the compartments in the core of the Acb are illustrated. Photomicrographs show anterogradely labeled fibers and CaB immunohistochemistry. Fibers from the mid ABpc (injection 94251P; area shown in *C* as indicated in Fig. 8*B*) are preferentially found in the CaB-rich matrix of the core, and avoid weakly CaB-immunoreactive patches. Scale bars: *A*, *B*, 150 μ m; *C*, *D*, 50 μ m.

Kita and Kitai, 1990; McDonald, 1991a,b; Brog et al., 1993; Shinonaga et al., 1994; Wright and Groenewegen, 1995). In addition, caudal parts of the Bmg at the midrostrocaudal level of the BAC give rise to substantial projections to central parts of the Acb located between the medially and laterally positioned projection areas of the rostral Bmg and caudomedial Bmg, respectively (Fig. 12). This is in agreement with the results of Russchen and Price (1984) and McDonald (1991b), but not with those of Shinonaga et

al. (1994), who suggested that midrostrocaudal parts of the basal nucleus project to lateral parts of the Acb. The presently found topography within the Bmg projections to the Acb (i.e., rostral and lateral parts to the lateral, and caudal and medial parts to the central and ventral Acb) is consistent with the fact that the medial Bmg is continuous with its caudal parts (see Fig. 4*B,C*) and with McDonald's (1991b) findings of distinct differences between the striatal projections of the medial and lateral parts of the Bmg.

Figure 8. Patterns of fiber labeling in the Acb after a PHA-L injection in the midrostromcaudal (*mid*) parvocellular accessory basal nucleus (*ABpc*; 94251P). *A–D*, Drawings are at four different caudal-to-rostral levels and are based on adjacent CaB- and Nissl-stained sections as described in the legend of Figure 5. The region indicated in *B* is shown in the photomicrograph of Figure 7C. Scale bar, 1 mm.

Figure 9. Patterns of fiber labeling at four different caudal-to-rostral levels of the Acb after a BDA injection in the caudal magnocellular basal nucleus (*Bmg*; 95013B). *A–D*, Drawings are based on adjacent CaB- and Nissl-stained sections as described in the legend of Figure 5. The region indicated with a *rectangle* in *A* is shown in the photomicrograph of Figure 10A. Scale bar, 1 mm.

Figure 10. Fibers from the Bmg are located preferentially in patches of the core/CPv. Photomicrographs are of sections stained for anterograde tracer and CaB IR. *A*, Fibers from the caudal Bmg (95013B) innervate a patch in the medial core/CPv located laterally along the ventricle, as indicated in Figure 9*A*. *B*, Projections from the rostral Bmg (94070P) reach a patch located rostrally and laterally in the core/CPv (area as indicated in Fig. 11*C*). Fibers in the ventrolateral matrix (*arrows*) extend into this patch. Very few fibers are seen dorsally in the matrix. *C*, Patch located more dorsally in the CPv compared with those shown in *B* and *D*. Fibers extend into this patch (94070P) from the lateral matrix (*arrows*). Within the patch, fibers are reduced in density medially compared with laterally. *D*, Patch in the caudal core/CPv (as indicated in Fig. 11*A*) innervated by the Bmg (94070P). Scale bars: *B*, *C*, 50 μ m; *A*, *D*, 50 μ m.

Little information is available regarding the precise topography of AB afferents in the Acb; it has been reported that the AB projects to both the medial (Brog et al., 1993) and ventrolateral Acb (McDonald, 1991b). The location of the ABmg [Turner and Zimmer's (1984) AB₂ and the BLv of Alheid et al. (1995)] with respect to the basal nuclei, and the fact that the projections of the ABmg to the Acb overlap with and are topographically related to those of the midrostrocaudal ABpc, supports the inclusion of the ABmg in the AB complex. As shown in the present study, the projection patterns of the AB are complementary to those from the Bmg and Bpc, and also express a medial-to-lateral topography in the Acb based on the caudal-to-rostral position of the projecting neurons (Fig. 12). This explains the differences in the projection patterns as inferred from retrograde studies (McDonald, 1991b; Brog et al., 1993).

Immunohistochemical and cellular relationships of BAC afferents in the Acb

It has been demonstrated previously that basal amygdaloid projections to the dorsal striatum (Ragsdale and Graybiel, 1988; Kita and

Kitai, 1990) and Bpc projections to the medial Acb (Wright and Groenewegen, 1995) respect compartmental boundaries. The present study indicates that such highly selective relationships are a general organizational principle of the BAC–Acb afferent system (Figs. 12, 13). Thus, for example, projections from the caudal Bmg and midrostrocaudal AB project to the same compartments in the caudal shell, but to complementary compartments in the core/CPv.

Whereas the BAC afferents tend not to overlap in the caudal Acb shell, AB and caudal Bmg afferents overlap significantly in the CaB-immunoreactive cell clusters of the ventral shell. This suggests that the neurons in the ventral shell consolidate or integrate incoming signals from the BAC. In the core/CPv, the situation is quite different because the AB projects primarily to matrix and the Bmg predominantly to patches. It is also noteworthy that none of the 43 BAC injections examined resulted in significant projections to the centrally located, moderately cell-dense zone of the medial shell. This zone harbors the low CaB-immunoreactive cone-shaped area (Voorn et al., 1989) and receives dense paraventricular thalamic and

Figure 11. Patterns of fiber labeling at four different caudal-to-rostral levels of the Acb after an injection of PHA-L in the rostral Bmg (94070P). *A–D*, Drawings are based on adjacent CaB- and Nissl-stained sections as described in Figure 5. The regions marked with rectangles in *A* and *C* are shown in the photomicrographs of Figure 10, *B* and *D*. Scale bar, 1 mm.

hippocampal subicular projections (Herkenham et al., 1984; Groenewegen et al., 1987, in press; Berendse and Groenewegen, 1990; Wright and Groenewegen, 1995). Thus, the inputs to the caudal half of medial shell may be strictly segregated between two major projections areas, each of which have different output targets (see below and Fig. 12*B*). The fact that prelimbic cortical inputs avoid the moderately cell-dense zone but overlap with amygdaloid inputs supports this conjecture (Berendse et al., 1992b; Wright and Groenewegen, 1995).

Implications for Acb compartmentalization

The highly specific relationships of the BAC inputs with the above-mentioned histochemical and cellular features support the notion that the Acb is a multicompartamental structure (Brog et al., 1993; Jongen-Rêlo et al., 1993; Wright and Groenewegen, 1995) with several rostrocaudally extensive zones in the shell and a patch/matrix organization in the core/CPv (Figs. 12, 13).

Compartments of the Acb have been defined as specific regions with characteristic histochemical, cytoarchitectural, and connectional features. Thus, the medial shell has been divided into a cell cluster zone and a moderately cell dense zone (Wright and Groenewegen, 1995). The present results confirm this and further suggest that a ventral cell cluster zone exists that receives projections from the caudal ABpc, caudal Bmg, midrostrocaudal ABpc, and ABmg. This zone provides efferents to the MEA and ventrolateral PAG, as well as to the dopaminergic lateral VTA and medial SN pars compacta (SNc) (Berendse et al., 1992a; Groenewegen et al., in press). The lateral shell also appears to represent a specific zone, given its characteristic moderate CaB IR and the specific afferent projections from the rostral Bmg. In several studies it has been reported that the Bmg provides input to lateral parts of the Acb (Kita and Kitai, 1990; McDonald, 1991a,b), including the shell (Brog et al., 1993), but without notion of the specific relationships with the histochemical features of the lateral shell. Unfortunately, without knowledge of the precise outputs of this area, our designation of the lateral shell as a specific compartment remains tentative.

Likewise, although there are specific relationships between the BAC afferents and the patterns of CaB IR in the rostral Acb, little is known about the relationships of output cells with the histochemical features of the rostral Acb. Nevertheless, in the most rostral parts of the Acb, including the rostral pole (Zahm and Brog, 1992), it appears that the CaB-poor central area and CaB-rich peripheral areas designate different compartments. This is supported by the findings that these areas receive inputs from specific combinations of BAC nuclear divisions (Fig. 13) and have different output patterns (for example, to the entopeduncular nucleus and the lateral preoptic area, respectively) (Zahm and Heimer, 1993).

In the core/CPv, afferents from the BAC have strong preferences for either the CaB-poor patches or the CaB-rich matrix (present results) (Kita and Kitai, 1990; Wright and Groenewegen, 1995). Thus, fibers from the basal nucleus (Bpc and Bmg) are positioned to influence the entire patch compartment of the core/CPv, but only very restricted portions of the matrix. In contrast, the midrostrocaudal ABpc and the ABmg together project preferentially to the entire matrix area surrounding the anterior commissure and tend to avoid the patches. Because the patch compartment sends output to the dopaminergic SNc and the matrix to the nondopaminergic SN pars reticulata (SNr) (Gerfen et al., 1987; Berendse et al., 1992a), it is probable that specific parts of the basal amygdala and AB can selectively influence the dopaminergic and the nondopaminergic SN, respectively.

AFFERENTS

EFFERENTS

Figure 12. Relationships of BAC afferents and caudal Acb outputs with the compartments of the ventral striatum. The rostrocaudal level of this drawing corresponds to the level of Figures 1B and 24. Only major amygalostratial projections and compartmental preferences are indicated. *A*, Topographical and compartmental relations of BAC-Acb afferents. In the shell, stipples indicate cell clusters (or cell-dense areas), stars indicate the CaB-immunoreactive cells in these zones, and the light gray shading indicates the moderate CaB IR of the lateral shell. In the core/CPv, patch and matrix compartments are indicated with light and dark shading, respectively. *B*, Relations of specific output cell populations with the immunohistochemical and cytoarchitectural features of the Acb, based on Berendse et al. (1992a) and Groenewegen et al. (1995). The question mark indicates that the output targets of the lateral shell are unknown. The arrowhead with a question mark indicates uncertainty about the compartmental origin of Acb projections to the ventral pallidum (VP) and lateral hypothalamus (LH).

Functional implications for amygalostratial systems

The amygdala is an essential component for evaluating the biological relevance of sensory information (internal and external) and subsequently orchestrating the different bodily activities involved in behavioral responses (Weiskrantz, 1956; LeDoux, 1991, 1992; Everitt and Robbins, 1992). The external and internal signals necessary for making such associations probably reach the amygdala via the sensory thalamus and cortex (LeDoux et al., 1990; LeDoux, 1991, 1992; Turner and Herkenham, 1991). In investigating the mechanisms by which the amygdala orchestrates behavior, studies have emphasized the connections of the central amygdaloid nucleus with ventral forebrain structures (e.g., hypothalamus, BNST, and extended amygdala) that can influence the endocrine pituitary and autonomic brainstem centers (Swanson and Mogenson, 1981; Davis, 1986, 1989; Ray et al., 1987; Alheid and Heimer, 1988; LeDoux et al., 1988). More recently, interest has focused on the influence of the basolateral amygdala

(i.e., the basal, accessory basal, and lateral nuclei) on brainstem motor centers, such as the PAG, MEA, and dopaminergic SNc via the ventral forebrain, as well as the Acb (Mogenson, 1987; Holstege, 1991; LeDoux, 1991; Turner and Herkenham, 1991; Everitt and Robbins, 1992). A series of behavioral studies have demonstrated that the basolateral amygdala and the Acb must be intact for rats to preferentially act on associations made between external sensory cues and biologically important goals, such as nourishment (in the case of hunger or thirst) and sexual reproduction (Cador et al., 1989; Everitt et al., 1989, 1991).

Our studies delineate the precise routes by which the BAC, via the Acb, may influence the above-mentioned brainstem motor areas, and further indicate that the BAC can modify both motor and reward/incentive systems via cells in the Acb projecting to the nondopaminergic SNr and the lateral parts of the VTA, respectively (Fig. 12). In addition, the present results suggest that

AFFERENTS

Figure 13. Summary of the BAC projections to the rostral parts of the Acb. Immunohistochemical and cytoarchitectural relationships are as in Figure 12. The lines representing the afferent projections are thinner in this figure compared with those in Figure 12 to indicate that fewer BAC projections are found in the rostral Acb. *A*, Illustration of amygdaloid afferents at the level of the Acb where the rostral end of the core/CPv are visible. *B*, Afferent relationships in the most rostral Acb area examined.

specific subregions of the BAC relay, via the compartments in the Acb, different aspects of sensory information to different autonomic and motor nuclei of the brainstem. For example, the AB may relay multimodal sensory information primarily of auditory and visceral origin (LeDoux, 1991; Turner and Herkenham, 1991) via the shell of the Acb to the lateral VTA, and via the matrix of the core to the SNr. The Bmg and Bpc probably transmit primarily viscerosensory signals (LeDoux, 1991; Turner and Herkenham, 1991) via the shell to the ventrolateral PAG, MEA, and lateral VTA, and via the patches in the core to the dopaminergic SNc.

Finally, although the Acb is considered an important gateway to autonomic and motor centers in the brainstem, it is also positioned to interact with the prefrontal cortex via the VP or SNr and the medial thalamus (Alexander et al., 1990; Deniau et al., 1994; Groenewegen and Berendse, 1994). It is currently difficult to speculate which prefrontal cortical areas might be accessible to the BAC via the Acb-VP route, because the precise organization of the cells in the Acb that project to the VP is unknown. However, based on our present anatomic data (Fig. 12) and on recent physiological evidence (Deniau et al., 1994), the BAC may have access to the prelimbic prefrontal cortex via projections from the AB to the matrix of the

core and, subsequently, via the SNr and the medial thalamus. Notably, the projections from the prelimbic cortex area reach autonomic centers in brainstem and spinal cord (Hurley-Gius and Neafsy, 1986; Sesack et al., 1989; Neafsy, 1990; Hurley et al., 1991). Thus, it also must be considered that the BAC-Acb system may also influence autonomic functions via the prefrontal cortex. Further studies will be necessary to establish whether other routes through the Acb exist whereby the BAC could modulate motor and endocrine activities of the prefrontal cortex.

REFERENCES

- Alexander GE, Crutcher MD, DeLong MR (1990) Basal ganglia-thalamocortical circuits: parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions. In: *The prefrontal cortex: its structure, function and pathology* (Uylings HBM, van Eden CG, DeBruin JPC, Corner MA, Feenstra MGP, eds), pp 119-146. Amsterdam: Elsevier.
- Alheid GF, Heimer L (1988) New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striato-pallidal, amygdaloid, and corticopetal component of the substantia innominata. *Neuroscience* 27:1-39.
- Alheid GF, de Olmos JS, Beltramini CS (1995) Amygdala and extended amygdala. In: *The rat nervous system*, 2nd Ed (Paxinos M, ed), pp 495-578. New York: Academic.
- Berendse HW, Groenewegen HJ (1990) Organization of the thalamo-striatal projections in the rat, with special emphasis on the ventral striatum. *J Comp Neurol* 299:187-228.
- Berendse HW, Groenewegen HJ, Lohman AHM (1992a) Compartmental distribution of ventral striatal neurons projecting to the mesencephalon in the rat. *J Neuroscience* 12:2079-2103.
- Berendse HW, Galis-de Graaf Y, Groenewegen HJ (1992b) Topographic organization and the relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. *J Comp Neurol* 316:314-347.
- Brog JS, Salyapongse A, Deutch AY, Zahm DS (1993) The patterns of afferent innervation of the core and shell of the "accumbens" part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. *J Comp Neurol* 338:255-278.
- Cador M, Robbins TW, Everitt BJ (1989) Involvement of the amygdala in stimulus-reward associations: interactions with the ventral striatum. *Neuroscience* 30:77-86.
- Davis M (1986) Pharmacologic and anatomic analysis of fear conditioning using the fear-potentiated startle paradigm. *Behav Neurosci* 100:814-824.
- Davis M (1989) Sensitization of the acoustic startle reflex by footshock. *Behav Neurosci* 103:495-503.
- Deniau JM, Menetrey A, Thierry AM (1994) Indirect nucleus accumbens input to the prefrontal cortex via the substantia nigra pars reticulata: a combined anatomical and electrophysiological study in the rat. *Neuroscience* 61:533-545.
- Everitt BJ, Robbins TW (1992) Amygdala-ventral striatal interactions and reward related processes. In: *The amygdala: neurobiological aspects of emotion, memory, and mental dysfunction* (Aggleton JP, ed), pp 401-429. New York: Wiley.
- Everitt BJ, Cador M, Robbins TW (1989) Interactions between the amygdala and ventral striatum in stimulus-reward associations: studies using a second order schedule of reinforcement. *Neuroscience* 30:63-75.
- Everitt BJ, Morris KA, O'Brien A, Robbins TW (1991) The basal amygdala-ventral striatal system and conditioned place preference: further evidence of limbic-striatal interactions underlying reward-related processes. *Neuroscience* 42:1-18.
- Geneser FA (1987) Distribution of acetylcholinesterase in the hippocampal region of the rabbit. II. Subiculum and hippocampus. *J Comp Neurol* 262:90-104.
- Gerfen CR, Sawchenko PE (1984) An anterograde neuroanatomical tracing method that shows detailed morphology of neurons, their axons and terminals: immunohistochemical localization of an anterogradely transported plant lectin, *Phaseolus vulgaris*-leucoagglutinin (PHA-L). *Brain Res* 290:219-238.
- Gerfen CR, Herkenham M, Thibault J (1987) The neostriatal mosaic. II. Patch- and matrix-directed mesostriatal dopaminergic and non-dopaminergic systems. *J Neurosci* 7:3915-3934.

- Graybiel A, Ragsdale Jr CW (1978) Histochemically distinct compartments in the striatum of the human, monkey, and cat demonstrated by acetylcholinesterase staining. *Proc Natl Acad Sci USA* 75:5723–5726.
- Groenewegen HJ, Berendse HW (1994) Anatomic relationships between the prefrontal cortex and the basal ganglia in the rat. In: *Motor and cognitive functions of the prefrontal cortex* (Thierry A-M, Glowinski J, Goldman-Rakic PS, Christen Y, eds), pp 51–77. Berlin: Springer.
- Groenewegen HJ, Vermeulen-Van der Zee E, te Kortschot A, Witter MP (1987) Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of *Phaseolus vulgaris*-leucoagglutinin. *Neuroscience* 23:103–120.
- Groenewegen HJ, Wright CI, Beijer A (1996) The nucleus accumbens: gateway for limbic structures to reach the motor system? In: *The emotional motor system* (Holstege G, Bandler R, Saper CB, eds). Amsterdam: Elsevier, in press.
- Heimer L, Zahm DS, Churchill L, Kalivas PW, Wohltmann C (1991) Specificity in the projection patterns of the accumbal core and shell in the rat. *Neuroscience* 41:89–125.
- Herkenham M, Moon-Edley S, Stuart J (1984) Cell clusters in the nucleus accumbens of the rat, and the mosaic relationship of opiate, acetylcholinesterase, and subcortical afferent terminations. *Neuroscience* 11:561–593.
- Holstege G (1991) Descending motor pathways and the spinal motor systems. Limbic and non-limbic components. In: *Role of the forebrain in sensation and behavior* (Holstege G, ed), pp 307–410. Amsterdam: Elsevier.
- Hurley KM, Herbert H, Moga MM, Saper CB (1991) Efferent projections of the infralimbic cortex of the rat. *J Comp Neurol* 308:249–276.
- Hurley-Gius KM, Neafsy EJ (1986) The medial frontal cortex and gastric motility: microstimulation results and their possible significance for the overall organization of rat frontal and parietal cortex. *Brain Res* 365:241–248.
- Jongen-Rêlo A, Groenewegen HJ, Voorn P (1993) Evidence for a multicompartmental histochemical organization of the nucleus accumbens in the rat. *J Comp Neurol* 337:267–276.
- Jongen-Rêlo A, Voorn P, Groenewegen HJ (1994) Immunohistochemical characterization of the shell and core territories of the nucleus accumbens in the rat. *Eur J Neurosci* 6:1255–1264.
- Kelley AE, Domesick VB, Nauta WJH (1982) The amygdalostratial projection in the rat—an anatomical study by anterograde and retrograde methods. *Neuroscience* 7:615–630.
- Kita H, Kitai ST (1990) Amygdaloid projections to the frontal cortex and striatum in the rat. *J Comp Neurol* 298:40–49.
- Krettek JE, Price JL (1977) Projections from the amygdaloid complex to the cerebral cortex and thalamus in the rat and cat. *J Comp Neurol* 172:687–722.
- LeDoux JE (1991) Emotion and the limbic system concept. *Concepts Neurosci* 2:169–199.
- LeDoux JE (1992) Brain mechanisms of emotion and emotional learning. *Curr Opin Neurobiol* 2:191–197.
- LeDoux JE, Iwata J, Cicchetti P, Ried DJ (1988) Differential projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. *J Neurosci* 8:2517–2529.
- LeDoux JE, Farb CR, Ruggiero DA (1990) Topographic organization of neurons in the acoustic thalamus that project to the amygdala. *J Neurosci* 10:1043–1054.
- McDonald AJ (1991a) Organization of amygdaloid projections to the prefrontal cortex and associated striatum in the rat. *Neuroscience* 44:1–14.
- McDonald AJ (1991b) Topographic organization of amygdaloid projections to the caudate putamen, nucleus accumbens, and related striatal-like areas of the rat brain. *Neuroscience* 44:15–33.
- Mogenson GJ (1987) Limbic motor integration. *Prog Psychobiol Physiol Psychol* 12:117–170.
- Mogenson GJ, Jones DL, Yim CY (1980) From motivation to action: functional interface between the limbic system and the motor system. *Prog Neurobiol* 14:69–97.
- Nauta WJH, Smith GP, Faull RL, Domesick VB (1978) Efferent connections and nigral afferents of the nucleus accumbens in the rat. *Neuroscience* 3:385–401.
- Neafsy EJ (1990) Prefrontal cortical control of the autonomic nervous system: anatomical and physiological observations. In: *The prefrontal cortex: its structure function and pathology* (Uylings HBM, van Eden CG, DeBruin JPC, Corner MA, Feenstra MGP, eds), pp 185–222. Amsterdam: Elsevier.
- Paxinos M, Watson C (1986) *The rat brain in stereotaxic coordinates*, 2nd Ed. Sydney: Academic.
- Pennartz CMA, Dolleman-Van der Weel MJ, Lopes da Silva FH (1992) Differential membrane properties and dopamine effects in the shell and core of the rat nucleus accumbens studies *in vitro*. *Neurosci Lett* 136:109–112.
- Price JL, Russchen FT, Amaral DG (1987) The limbic region. II. The amygdaloid complex. In: *Handbook of chemical neuroanatomy*, Vol 5, Integrated systems of the CNS, Pt I, Hypothalamus, hippocampus, amygdala, retina (Björklund A, Hökfelt T, Swanson LW, eds), pp 279–388. Amsterdam: Elsevier.
- Ragsdale CW, Graybiel AM (1988) Fibers from the basolateral nucleus of the amygdala selectively innervate striosomes in the caudate nucleus of the cat. *J Comp Neurol* 269:506–522.
- Ray A, Henke PG, Sullivan RM (1987) The central amygdala and immobilization stress-induced gastric pathology in rats: neurotensin and dopamine. *Brain Res* 409:389–402.
- Russchen FT, Price JL (1984) Amygdalostratial projections in the rat. Topographical organization and fiber morphology shown using the lectin PHA-L as an anterograde tracer. *Neurosci Lett* 47:15–22.
- Sesack S, Deutch AY, Roth RH, Bunney BS (1989) Topographic organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with *Phaseolus vulgaris* leucoagglutinin. *J Comp Neurol* 290:213–242.
- Shinonaga Y, Takada M, Mizuno N (1994) Topographic organization of collateral projections from the basolateral amygdaloid nucleus to both the prefrontal cortex and the nucleus accumbens. *Neuroscience* 58:389–397.
- Sternberger LA (1979) *Immunocytochemistry*, 2nd Ed. New York: Wiley.
- Swanson LW, Mogenson GJ (1981) Neural mechanisms for the functional coupling of autonomic, endocrine, and somatomotor responses in adaptive behavior. *Brain Res Rev* 3:1–34.
- Tago H, Kimura H, Maeda T (1986) Visualization of detailed acetylcholinesterase fiber and neuron staining in rat brain by a sensitive histochemical procedure. *J Histochem Cytochem* 34:1431–1438.
- Turner BH, Herkenham M (1991) Thalamoamygdaloid projections in the rat: a test of the amygdala's role in sensory processing. *J Comp Neurol* 313:295–325.
- Turner BH, Zimmer J (1984) The architecture and some of the interconnections of the rat's amygdala and lateral periallocortex. *J Comp Neurol* 227:540–557.
- Veenman CL, Reiner A, Honig MG (1992) Biotinylated dextran amine as an anterograde tracer for single- and double-labeling studies. *J Neurosci Methods* 41:239–254.
- Voorn P, Gerfen CR, Groenewegen HJ (1989) Compartmental organization of the ventral striatum of the rat: immunohistochemical distribution of enkephalin, substance P, dopamine and calcium binding protein. *J Comp Neurol* 289:189–201.
- Weiskrantz I (1956) Behavioral changes associated with ablation of the amygdaloid complex in monkeys. *J Comp Physiol Psychol* 49:381–391.
- Wright CI, Groenewegen HJ (1995) Patterns of convergence and segregation in the medial nucleus accumbens of the rat: relationships of prefrontal cortical, midline thalamic and basal amygdaloid afferents. *J Comp Neurol* 361:383–403.
- Zaborszky L, Alheid GF, Beinfeld MC, Eiden LE, Heimer L, Palkovits M (1985) Cholecystokinin innervation of the ventral striatum: a morphological and radioimmunological study. *Neuroscience* 14:427–453.
- Zahm DS, Brog JS (1992) On the significance of subterritories in the “accumbens” part of the rat ventral striatum. *Neuroscience* 50:751–767.
- Zahm DS, Heimer L (1990) Two transpallidal pathways originating in the rat nucleus accumbens. *J Comp Neurol* 302:437–446.
- Zahm DS, Heimer L (1993) Specificity in the efferent projections of the nucleus accumbens in the rat: comparison of the rostral pole projection patterns with those of the core and shell. *J Comp Neurol* 327:220–232.