Cholinergic Innervation of the Amygdaloid Complex in the Human Brain and its Alterations in Old Age and Alzheimer’s Disease

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ABSTRACT

The cholinergic innervation of the human amygdaloid complex was studied immunohistochemically with a choline acetyltransferase (ChAT) antibody in eight brains: five control and three with Alzheimer’s disease (AD). All amygdaloid nuclei displayed ChAT-immunopositive axons and varicosities. The density of these axons reached levels that were higher than in any other part of the forebrain except for the striatum. The highest level of ChAT-immunopositive profiles was seen in the basolateral nucleus and the second highest in the lateral part of the central nucleus. The basomedial, accessory basal, and cortical nuclei, the amygdalo-hippocampal and cortico-amgydaloid transition areas, as well as the anterior amygdaloid area, showed a moderate density of ChAT-positive varicosities and fibers. The lateral nucleus displayed a relatively low density of cholinergic innervation, and there were only rare ChAT-positive fibers in the medial nucleus. Although the level of cholinergic innervation in the lateral nucleus was relatively lower than in many of the other amygdaloid nuclei, it was approximately equivalent to that of entorhinal cortex, a region that receives one of the heaviest cholinergic inputs in the cerebral cortex. The distribution of the cholinergic fibers as studied by ChAT immunohistochemistry was nearly identical to that observed with AChE histochemistry. Quantitative densitometry in control specimens showed that there was no decline of amygdalo-hippocampal cholinergic input when middle-aged subjects were compared with senescent subjects. In AD there was a severe and regionally selective depletion of this innervation in the amygdaloid complex. The cortical, accessory basal, and lateral nuclei displayed the most severe loss of ChAT-positive profiles, whereas the basolateral, and especially the central, nuclei displayed relatively little change. There was no consistent relationship between the loss of cholinergic fibers and the density of amyloid plaques and neurofibrillary tangles in amygdaloid nuclei.

Key words: immunohistochemistry, choline acetyltransferase, acetylcholinesterase, limbic system, dementia, acetylcholine

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the distribution of this innervation (Ben-Ari et al., '77; Hoover et al., '78; Girgis, '80; Svendsen and Bird, '85; Hellendall et al., '86).

Choline acetyltransferase immunocytochemistry provides the most specific visualization of cholinergic pathways and has been used to study the cholinergic input into the amygdala of the rat (Hellendall et al., '86; Ichikawa and Hirata, '86), cat (Kimura et al., '81; Vincent and Reiner, '87), and monkey (Amaral and Bassett, '89), but not of the human brain. The purpose of this study is to examine the differential distribution of choline acetyltransferase-positive (cholinergic) fibers within the anatomical subdivisions of the human amygdaloid complex. As the basal forebrain cholinergic system and limbic structures show severe pathological changes in Alzheimer's disease, we also investigated the alterations of amygdaloid cholinergic innervation associated with this disease.

METHODS

The distribution of cholinergic markers was investigated in eight specimens (Table 1). All specimens were collected at autopsy and in accordance with a protocol approved by the Human Studies Committee at Beth Israel Hospital. Five brains were obtained from mentally normal individuals who died at the ages of 55 (two), 71, 72, and 84 years, and three brains from patients with AD who died at the ages of 67, 82, and 87 years. In the latter three cases the diagnosis of AD was made clinically and established pathologically with Thioflavin-S histofluorescence according to the NINCDS (McKahn et al., '84) and Khatchaturian (85) criteria. The duration of dementia varied from 9 to 11 years. Extensive clinical information was available for each subject. The cause of death was cardiovascular or respiratory.

All eight brains were fixed (in 4% buffered paraformaldehyde for 24–40 hours), cryoprotected, and cut coronally at a thickness of 40 µm as previously described (Mesulam and Geula, '82). Several sets of sections were obtained, each spanning the entire amygdala at 1–2 mm intervals. The angle of cut was comparable in all cases. One set of sections was stained with cresyl violet for cytoarchitectonic identification, a second matching set with a modified Koele–Friedenwald histochemical procedure (Mesulam and Geula, '88) for acetylcholinesterase (AChE), and a third set with choline acetyltransferase (ChAT) immunocytochemistry with a polyclonal antibody raised against human placental ChAT (German et al., '85). An additional set of sections immunostained for ChAT was counterstained with neutral red in order to assess, within the same section, the relationship between cholinergic fibers and nuclear subdivisions of the amygdaloid complex.

For AChE histochemistry, sections were mounted on glass slides, air dried, rinsed 6 times (30 seconds each) in 0.1 M acetate buffer at pH 5.5, and incubated for 3.5–6 hours on a shaker at room temperature in a solution made by adding 72 mg ethopropazine, 750 mg glycine, 500 mg copper sulfate, 2,400 mg acetylthiocholine iodide, and 6,800 mg sodium acetate (NaC2H3O2•3H2O) to 1,000 ml distilled water. The solution was titrated to pH 5.5 with glacial acetic acid. The slides were then washed 6 times (30 seconds each) in the acetate buffer and placed for 1 minute in a developer made by dissolving 19.2 g sodium sulfide (Na2S•9H2O) in 500 mL of 0.1 N HCl (resultant solution adjusted to pH 7.8 by titrating with 10 N HCl). Sections were rinsed again 6 times (30 seconds each in the acetate buffer) and placed in an intensification solution of 1% (w/v) aqueous silver nitrate (AgNO3). The sections were rinsed, dehydrated in graded ethanol, cleaned in xylene, and coverslipped with Permount.

Immunohistochemistry was performed following the avidin-biotin ABC procedure (Hsu et al., '81). Sections were incubated in a solution of primary antibody (1/1,000 dilution in a vehicle of PBS with 10% normal goat serum and 0.5% Triton X-100) overnight at 4°C. Diaminobenzidine (DAB) was used as chromogen. The DAB polymers were intensified by using a modified Fontana–Masson method (Masson, '28; Kitt et al., '88). The sections were incubated in a 2.5% silver nitrate solution, then placed in a 0.2% gold chloride solution, and fixed in 5% sodium thiosulfate.

Two types of controls for ChAT immunocytochemistry were used. In one set of control sections the specific ChAT antibody was replaced with an irrelevant IgG. An additional absorption control was obtained by preincubating the primary antibody in the presence of purified human ChAT before using it for immunocytochemistry. For these absorption controls, 10 µl of purified ChAT (1 µg/µl) was added to 5 µl of ChAT antibody (2 µg/ml) and placed in 100 µl of the same vehicle. This solution was left for 2 hours at room temperature and overnight at 4°C. The volume was then brought to 5 ml with vehicle, resulting in a final concentration of 2 µg/ml of ChAT and a 1/1,000 dilution of ChAT antibody. This solution was used for immunostaining of ChAT-adsorbed control sections. In tissue from patients with AD, the amygdala was also examined in sections stained for Thioflavin-S histofluorescence.

### Table 1. Subjects for Choline Acetyltransferase Immunocytochemistry

<table>
<thead>
<tr>
<th>Case</th>
<th>Gender</th>
<th>Age (yr)</th>
<th>Death to fixation (hr)</th>
<th>Diagnosis</th>
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<tr>
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<td>M</td>
<td>55</td>
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<td>Control</td>
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<tr>
<td>6</td>
<td>F</td>
<td>67</td>
<td>4.5</td>
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</tr>
<tr>
<td>7</td>
<td>F</td>
<td>82</td>
<td>6</td>
<td>AD (9 yr)</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>87</td>
<td>12</td>
<td>AD (10 yr)</td>
</tr>
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*The numbers in parentheses indicate disease duration.*
The density of ChAT-immunoreactive axons in the amygdaloid complex in the control brains was charted semiquantitatively with the help of a microplotter digitally connected to the movable stage of a microscope (Microplotter 4000+, Dilog Instruments, Tallahassee, FL). Nuclear subdivisions of the amygdaloid complex were identified in sections stained with cresyl violet and AChE histochemistry, and then in ChAT stained sections. The classification proposed by Crosby and Humphrey (41) was used for nuclear designations, and several other studies were also useful for delineating boundaries (Krettek and Price, '78; Price et al., '87).

For qualitative comparisons of cholinergic innervation in controls and patients with AD, ChAT immunostained sections were first subjected to a general survey. Microphotographs at 130× magnification were then taken at representative sites in each nucleus from non-AD and AD brains at comparable amygdaloid levels. For quantitative comparisons, representative areas of the basolateral, central, and lateral nuclei were photographed at 600× in each of the eight specimens and the photographs were digitized for densitometry. These photographs were taken at a level corresponding to Figure 3C, where all three relevant nuclei could be identified in the same tissue section. At this level, the density of ChAT' innervation in the lateral nucleus is higher than at more rostral levels of this nucleus. In the central nucleus, the photograph was taken at the border of the lateral (Cel) and medial (Cem) sectors, and was therefore not representative of the highest densities that exist in the nucleus. These choices were dictated by the practical need to have optimally stained material for equivalent sectors and levels of each of the three nuclei in all eight cases. Thus, the densitometry results provide systematic comparisons among the eight cases but do not provide a representative quantification of overall innervation densities in the three relevant nuclei. The photographs were digitized by an image analyzer (Imaging Research Inc., St. Catharines, Ontario, Canada), and the number of supra-threshold pixels (containing ChAT-positive axons and varicosities) was calculated automatically and expressed as a percentage of the total imaged area which covered 245,280 pixels. Illumination during photography and exposures during printing were the same for all 24 frames. Unevenness of background illumination was corrected by the image analyzer. During digitization, we placed fiduciary gray and white cards at the edges of each of the 24 photographs. These were used to establish uniform threshold and background levels. In seven of the cases, the photographs were number coded and the investigator doing the image analysis was blinded with respect to age, diagnosis, or nucleus of origin.

An additional brain (from a 25-year-old individual) was embedded in celloidin, cut into 35 μm thick sections, and stained with cresyl violet and Loyez myelin stains for the identification of anatomical landmarks.

**RESULTS**

**Amygdaloid nuclei**

Nuclear boundaries within the amygdaloid complex were easily delineated with the help of Nissl, myelin, and AChE stains (Figs. 1 and 2). As proposed by Crosby and Humphrey (41), we divided the amygdaloid complex into two groups, laterobasal and corticomedia. The laterobasal group includes the lateral (L), the basolateral (BL), the basomedial (BM), and the accessory basal (ABl and ABm) amygdaloid nuclei. These nuclei are separated from each other by weakly myelinated fiber bundles and, on occasion, by intercalated cell masses (IC in Fig. 2A). The nomenclature of these nuclei is based on phylogenetic rather than spatial considerations. Thus the designations of “lateral” and “medial” do not necessarily reflect true anatomical relationships in the human brain.

The corticomedia group includes the cortical (Co), the medial (M), and the central (Ce) nuclei. The nuclei in this group are relatively small and less well delimited from the substantia innominata and the cerebral cortex of the medial temporal lobe. In addition to these main nuclei, the medial amygdaloid region also includes three other poorly delimited areas, designated the anterior amygdaloid area (AAA), the cortico-amygdaloid transition area (CAT), and the amygdalo-hippocampal area (AHA) (Figs. 1 and 2).

**Cholinergic innervation of the amygdala in the normal brain**

The intensified ChAT immunoocytochemical staining yielded a brownish-black, granular reaction product. The ChAT immunostaining of the amygdaloid complex was consistent in all five control brains examined. All nuclei of the amygdaloid complex contained ChAT-immunoreactive axons and punctate varicosities (Figs. 3–6). There was substantial regional variation in the density of these axons. In some regions ChAT-immunoreactive varicosities were so dense that individual fibers were not identifiable [e.g., basolateral nucleus (BL) in Fig. 4B]. The varicosities were interpreted to represent preterminal specializations. On occasion, varicose axons formed complex dense clusters (Figs. 5 and 6). In counterstained sections, varicosities along ChAT-positive axons were intimately associated with the cell body of amygdaloid neurons. The immunopositive axonal profiles were not seen in control sections obtained by substituting ChAT with an irrelevant IgG or after preincubating the antibody with pure ChAT (Fig. 7). A diffuse or punctate light perikaryal staining observed in various amygdaloid nuclei was not blocked in sections processed with ChAT-adsorbed antibody and was therefore considered to be nonspecific. No consistent evidence could be obtained that the amygdala contained intrinsic ChAT-positive perikarya, except for a few aberrant neurons of the nucleus basalis (Ch4) that were located at the boundary of the amygdala with the substantia innominata.

**Laterobasal group**

**Basal nucleus.** The basal amygdaloid nucleus can be divided into basolateral (BL) and basomedial (BM) subdivisions. The basolateral nucleus contained the highest density of ChAT-immunoreactive profiles and was sharply delineated from adjacent nuclei on the basis of its immunoreactivity (Figs. 3, 4B, 5A, and 8A). Varicosities were very dense and individual fibers were difficult to discern except under the highest magnifications (Figs. 4B and 5A). The very high density of ChAT-positive profiles remained unchanged along the anter-posterior extent of the basolateral nucleus. The density of cholinergic fibers decreased gradually in the transition from the basolateral nucleus to the basomedial nucleus (Figs. 3 and 8A). The basomedial component of the basal nucleus can be divided into a deep (BMD) and a superficial (BMS) portion. The superficial portion was confluent with the corticoamygdaloid area (CAT) medially and lateral nucleus (L) laterally. The deep portion merged into the accessory basal nucleus (Fig. 8A).
Fig. 1. Coronal sections through the amygdala in a 25-year-old normal human brain stained with cresyl-echt violet (A) and the Loyez myelin stain (B). Dorsal is towards the top, medial to the left. Magnification: 6×.
The deep and superficial portions of the basomedial nucleus had a high to moderate density of ChAT-positive profiles (Fig. 3). The density of cholinergic fibers in the basomedial nucleus did not show substantial variations in its anteroposterior extent.

Accessory basal nucleus. This nucleus has traditionally been subdivided into a "lateral" (ABI) and a "medial" (ABm) division. Both divisions displayed a moderate density of ChAT-positive varicosities and fibers (Figs. 3B, 5B, and 8A). Thin fibers with varicosities predominated, and there were occasional thick nonvaricose fibers (Fig. 5B). The density of ChAT immunopositivity was not uniform within the nucleus, and patches of slightly more intense staining could be identified (Figs. 3B,C and 8A). The lateral accessory basal nucleus (ABI) was separated from the basolateral nucleus by an intervening, mostly immunonegative, and thinly myelinated fiber bundle (Fig. 1B). The medial accessory basal nucleus (ABm) merged into the basomedial nucleus (BM) ventrally and the cortical nucleus (Co) medially (Fig. 8A). At anterior and midamygdaloid levels, a dense collection of ChAT-positive fibers was located at the junction of the accessory basal, cortical, and basomedial nuclei (Fig. 8A). In sections stained with cresyl violet this dense matrix corresponded to intercalated cell masses (IC in Figs. 2 and 8A).

Lateral nucleus. In comparison with the other amygdaloid nuclei, the lateral nucleus (L) had a low density of ChAT-positive fibers (Figs. 3, 5C, and 8A). Most of these were thin and varicose, while others were thick and straight (Fig. 5C). In general, the density was higher in the posterior levels of the nucleus. There were also ventral and lateral patches with a relatively higher density of ChAT-positive fibers (Fig. 3).

Cortico medial group.

Cortical nucleus. The cortical nucleus (Co) displayed a moderate density of mostly varicose and thin ChAT-positive fibers (Figs. 3 and 8A). The density was higher in the periphery of the nucleus but in a patchy fashion (Fig. 8A). The density of ChAT-positive profiles was slightly higher in the posterior parts of the nucleus (Fig. 3).

Medial nucleus. Among all amygdaloid nuclei the medial nucleus (M) had the lowest density of ChAT-immunonegative profiles (Fig. 3). Only few ChAT-positive varicose axons were detected.

Central nucleus. This nucleus had a clearly delimited and oval lateral sector (Cell), which was surrounded by an
immunonegative fiber bundle and a less well-delineated medial part (Cem), which merged into neighboring structures such as the substantia innominata (Figs. 1, 2, and 8A). The density of varicose ChAT-immunoreactive profiles was high within the lateral part of the central nucleus (Cel), but slightly less intense within its medial part (Cem; Figs. 3, 4A, and 8A). The density was slightly higher and more patchy in the more posterior levels, especially in the lateral part of the nucleus.

Transitional areas. The anterior amygdaloid area (AAA) showed a moderate to low level of ChAT immunostaining (Fig. 3A). The amygdalo-hippocampal (AHA) and cortic amygdaloid transition (CAT) areas had a moderate density of ChAT-positive fibers (Fig. 3). The staining in the cortico amygdaloid transition area was variable and patchy.

In overall ranking, the basolateral nucleus had the highest density of ChAT-positive fibers and varicosities, followed by the lateral part of the central nucleus. The accessory basal, the basomedial, and the cortical nuclei displayed the third highest level of immunoreactivity followed by the amygdalo-hippocampal area, the anterior amygdaloid area, the cortico amygdaloid transition area, and the lateral nucleus. The medial nucleus displayed the lowest density of ChAT-positive fibers. The density of cholinergic axons in the lateral nucleus was approximately equal to the highest density encountered in the entorhinal area. The density of ChAT and AChE reactivity reached much higher levels in the amygdala than in the hippocampal complex (Fig. 2).

There was a good overlap between ChAT-immunocytochemistry and AChE-histochemistry, and both markers displayed a similar pattern of differential staining intensity within the subnuclei of the amygdaloid complex (Figs. 2 and 3).

Alterations in the cholinergic innervation of the amygdaloid complex in Alzheimer’s disease

The amygdaloid complex showed considerable atrophy and loss of cholinergic axons in the three brains of patients with AD (Figs. 8–10). In ChAT immunostained sections, the most severe depletion of immunopositive profiles was observed in the lateral, cortical and the accessory basal nuclei, especially with respect to thin, varicose axons (Figs. 8–10). There was probably also some fiber loss in the medial nucleus, but since the density of cholinergic fibers was very low in control brains, the amount of fiber loss in this nucleus was difficult to assess. The ChAT-immunoreactive profiles were depleted to a lesser degree in the basolateral
Fig. 4. Choline acetyltransferase (ChAT) immunohistochemistry in a 55-year-old subject (Case 1). A: ChAT immunopositive axons and varicosities in the central nucleus (Ce). The curved arrow points to ChAT-immunopositive neurons belonging to Ch4 cell group. ChAT-positive axons (probably representing Ch4 efferents) are streaming towards the amygdala. B: The basolateral (BL) nucleus contains a very high density of ChAT-positive axons and varicosities, whereas the density is substantially lower in the accessory basal nucleus (AB). Dorsal is towards the top, medial to the left. Magnification: 94×.
Fig. 5. Choline acetyltransferase (ChAT) immunohistochemistry in a 71-year-old subject (Case 3). A: Very high density of ChAT-immunopositive varicosities is seen in the basolateral nucleus (BL). B: The accessory basal nucleus (AB) displays a moderate density of ChAT-positive axons and varicosities. C: Relatively low density of ChAT-positive axons is seen in the lateral nucleus (L). Individual fibers and varicosities are clearly visible. The perikaryal staining is nonspecific. D: ChAT-positive axons in layer 2 (L2) and layer 3 (L3) of entorhinal cortex (ENT). Note that the density of ChAT-positive axons in the lateral nucleus in C is comparable to that of entorhinal cortex in D. Magnification in A–D: 333×.
Fig. 6. Choline acetyltransferase (ChAT) immunohistochemistry in a 71-year-old subject (Case 3). A: Varicosities of ChAT-positive axons (curved arrow) in the lateral nucleus (L) give the axons a beaded appearance. B: Dense clusters with complex structures formed by ChAT-positive axons and varicosities (curved arrows) in the deep portion of the basomedial nucleus (BMd). Magnification in both frames is 1,170x.
nucleus and appeared relatively preserved in the lateral part of the central nucleus (Figs. 8–10).

The digitized densitometry of ChAT-positive profiles showed that there was no consistent difference in the density of cholinergic innervation when the two middle-aged specimens (55 years old in each case) were compared to the three older specimens (71, 72, and 84 years old). Compared to the older specimens, the AD cases (67, 82, and 87 years old) showed almost no change of cholinergic innervation in the central nucleus, a mild depletion in the basolateral nucleus, and a very severe depletion in the lateral nucleus (Table 2; Figs. 11 and 12).

Thioflavin-S immunofluorescence in two of the AD specimens (cases 6 and 8) showed that the density of plaques and tangles was higher in the lateral than in the central or basolateral nucleus. In case 7, however, the number of tangles was slightly higher in the basolateral nucleus than in the lateral nucleus (Fig. 13), even though the cholinergic depletion in this case, as in the others, was far more severe in the lateral nucleus (Table 2; Fig. 12). The basolateral nucleus seemed to have fewer dense-core, neuritic plaques than the other nuclei, but the overall number of plaques was equivalent to that of the lateral nucleus. We were therefore unable to identify a simple relationship between indices of AD pathology and the degree of cholinergic depletion.

**DISCUSSION**

Choline acetyltransferase (ChAT) immunohistochemistry revealed that the cholinergic innervation of the human amygdaloid complex is one of the heaviest in the entire forebrain. This innervation is differentially distributed among the various amygdaloid nuclei. The basolateral nucleus displays the highest level of ChAT-positive axons, and this level is only slightly less than that of the striatum, which shows one of the highest levels of ChAT-immunostaining in the brain. Even amygdaloid nuclei with relatively low levels of ChAT-immunostaining have a very high cholinergic innervation when compared with the innervation of the cerebral cortex. The lateral nucleus, for example, has a relatively low density of cholinergic innervation when compared with other amygdaloid nuclei, but displays a level of innervation which is comparable to that of layers II and III in the entorhinal cortex, a region that contains one of the heaviest cholinergic innervations in the cerebral cortex (Mesulam et al., '92). In most amygdaloid nuclei, the density of cholinergic innervation was substantially higher than that of the hippocampus. The amygdala therefore receives an exceptionally intense cholinergic input. Tracer experiments in the monkey suggest that the majority of this input originates from the anterolateral sector of the Ch4 nucleus basalis complex (Mesulam et al., '83).

The regional variations in the pattern of ChAT-positive immunostaining were comparable to those revealed by AChE histochemistry (Svenston and Bird, '85). This is in keeping with recent findings in the human cerebral cortex, which revealed an excellent overlap between AChE-rich and ChAT-positive fibers (Mesulam and Geula, '92). The differential ChAT-immunostaining of amygdaloid nuclei that we observed in the human brain was also nearly identical to that described in the monkey (Amaral and Bassett, '89).
Fig. 8. Choline acetyltransferase (ChAT) immunohistochemistry. A: Low power photomicrograph showing the various amygdaloid nuclei at a midamygdaloid level in a control brain from a 71-year-old man with no history of dementia (case 5). The darkly stained basolateral nucleus (BL) is clearly delimited from the adjacent accessory basal (ABI, ABm) and lateral (L) nuclei, whereas the transition from basolateral to the deep portion of the basomedial (BMD) nucleus is more gradual. The cortical nucleus (Co) shows a moderate level of staining, which becomes slightly more intense along the dorsomedial rim. The medial nucleus (M) has a very low density of ChAT-immunostaining. The central nucleus (Cel, Cem) shows the second highest density of staining, but in a patchy distribution. The dense fiber patch in the ventromedial ABm corresponds to intercalated cell masses (IC), a frequent location of such cell groups. B: Photomicrograph from a 82-year-old patient with AD (case 7), taken at approximately the same midamygdaloid level as in A. There is a major decrease in the size of the amygdaloid complex. The cortical (Co) and accessory basal (ABI and ABm) nuclei show the most severe depletion of cholinergic fibers. The central (Cem and Cel), basolateral (BL), lateral (L), and deep basomedial (BMD) nuclei show less change. The intercalated cell mass seen in the ABm nucleus in the control brain (A) is not a consistent landmark in all cases and is not visible in the AD brain. In both frames dorsal is towards the top and medial to the left. The dark subpial band of staining is mostly nonspecific. Magnification: 8.5 x.

The amygdala is a major component of the limbic system. Its diverse and complex behavioral affiliations are closely related to the realms of emotion, drive, memory, and autonomic control (Halgren, '81). Bilateral removal of the amygdala in monkeys, for example, produces striking behavioral changes, including emotional placidity, tameness,
Fig. 9. Choline acetyltransferase (ChAT) immunohistochemistry. Photomicrographs taken in a 71-year-old control brain (A,C,E) and in the brain of a 67-year-old patient with AD (B,D,F). A,B: The very dense ChAT-immunostaining in the control basolateral nucleus (BL) is slightly reduced in AD. C,D: The density of ChAT-positive fibers and varicosities in the central nucleus (Ce) in the control brain (C) is approximately equivalent to that observed in the brain of a patient with AD (D). E,F: The density of ChAT-positive axons in the lateral nucleus (L) in the control brain (E) is substantially reduced in AD. Occasional small clusters of ChAT-positive axons in an almost normal density (as shown in the bottom of frame F) can be seen in the lateral nucleus but are not representative of the generally much reduced density that this nucleus displays in AD. Magnification: 130×.
excessive mouthing of objects, and altered sexual behavior (Weiskrantz, '56). These behavioral changes were initially observed by Klüver and Bucy ('39) as a consequence of bilateral anterior temporal lobectomies in monkeys. The behavioral changes in these animals may reflect an impaired ability to form associations between stimuli and reinforcement (Weiskrantz, '56; Jones and Mishkin, '72), or to attach motivational significance to external stimuli (Mesulam, '86). Experiments based on electrophysiological recordings (Kling et al., '79; Rolls, '81, '92; Ono et al., '83) and cerebral blood flow measurements (LeDoux et al., '83) provide additional evidence for the involvement of the amygdala in emotion and drive, especially in attaching emotional valence and motivational significance to external stimuli and experiences. Additional support for the pivotal role of the amygdala in the processing of emotion comes from the observations of Gloer et al. ('82) in patients with temporal lobe epilepsy. These studies revealed that recall of emotionally charged experiences was more readily evoked by the stimulation and spontaneous discharges in the amygdala than in other temporal lobe regions (Gloer et al., '82).

The amygdala has also been implicated in memory and learning, especially in mediating polysensory-to-limbic associations and in sustaining the learning of emotionally charged material (Murray and Mishkin, '85; Gaffan and Harrison, '87; Gaffan et al., '88, '89; Gaffan and Murray, '90; LeDoux et al., '90). In contrast to hippocampal lesions, which can by themselves induce severe deficits of memory and learning, isolated amygdaloid lesions do not seem to cause severe amnestic disorders either in monkeys (Mishkin, '78) or in humans (Narabayashi et al., '63; Small et al., '77). Combined amygdala-hippocampal lesions, however, result in more severe memory deficits than lesions in either structure (Mishkin, '78; Phillips and Mishkin, '84). As pivotal components of the limbic system, the hippocampus and amygdala have substantial interconnections (Saunders and Rosene, '88; Saunders et al., '88). They are thus likely to have complementary roles in learning and memory, but the former structure seems to play a more critical role in memory, whereas the latter plays a more critical role in

Fig. 10. Choline acetyltransferase (ChAT) immunocytochemistry in the same control (A,C) and AD (B,D) brains as in Figure 9. A,B: The density of thin, varicose ChAT-positive fibers in the accessory basal nucleus (AB) in the control brain is severely reduced in AD. C,D: The cortical nucleus (Co), which normally has a moderate density of thin, varicose ChAT-positive fibers in the control brain, shows a severe depletion of these fibers in AD. Magnification: 130 x.
mood and affect (Mesulam, '88). It has also been shown that stimulation of the amygdala results in alterations in autonomic tone (Anand and Dua, '56) and influences the hypothalamic regulation of hormonal levels (Zolovick, '72).

The primate amygdala has been shown to have extensive connections with association cortex (Herzog and van Hoesen, '76; Aggleton et al., '80; Turner et al., '80; Mufson et al., '81; Porrino et al., '81; Van Hoesen, '81; Amaral and Price,
CHOLINERGIC INNERVATION OF THE HUMAN AMYGDALOID COMPLEX

Fig. 12. The density of cholinergic innervation in all eight cases. The numbers along the vertical axis indicate the percentage of pixels that were above threshold (i.e., that contained ChAT-positive axons and varicosities) in the three nuclei where these measurements were obtained.

TABLE 2. Proportional Area Covered by Choline Acetyltransferase-
Positive Neurites

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</tbody>
</table>

1This table reflects the percentage of pixels that contained ChAT-positive neurites in the three groups of subjects. The numbers in parentheses refer to the case numbers in Table 1.

The responsiveness of the amygdala to cholinergic stimulation was demonstrated almost 30 years ago in the course of experiments where the cholinergic agonist carbamol was injected into the amygdala of rats (Grossman, '83; '72; Iglic et al., '70). Such injections caused continuous epileptic activity, overt psychomotor seizures, extreme aggressiveness, and abnormalities in escape-avoidance learning. The amygdala is a region that also has one of the lowest thresholds to kindling and seizure activity. The particularly intense cholinergic innervation of the amygdala may contribute to this susceptibility.

The amygdaloid complex displays severe pathology in AD (Scott et al., '92). Morphometric studies in AD revealed that the medial, cortical, and the central (medial part) nuclei had the highest amount of atrophy and decrease in cell-packing density, whereas the basomedial and lateral nuclei showed the least changes (Herzog and Kemper, '80). Brockhaus ('88) described the distribution of senile plaques and neurofibrillary tangles in a single case. He reported severe involvement of the cortical, accessory basal, ventral basolateral, and deep basomedial nuclei, with sparing of the dorsal basolateral as well as the lateral nucleus, and little involvement of the central, medial, and superficial basomedial nuclei. Subsequent studies investigating the distribution of neurofibrillary tangles, neuritic plaques (Brady and Mufson, '90, Kromer-Vogt et al., '90), and Aβ-immunoreactive fibers (Unger et al., '91) have confirmed these early findings. In the Kromer-Vogt et al. ('90) study, for example, the lateral, laterobasal, central, and medial nuclei generally contained fewer plaques and tangles than the other nuclei.

The loss of cholinergic innervation does not display the same pattern; we found that the loss of cholinergic innervation was severe in the lateral, accessory basal, and cortical nuclei, whereas it was much milder in the basolateral and central nuclei. Furthermore, we were unable to detect a consistent relationship between plaque or tangle densities, and the pattern of cholinergic denervation. These observations indicate that there is no obvious relationship between the traditional neuropathological markers of AD and the differential cholinergic depletion in amygdaloid nuclei.

The widespread depletion of cholinergic innervation in the cerebral cortex is one of the earliest and most prominent components in the neuropathology of AD. Much of the existing literature has emphasized the loss of cholinergic innervation in the cerebral cortex, including the hippocampal formation. Our study shows that there is also a severe and regionally selective depletion of cholinergic input in the amygdala. This depletion may contribute to the severe abnormalities of memory, mood, and motivation that are seen in patients with AD. The substantial loss of cholinergic innervation that we observed in the amygdala is consistent with biochemical investigations that have repeatedly shown a severe depletion of choline acetyltransferase activity in the amygdala of patients with AD (Davies and Maloney, '76; Rossor et al., '82; Esiri et al., '90).

Our results also indicate that the cholinergic innervation of the amygdala does not show an age-related decline during late adulthood. This is consistent with the biochemical findings of Hornykiewicz et al. ('90) and adds further credence to our previous studies showing that the age-related changes of cholinergic innervation in cortical and limbic structures is regionally specific and quite modest (Geula and Mesulam, '89).
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LITERATURE CITED


