ABSTRACT

The distribution of GABA-like (GABA-Li) immunoreactivity in the rat amygdaloid complex was studied by using an anti-GABA antibody. GABA-Li positive neurons and processes were present in every nucleus of the complex. Three patterns of immunoreactivity were revealed: (1) the intercalated masses and the lateral olfactory tract nucleus exhibited the most intense staining of the neuropil, and virtually every neuron was labeled, (2) the central and medial nuclei contained intensely labeled neuropil and moderately labeled neurons, and (3) in the remaining nuclei, the neuropil was weakly labeled, and relatively numerous GABA-Li neurons were present. Our results suggest that: (1) the intercalated masses and lateral olfactory tract nucleus consist of large aggregates of GABA-Li immunoreactive neurons, and (2) the lateral, basal dorsal, and the posterior cortical nuclei may constitute a significant source of GABAergic connections to other amygdaloid nuclei, in particular to the medial and central nuclei.

Key words: immunocytochemistry, GABAergic system, amygdala

The mechanism by which amygdaloid nuclei influence the activity of other limbic centers and coordinate motivational states and emotions is poorly understood (Eleftheriou, '72; Ben-Ari, '81). Amygdaloid nuclei control important biological functions; they influence alimentary, defensive, aggressive, neurosecretory, and sexual functions as well as memory and learning processes (Eleftheriou, '72; Ben-Ari, '81). Experiments performed two decades ago had shown the existence of two functional regions within the amygdaloid complex: an excitatory (centromedial) and an inhibitory (basolateral) part (Kaada, '72; Fonberg, '81). The anatomical basis of these opposite activities has not been fully elucidated.

The inhibitory transmitter α-aminobutyric acid (GABA) plays an important role in the modulation of neuronal activity. However, the organization of the GABAergic system in the amygdala has not been investigated in detail. Thus, at present, it is not clear how GABA-containing neurons are involved in mediating the inhibitory or excitatory actions of the amygdaloid stimulation. The basolateral (inhibitory) part of amygdaloid complex is known to have massive, reciprocal connections with different cortical fields, and during evolution it has increased in size dramatically in parallel with the extensive development of the neocortex (Johnston, '23; Koikegami, '63; Whitlock and Nauta, '66; Druga, '70; Jacobson and Trojanowski, '75; Herzog and Van Hoesen, '76; Kosmal, '76; Krettek and Price, '77; Hall, '63; Aggleton et al., '80; Kosmal and Dabrowska, '80; Muesen et al., '81; Ottersen, '82). The basolateral region also has axonal projections to the centromedial zone (Krettek and Price, '78a; Noble et al., '81a,b; Ottersen, '82; Price and Amaral, '84). Furthermore, direct connections emerging from the basolateral part and extending to the hypothalamus and other brainstem centers, which may mediate its inhibitory effect, are less numerous than the projections emerging from the centromedial (excitatory) region (Cowan et al., '65; Leonard and Scott, '71; De Olmos, '72; Krettek and Price, '78b; Aggleton et al., '80; Post and Mai, '80; Mehler, '80; Noble et al., '81a,b; Price, '81a,b). This raises the question of whether the amygdala mediates its inhibitory effect on the hypothalamus and other subcortical limbic centers by means of direct long axonal connections or by means of an inhibition of the centromedial zone.

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We describe in the present study the distribution of GABA-like (GABA-Li) immunoreactive neurons and processes in the amygdala.

**MATERIALS AND METHODS**

The distribution of GABA-like immunoreactivity in the amygdaloid complex has been studied by using specific anti-GABA antibodies (Seguela et al., '84; Geffard et al., '85). Male Wistar rats (n = 9) were anesthetized with pentobarbital, and perfusion-fixation was performed by intracardiac injection of saline followed by 5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.6). After postfixation (c. 1 h) in the same fixative, 50-μm vibratome sections were cut and used for the immunohistochemical procedure (Seguela et al., '84). The concentration of the antisera was 1/3,000 to 1/5,000 stained all sera at a concentration of 1:3,000 or 1:5,000 stained all controls of the antibody specificity included incubation of tissue elements weakly and unselectively. The sections incubated without primary serum did not show the stain. The excess of immunogen or nonimmune serum; other sections were incubated without the primary antiserum. The control sera at a concentration of 1:3,000 or 1:5,000 stained all tissue elements weakly and unselectively. The sections incubated without primary serum did not show the stain.

**RESULTS**

The terminology of the amygdaloid nuclei established by Johnston ('23) has been used with modifications resulting from more recent morphological studies and acetylcholinesterase stains (Nitecka, '75; Price, '81a). The simplified diagrams in Figure 1 show the amygdaloid nuclei of the rat in frontal sections. Diagrams in Figure 2 illustrate the pattern of distribution of GABA-Li neurons in amygdaloid nuclei; the location of every GABA-Li neuron is depicted by camera lucida. The general distribution of GABA-Li neurons and neuropil in the amygdaloid nuclei are shown in the photomicrographs of Figures 3-5. It is evident from these figures that every amygdaloid nucleus contained GABA-Li material. However, the pattern and location of the neurons varied, as did their shapes, the density of their distribution, and the intensity of the staining in the neuropil.

Relying on the organization of GABA-Li neurons, we have differentiated three groups of nuclei: group I (lateral olfactory tract nucleus and intercalated nuclei) with intensely stained neurons and heavy labeling in the neuropil (Figs. 3-7); group II (central and medial nuclei and anterior amygdaloid area) with moderately labeled neurons and intensely labeled neuropil (Figs. 3-5, 8); group III (remaining nuclei) with weakly labeled neuropil and scattered clusters of GABA-Li neurons (Figs. 3-5, 8, 9).

**Nuclei of group I**

**Intercalated nuclei (Figs. 3-6).** The small neurons of the intercalated masses were intensely labeled. Relaying on the counterstained sections, it was conspicuous that virtually every neuron contained GABA-Li material (Figs. 3-6). The neuropil was also intensely stained. This corresponded probably to fine branches of the dendrites originating from GABA-Li perikarya located in the intercalated nuclei. The intensely labeled neuropil often extended beyond the regions of aggregated perikarya (Figs. 5, 6). As a consequence of this organization, histological sections made through the periphery of the intercalated nuclei showed spots of intensely labeled neuropil within adjacent amygdaloid nuclei (Fig. 5, big arrows).

**Lateral olfactory tract nucleus (Fig. 7).** Three subdivisions of the lateral olfactory tract nucleus could be identified: dorsal, intermediate, and ventral areas (Fig. 7). In the dorsal area, scattered GABA-Li perikarya were conspicuous and the neuropil was poorly labeled. The intermediate area contained an intensely stained neuropil and numerous large, pyramidal neurons with intense GABA-Li material. Virtually every neuron of the large intermediate zone was labeled (Fig. 7A, B). The dendrites of these neurons were always directed ventrally and the initial segments of the apical dendrites were often conspicuous (Fig. 7B). In contrast, very few GABA-Li neurons were observed in the ventral area, but the neuropil was intensely labeled (Fig. 7A).

**Nuclei of group II**

**Central nucleus (Figs. 3-5, 8A,B).** The most prominent feature of this nucleus was the presence of an intensely labeled neuropil and densely packed GABA-Li neurons. The labeling of the neuropil was not homogeneous; small, very intensely stained areas were conspicuous especially in the medial part of the nucleus (Fig. 5, big arrow).

Three areas showing different arrangement of GABA-positive neurons could be identified in frontal sections. Two regions marked as "x" and "y" in Figure 5 correspond to the medial and central part of the nucleus, respectively (Johnston, '23; Hall and Geneser-Jensen, 71; Hall, 72; Nitecka, '75; Price, '81a). In region "x", which probably corresponds to a caudal extension of the anterior amygdaloid nucleus (Johnston, '23; Halland Geneser-Jensen, 71; Hall, 72; Nitecka, '75; Price, '81a), clusters of medium-size neurons and single larger neurons were arranged in irregular rows. These are probably situated along the bundles of
fibers of the stria terminalis (Fig. 5). In region "y", large clusters (c. 10 cells) of medium-size, moderately stained perikarya were arranged irregularly (Figs. 5, 8A—open arrows). Initial segments of dendrites emerging from these neurons were often stained (Fig. 8B). In some cases because of the intense labeling of the neuropil, it was difficult to observe the moderately labeled cell bodies. GABA-Li punctate structures, presumably boutons, outlined GABA-Li negative (Fig. 8B, small arrow) or GABA-Li positive perikarya or dendrites (Fig. 8B, large arrow). In region "z", the arrangement of GABA-Li positive perikarya is reminiscent to that seen in the adjacent striatum (Fig. 5). Labeled, medium-size perikarya were arranged in small clusters (4–5 neurons), which were less densely packed than in region "y". Acetylcholinesterase histochemistry also suggests similar subdivisions of the central nucleus (Hall and Geneser-
Fig. 3. Photomontage to illustrate the pattern of GABA-Li material in a frontal section the anterior part of the amygdala. The arrows indicate the intercalated masses. Scale bars=200 μm.

Fig. 4. Photomicrograph illustrating the pattern of GABA-Li material in the caudal part of the amygdala. The arrows indicate the intercalated masses. Asterisks indicate artifacts. Scale bar=200 μm.
Fig. 5. Photomontage of GABA-Li and acetylcholinesterase activity patterns in the central portion of the amygdala. x,y,z indicates the three areas of the central nucleus with different patterns of GABA-Li material; the pattern of the acetylcholinesterase activity is shown in the boxed photomicrograph. Small arrows indicate the intercalated masses and large arrows the neuropil with intense GABA-Li neurons. Scale bars=200 μm; 1 mm for the boxed photomicrograph.

Jensen, '71; Hall, '72; Nitecka, '75; Ben Ari et al., '77; also see Fig. 5). Other lines of evidence suggest that these subdivisions of the central nucleus have also different patterns of connections (Jones and Burton, '76; Norgen, '76; McBride and Sutin, '77; Ottersen and Ben-Ari, '78; Veening, '78; Nitecka et al., '79, '80; Saper and Loewy, '80). Areas “y” and “z” are thought to be connected, respectively, with the viscerosensory brainstem centers and with the somatosensory nuclei of the posterior thalamic region.

Medial nucleus (Figs. 3-5). The neuropil of the medial nucleus contained relatively high levels of GABA-Li material, particularly in the caudal regions of the nucleus (Fig. 4). As in the central nucleus, small areas with intense labeling of neuropil were observed notably in the vicinity of the intercalated masses (Fig. 5, large arrow). Also, clusters of small, intensely labeled perikarya were scattered in

Fig. 6. Photomicrographs to illustrate GABA-Li perikarya and neuropil of the intercalated masses (I). Note the location of the largest aggregates of intercalated masses and the numerous large GABA-Li perikarya of the anterior pole of the basal dorsal nucleus. Note the densely packed, small GABA-Li perikarya of the intercalated masses. Higher magnification to illustrate GABA-positive neurons in the intercalated masses is shown in the insert. Scale bars=100 μm.
Nuclei of group III

Lateral nucleus (Figs. 3–5). GABA-Li neurons were homogeneously distributed throughout the entire extent of the nucleus. Their labeling varied in intensity from moderate to strong. Most of the GABA-Li neurons were of medium size (15–20 μm), a few large neurons (30 μm) were, however, present. Medium-size, labeled neurons were grouped in clusters of 2–4 neurons (Fig. 5). The neuropil was labeled weakly or moderately.

Anterior amygdaloid area (Fig. 7). The distribution of GABA-immunoreactivity in this region was similar to that seen in the medial nucleus. The neuropil was intensely stained with few GABA-positive cell bodies.

Basal dorsal nucleus (Figs. 3–5, 8C,D). The distribution of GABA-Li neurons and neuropil was comparable to that seen in the lateral nucleus. Large, intensely labeled neurons were especially numerous in the anterior part of the nucleus (Fig. 6A). They were sparsely distributed and were not concentrated in clusters (Fig. 9A). Large pyramidal-shaped neurons with labeled initial dendrites were often conspicuous (Fig. 8C, arrowhead). GABA-Li punctate structures were occasionally noted in contact with GABA-Li perikarya (Fig. 8C). Also, GABA-positive punctate structures often outlined the shape of unlabeled, large-size cell bodies (Fig. 8C).

Posterior part of the cortical nucleus (Figs. 4, 9B). Scattered clusters of medium-size labeled neurons were prominent features of this region. Large, heavily stained neurons with a pyramidal shape were also conspicuous; the initial segments of the dendrites were often also labeled (Fig. 9B, open arrow).
Basal ventral nucleus (Figs. 3, 9A). GABA-Li perikarya of medium size and a few large ones were conspicuous in this nucleus. Clusters of medium-size neurons were distributed within the nucleus and the neuropil was poorly labeled. This distribution is similar to that seen in layer III of the adjacent piriform cortex. Interestingly, the border between the basal ventral nucleus and this layer of the piriform cortex was ill-defined (Fig. 3).

Anterior part of the cortical nucleus (Figs. 3, 9A). The distribution and intensity of GABA-Li labeling was similar to that noted in the adjacent layers I and II of the piriform cortex (Fig. 3). In the most superficially located zone, only a few neurons with GABA-Li immunoreactivity were observed; a similar pattern was present in layer I of the piriform cortex. Like layer II of the piriform cortex, the more deeply located neuropil was more heavily labeled and contained clusters of medium-size, GABA-positive neurons (Figs. 3, 9A).

The stria terminalis and its bed nucleus (Fig. 10). GABA-Li neurons of medium size were conspicuous in the bed nucleus of the stria terminalis, i.e., both in its rostral parts (Fig. 10A) and in the more caudal part where the stria terminalis and its bed nucleus are located above the thalamus (Fig. 10A, B). Bundles of GABA-Li fibers were present in every component of the fiber tract. They were evenly distributed throughout the commissural component of the stria terminalis. In the other components of the stria terminalis, GABA-Li fibers occupied mainly the dorsolateral and ventromedial parts of the fiber tract (Fig. 10B).

DISCUSSION

Earlier studies suggest that GABA-like immunoreactivity is a good marker of neurons thought to use GABA as a transmitter. Thus, a recent study using antisera raised against GABA conjugated to bovine serum albumin with glutaraldehyde (Storm-Mathisen and Ottersen, '86) indicates that the distribution of GABA-Li immunoreactivity closely matches that of the GABA synthesizing enzyme, glutamic acid decarboxylase (GAD) (Ottersen and Storm-Mathisen, '84). The GABA antisera labeled most of the neurons of the reticular nucleus of the thalamus, the medium-size cells of the caudate-putamen, and the stellate and
unpublished observations). Therefore, the immunoreactive material probably reflects GABAergic systems, although the use of this term is necessarily provisional, depending on the specificity of the antibodies.

Organisation of GABA-Li material in the amygdala

The distribution of GABA-Li immunoreactivity shown in the present study is in agreement both with brief recent reports using anti-GABA antibodies from another source (McDonald, '85; Ottersen et al., '86) and with earlier biochemical measurement of the levels of GAD and GABA in microdissected nuclei (Ben-Ari et al., '76). Furthermore, this distribution is in general agreement with that obtained with GAD immunocytochemistry (Mugnaini and Oertel, '85). The principal features of this distribution can be summarised as follows:

1. The central and medial nuclei, as well as the anterior amygdaloid area, are the main targets of the GABA-Li inputs to the amygdaloid nuclei. They show very strong GABA-Li labeling in the neuropil with a high density of GABA-Li punctate structures—presumably boutons (Ottersen et al., '86; present study). As mentioned earlier, these nuclei contain the highest amount of GAD and GABA (Ben-Ari et al., '76) and a dense plexus of GAD immunoreactive material is conspicuous in the neuropil (Mugnaini and Oertel, '85). In the cat, numerous symmetrical axodendritic synapses have been observed in these nuclei (Hall, '72; Juraniec and Narkiewicz, '77; Narkiewicz et al., '77). The majority of these synapses are likely to be GABAergic, although asymmetrical synapses can also be GAD-positive (e.g., Sotelo et al., '86).

Three sources could be responsible for this GABAergic innervation: (1) Anatomical observations suggest that the medial and central nuclei receive most of the intra-amygdaloid (and interamygdaloid) connections (Krettek and Price, '78a; Nitecka et al., '81a,b; Ottersen, '82; Price and Amaral, '84). These fibers arise primarily from the basolateral region (Hall, '72; Kamal and Tombol, '75; Krettek and Price, '78a; Nitecka et al., '81a,b; Price and Amaral, '84; Smith and Milhouse, '85). Since the lateral and basal dorsal nuclei also showed a high density of labeled neurons (Ottersen et al., '85; McDonald, '85; present study), they constitute a possible source of the GABA-Li innervation to the central and medial nuclei. This is in keeping both with lesion studies, which do not support the existence of a major GABA input to the amygdaloid complex from external sources (Le Gal La Salle, '78), and with electrophysiological studies, which suggest the existence of inhibitory intra-amygdaloid connections (Le Gal La Salle, '76). It bears stressing that following blockade of the axonal transport by local injections of colchicine, there is a considerable enhancement of the proportion of cells labeled in the central nucleus. The use of colchicine, however, raises several problems and the significance of the enhancement of labeling produced by this treatment is not fully understood at present (e.g., Mugnaini and Oertel, '85). (2) Neurochemical data suggest that the bed nucleus of the stria terminalis constitutes another possible source of GABAergic innervation to the central nucleus. Thus, transection of the stria terminalis produces a small but significant reduction of GAD in the central, but not in other, amygdaloid nuclei (Le Gal La Salle et al., '78). Furthermore, the content of GABAergic markers in the microdissected stria terminalis is two times higher than that found in other fiber tracts (Ben-Ari et al., '76). Furthermore, transection of the stria terminalis pro-

Fig. 9. A. GABA-Li material in the basal ventral nucleus and the anterior part of the cortical nucleus. B. Posterior part of the cortical nucleus. The arrow indicates a group of cells reminiscent of the intercalated masses; open arrow, a GABA-Li large neuron. Scale bars = 100 μm.

basket cells of the cerebellum. A similar parallelism between GAD and GABA-Li material is also conspicuous with the anti-GABA antibodies used in the present study (Gamrani et al., '86; Seguela et al, '84; Nitecka and Ben-Ari,
duces a highly significant (>50%) reduction of GABAergic markers in the central and medial nuclei (Le Gal La Salle et al., '78). This observation is in keeping with the presence of numerous GABA-Li fibers in the stria terminalis (Ottersen et al., '86; present study). (3) The intensely GABA-positive neurons of the intercalated masses and lateral olfactory tract nucleus constitute a third possible source of GABAergic afference to the central and medial nuclei. These nuclei send their axons to the ipsi- and contralateral centromedial region of the amygdala (Kamal and Tömböl '75; Nitecka et al., '81: Millhouse '86; see also below).

2. The lateral olfactory tract nucleus and the intercalated masses show an intense labeling in the neuropil and the highest density of GABA-Li cell bodies in the amygdala. Ottersen et al. ('86) also found a high labeling in the neuropil (but see also Ottersen and Storm-Mathisen, '84); these authors, however, did not comment on a special reactivity of the cell bodies. This difference may be due to the use in the present study of secondary antibodies, which amplify the immunoreactive reaction and facilitate the visualization of GABA-Li cell bodies in regions in which the neuropil is also densely labeled (see Methods). It also bears stressing that neurons of the lateral olfactory tract nucleus are heavily labeled by retrograde transport of $^{3}$H aspartate (injected in the lateral nucleus), raising the possibility that the neurons are putatively glutamatergic or aspartergic (Price, '86; Ottersen and Storm-Mathisen, '86). However, the neurons labeled with $^{3}$H aspartate are mainly concentrated in the dorsal part of the nucleus and in the boundary of the intermediate region; i.e., there is little overlap with the regions enriched in GABA-Li material. Further histochemical studies should be performed to better comprehend the organization of this nucleus.

Although the functional significance of the intercalated cell masses has not been clarified, several observations raise the possibility that they modulate the sensory information that converges to the lateral and central nuclei of the amygdalae. Earlier studies have shown that the lateral and central nuclei receive direct connections, respectively, from the somatosensory nuclei of the posterior brainstem nuclei (Jones and Burton, '76; Jones et al., '76; Ottersen and Ben-Ari, '78, '79; Nitecka, '79; Nitecka et al., '79, '80; Ottersen, '81). Electrophysiological studies have also shown the convergence of sensory modalities or neurons of the lateral nucleus (Ben-Ari et al., '74) and the important alterations in unit activity that occur during sensory habituation and sensory-sensory conditioning procedures (Ben-Ari and Le Gal La Salle, '73, '74). Interestingly, the intercalated cell masses project to the central nucleus and to the basolateral region (De Olmos, '72; Kamal and Tömböl, '75; Nitecka et al., '81a,b; Millhouse, '86). Furthermore, fibers originating in the peripeduncular nucleus, which is considered to be the subcortical auditory center, terminate in the intercalated cell masses (Jones et al., '76). It is thus possible that by means of these connections, the intercalated cell masses modulate the elaboration in the central and lateral nuclei of appropriate responses to exteroceptive stimuli.

3. There is a close similarity of the anterior part of the cortical nucleus and the basal medial nucleus to the adjacent piriform cortex. Similar relationships are suggested from the observations of their cytoarchitecture and the location of the acetylcholinesterase activity in the piriform lobe (Johnston, '23; Koikegami, '63; Hall and Geneser-Jensen, '71; Hall, '72; Nitecka, '75). These regions also share in common the input from olfactory regions (Cowan et al., '65; Price and Powell, '70; Lammers, '72).

Summing up, the general pattern of GABA immunoreactivity in amygdaloid nuclei suggests that (1) GABA is located to a large extent in neurons giving rise to intra-amygdaloid connections, (2) GABA-containing terminals are

Fig. 10. Photomicrographs to illustrate the pattern of the GABA-Li material in the bed nucleus of the stria terminalis (A) and in the caudal suprathalamic segment of the stria terminalis (B). Note the presence of GABA-Li perikarya in the bed nucleus and within the stria terminalis bundle. GABA-Li positive fibers are present in every component of the stria terminalis. Scale bars=200 μm.
enriched in the central and medial (excitatory) nuclei; and (3) a large number of GABA-Li perikarya are present in the basolateral (inhibitory) region. This raises the possibility that by sending GABAergic connections to the central and medial nuclei, the basal and lateral nuclei are able to exert an important inhibitory effect on drives and emotions.

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LITERATURE CITED


