Immunohistochemical mapping of enkephalins, NPY, CGRP, and GRP in the cat amygdala

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Abstract

This immunohistochemical study shows a wide distribution of neuropeptides in the cat amygdala. Neuropeptide Y is present along the whole amygdaloid complex, and fibers and cell bodies containing neuropeptide Y are observed in all the nuclei studied. Leucine-enkephalin-, gastrin-releasing peptide/bombesin-, and calcitonin gene-related peptide-immunoreactive fibers and perikarya are observed only in discrete nuclei of the amygdaloid complex, whereas only fibers -but no cell bodies- containing methionine-enkephalin-Arg6-Gly7-Leu8 have been observed. No immunoreactivity has been found for γ-melanocyte-stimulating hormone, dynorphin A (1–17), or galanin. These data are compared with those reported in the amygdala of other mammals. © 1999 Elsevier Science Inc. All rights reserved.

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1. Introduction

The amygdaloid complex is a heterogeneous region that has been implicated in a variety of functions. These range from somatic-endocrine mechanisms, the modulation of defensive rage behavior [29], feeding, reproduction, and aggression to memory and learning [7]. Several neurologic disorders, such as Alzheimer’s and Huntington’s diseases, are due in part to deficiencies in the performance of the amygdala [7].

Some of the physiological functions in which the amygdala has been implicated are also performed by several neuropeptides. For example, calcitonin gene-related peptide and neuropeptide Y have cardiovascular effects [1,9,32]; the elicitation of analgesia has been reported for gastrin-releasing peptide/bombesin [26]; and enkephalins are implicated in nociception [22]. With immunohistochemical or radio-immunooassay techniques, these biologically active peptides, and others, have been detected in the amygdaloid complex of several species, including the rat [8,11,22,26,32], the monkey [23], and human [34].

However, only scarce data are available on the distribution of neuropeptides in the amygdala of the cat [21,27,28,30,31]. No previous information appears to be available in the literature concerning the distribution of immunoreactive structures containing calcitonin gene-related peptide, methionine-enkephalin-Arg6-Gly7-Leu8, gastrin-releasing peptide/bombesin, dynorphin A [1–17], leucine-enkephalin, galanin, γ-melanocyte-stimulating hormone, and neuropeptide Y in the amygdaloid complex of the cat. The reason for this work was: 1) to study the distribution of fibers and cell bodies containing those peptides in the cat amygdala; and 2) to compare our findings with previous studies reported on the presence of the above-cited peptides in the amygdaloid complex of other species (specifically rat, monkey, human).

2. Methods

Eight male adult cats (2–3 kg body weight), obtained from commercial sources, were used in this study. Each animal was kept in a cage under standard conditions of light (lights on at 6:00 a.m., off at 8:00 p.m.), temperature...
(25°C), and had free access to food and water. The animals remained in their cages for 10 days before experiments.

Four animals were deeply anesthetized with ketamine [40–50 mg/kg intraperitoneally (IP)], heparinized, and perfused via the ascending aorta with 500 ml of cold 0.9% NaCl. This pre-rinse was immediately followed by fixative (3 l of 4% paraformaldehyde) in 0.15 M phosphate-buffered saline (PBS) (pH 7.2). Brains were removed and post-fixed in the same fixative for 12 h at 4°C. Next, brains were cryoprotected by immersion in sucrose baths (10–30%) until they sank. Eighty μM-thick frontal sections were cut on a cryostat, collected in PBS, and kept at 4°C. In general, 6 or 7 serial sections were used for immunocytochemistry, whereas the remaining sections were stained for Nissl.

Under ketamine anesthesia 4 cats were placed in a stereotaxic apparatus for surgery. Body temperature was maintained at 37°C with a feedback-controlled heating pad. A hole was drilled in the skull and a glass micropipette was used for colchicine injections. The animals received unilateral injections of colchicine (300 μg diluted in 5 μl of saline solution) in the lateral cerebral ventricle to enhance the immunoreactivity of cell bodies. After a 2-day survival time, animals were again deeply anesthetized. The perfusion and tissue processing were identical to those described above.

2.1. Antisera and immunocytochemistry

Polyclonal primary antibodies were raised in rabbits against their respective immunogen, prepared by coupling the whole synthetic peptide to a carrier protein (human serum albumin) with glutaraldehyde. Rabbits were initially immunized with immunogens emulsified with Freund’s complete adjuvant and at 2-week intervals were given booster doses of Freund’s incomplete adjuvant. Plasma from rabbits was obtained 10 days after 3 such booster-injections and periodically thereafter. The immunologic properties of the antibodies used in this study have been reported previously [3,6,18–20].

Free-floating sections were processed for immunostaining as previously described [18–21]. Briefly, after several rinses in PBS (3 × 10 min), sections were incubated for 16–18 h at 4°C in the primary antisera against leucine-enkephalin (Leu-enk), methionine-enkephalin-Arg6-Gly2-Leu5 (Met-enk), dynorphin A [1–17] (Dyn), gastrin-releasing peptide/bombesin (GRP), galanin (Gal), γ-melanocyte-stimulating hormone (MSH), calcitonin gene-related peptide (CGRP) and neuropeptide Y (NPY) -diluted 1/1000 in PBS containing 0.3% Triton X-100 in all cases. Sections were then rinsed extensively in PBS (3 × 10 min) and incubated for 1 h at room temperature in secondary antisera (sheep anti-rabbit immunoglobulins coupled to horsedarish peroxidase) diluted 1/250 in PBS plus 0.3% Triton X-100. Finally, peroxidase was developed by the 3–3′ diaminobenzidine method. To determine the specificity of the immunostaining several histologic controls have been performed. Each primary antibody was pre-absorbed with it corresponding synthetic peptide and after immunohistochemical protocol no immunostaining appeared. Also, sections were incubated by omitting each primary antibody used in this study and no labeling was detected in any case.

The stereotaxic atlas of Jasper and Ajmone–Marsan [12] was used for mapping, whereas the nomenclature of the different amygdaloid nuclei was adapted from that of Krettek and Price [16]. The density of the immunoreactive fibers was graded into 4 categories: high, moderate, low, and scarce. To establish these categories, all the sections were studied under the optical microscope at the same magnification and compared with photographs of previously defined series of densities [5,18–20].

Cell body size was measured using a micrometer grid under the optical microscope at 20 × magnification. Characterization of cell bodies was carried out according to the criterion of Ljungdahl et al. [17]. Cell body profiles with the largest diameter below 15 μM were termed small; those with a diameter of 15–25 μM were termed medium-sized, and those with a diameter above 25 μM were termed large. The density of the immunoreactive neurons was rated as follows: > 20 cell bodies/section, high density; 10–20 cell bodies/section, moderate density; and < 10 cell bodies/section, low density.

3. Results

Figs. 1 and 2 show the distribution of Leu-enk-, GRP-, Met-enk-, CGRP-, and NPY-like-immunoreactive (Leu-enk-like-ir, and similar for the remaining neuropeptides) structures observed in the cat amygdala. The distribution of the different immunoreactive structures represented in these maps is based on the results obtained from the global mapping, including both control and colchicine-treated cats. In the case of NPY immunoreactive cell bodies were easily observed even in non-colchicine-treated cats, although there was not significant differences between animals pre-treated or not with colchicine. No labeling for Dyn, MSH, or Gal was found in the amygdaloid complex. Immunohistochemical labeling was always restricted to neurons. Glial cells were devoid of labeling.

3.1. Leu-enk

The central nucleus of the amygdala displayed Leu-enk-like-ir fibers in both the lateral (low density) and medial (moderate density) divisions, homogeneously located on the surface of the nuclei. These densities remained constant throughout the caudo-rostral extent of the amygdala (Figs. 1, 2). Fibers were similar in appearance in the two divisions of the central nucleus: short, thin, varicose, and unbranched. However, some large Leu-enk-like-ir fibers were observed in the lateral division. A low density of cell bodies contain-
Leu-enk was observed only in this division of the central nucleus. These weakly immunoreactive perikarya were small and round, and had two thin and short processes.

In the medial nucleus of the amygdala, scarce Leu-enk-like-ir fibers were detected (Fig. 3A). These fibers were similar in appearance to those observed in the central nucleus, and their density and distribution were similar at all caudo-rostral levels of the amygdaloid complex.

3.2. Met-enk

Fibers containing Met-enk were observed in all the amygdaloid nuclei except the lateral and basomedial nuclei of the amygdala (Figs. 1, 2). These Met-enk-like-ir fibers were short, thin, varicose, and unbranched, their density being constant at all caudo-rostral levels.

The lateral and the medial divisions of the central nucleus, as well as the anterior amygdaloid area, showed a low density of Met-enk-positive fibers. In these cases, the principal type of nerve elements containing Met-enk were thick puncta, intermingled with some fibers as described above. In the anterior amygdaloid area, both fibers and puncta were localized in the dorsalmost part of the nucleus (Fig. 3B).

Scarce Met-enk-like-ir fibers were detected in the medial nucleus and both the anterior and the posterior divisions of
the basolateral nucleus. Both the anterior and the posterior cortical nuclei showed scarce weakly immunoreactive Met-enk-positive puncta. No cell bodies containing Met-enk were observed in the amygdaloid complex.

### 3.3. NPY

All the amygdaloid nuclei contained NPY-like-ir cell bodies and fibers (Fig. 1, 2). In some nuclei, caudo-rostral changes in the density of positive structures were observed, except in the anterior amygdaloid area, in both the anterior and the posterior cortical nuclei and in the posterior division of the basolateral nucleus, where a similar pattern of densities of NPY-like-ir neurons and fibers was found throughout the amygdala.

In the anterior amygdaloid area and the cortical nuclei, low densities of NPY-positive cell bodies and moderate densities of fibers containing NPY were detected. In the dorsalmost part of these nuclei the fibers were thin, varicose, short and branched; but they were long and unbranched in the ventral part. In the anterior amygdaloid area these two types of fibers were as abundant as thick NPY-positive puncta. In this nucleus, cell bodies containing NPY were round and medium-sized and had at least two processes, whereas in the cortical nuclei (Fig. 3C) two types of NPY-like-ir neurons were found. One type had weakly labeled, polygonal, and medium-sized perikarya displaying one or two dendritic processes. The second type was medium or large, round or elongated, heavily stained, with at least three processes (in general 5–6). Some of these dendritic processes were branched.
The posterior division of the basolateral nucleus displayed a moderate density of both NPY-like-ir cell bodies and fibers. Fibers were short, very thin, and varicose, the branched and unbranched types being equally abundant. Some thick puncta containing NPY were also observed. Regarding cell bodies, three types were present in this nucleus. One type showed polygonal, medium-sized, and weakly stained perikarya, with one or two thin processes. The second type showed heavily labeled small, round cell bodies with two processes. The third type was large, polygonal cell bodies with three or four long and branched dendritic processes, heavily immunoreactive for NPY.

Three other amygdaloid nuclei displayed a similar density of NPY-like-ir fibers, but not cell bodies, throughout their caudo-rostral extent. The lateral division of the central nucleus (Fig. 3D) showed a low density of thin, varicose, and unbranched fibers. In general, they were short, although some long fibers were also present. In this nucleus, at A 10.5, a moderate density of NPY-like-ir cell bodies was observed, decreasing rostrally to low. Perikarya were round, medium-sized, and weakly stained and had one or two processes.

Both the medial and the basomedial nuclei displayed a similar moderate density of NPY-like-ir fibers. These fibers were thin and varicose and were in general short and unbranched in the basomedial nucleus and short and branched in the medial nucleus. This nucleus (Fig. 3E) showed a moderate density of NPY-containing cell bodies at rostral levels (A 13.0 and A 13.5). These neurons differed in this arrangement and morphology depending on their location. Perikarya located nearest the border of the nucleus were arranged in a line; they were triangular in shape, medium-sized, and weakly stained, and they showed one large process. Cell bodies containing NPY located in the innermost part of the medial nucleus were round or triangular, medium-sized, and characterized by at least three short processes, some of them being branched. More caudally (A 12.5), no NPY-like-ir perikarya were observed in the medial nucleus.

Regarding the basomedial nucleus, a high density of NPY-positive perikarya was detected from the caudalmost levels to A 10.5, decreasing rostrally to moderate (Fig. 3F). In general, these neurons were large, round or elongated, and showed two long processes, although some small and medium-sized round perikarya with one or two dendritic processes were also observed.

The remaining amygdaloid nuclei displayed caudo-rostral changes in the density of both cell bodies and fibers containing NPY. At caudal levels, the lateral nucleus (Fig. 3D) showed a moderate density of NPY-like-ir fibers, but from A 12.0 to A 14.0 this density decreased to low. These fibers were in general varicose, thin, short, and unbranched, but at A 14.0 some long and branched fibers were also found in the ventral part of the nucleus. At the caudalmost levels, a moderate density of NPY-like-ir perikarya was detected, decreasing to low from A 10.5. Cell bodies were round, medium-sized, and weakly stained. In general, dendritic processes were virtually absent, although in some cases one or two processes were observed.

At caudal levels, the medial division of the central nucleus (Fig. 4A) showed a high density of NPY-positive fibers, being moderate rostrally as from A 13.0. These fibers were thin, varicose, short, and branched. However, thick puncta and a few long and unbranched fibers were also found. A caudo-rostral decrease in the density of NPY-like-ir cell bodies was observed in this nucleus, from moderate to low, at A 12.0. These neurons were located at the border of the nucleus, mainly next to the lateral division of the central nucleus. Perikarya were round, medium-sized, and weakly stained and had a varying numbers of dendritic processes. At A 13.0, no cell bodies containing NPY were observed in the medial division of the central nucleus.

Finally, the anterior division of the basolateral nucleus showed a caudo-rostral increase (from moderate to high, at A 14.0) in the density of NPY-like-ir fibers, whereas the density of NPY-positive cell bodies decreased from moderate to low at the same anteriority. The three morphologic groups of neurons containing NPY described in the posterior division of the basolateral nucleus were also observed in the anterior division, but at A 14.0 (Fig. 4B) only one neuronal type was found. These cell bodies were round and medium-sized and had two or three processes. In this nucleus, NPY-like-ir fibers were generally long, thin, varicose, and branched, although a few unbranched fibers were also detected.

3.4. GRP

Only 3 amygdaloid nuclei showed GRP-like-ir structures (Figs. 1, 2). In the anterior and posterior cortical nuclei, a moderate density of GRP-positive fibers was detected. Puncta were the most abundant type, although some thin, varicose, short, and unbranched fibers were also observed (Fig. 4C).

The medial nucleus of the amygdala showed scarce GRP-like-ir puncta, whereas a moderate density of cell bodies containing GRP was found at A 12.0, decreasing rostrally to low from A 13.0. In general, these GRP-positive perikarya were round, medium-sized, and weakly labeled and had at least two processes (Fig. 4D).

3.5. CGRP

Only the medial (Fig. 4E) and the cortical (Fig. 4F) nuclei showed a low density of CGRP-like-ir cell bodies. Perikarya were round and medium-sized, and had two short processes. In the medial nucleus, scarce, thick puncta containing CGRP were detected, as well as some thick, unbranched, and non-varicose CGRP-like-ir fibers. They were homogeneously located on the surface of the medial nucleus, and their arrangement and distribution remained similar throughout the caudo-rostral extent of the amygdala.
The remaining amygdaloid nuclei displayed CGRP-like-ir nerve elements except the anterior amygdaloid area and the anterior and posterior cortical nuclei (Figs. 1, 2). Scarcely CGRP-positive thick puncta were observed in the lateral nucleus, whereas in the lateral division of the central nucleus and in both divisions of the basolateral nucleus a low density of thick CGRP-like-ir puncta was detected. However, a few thin, short, varicose, and unbranched fibers were also observed. Their density was similar at all caudo-rostral levels of the amygdala.

In the basomedial nucleus and in the medial division of the central nucleus, a caudo-rostral increase in the density of CGRP-like-ir fibers was observed. The caudalmost levels displayed a low density of CGRP-positive nerve elements, increasing to moderate rostrally from A 12.0. Two types of positive nerve elements, equally abundant, were observed in these two nuclei: thick puncta and thin, varicose, short, and generally unbranched CGRP-like-ir fibers.

4. Discussion

The present findings are original in that they report the distribution of Leu-enk-, Met-enk-, NPY-, GRP-, and CGRP-like-ir fibers and cell bodies in the cat amygdala according to immunoperoxidase immunocytochemistry. Considerable diversity in the immunohistochemical distribution of the above peptides was observed in the amygdaloid complex of the cat, providing a valuable body of information regarding the peptidergic content of this nuclear complex.
4.1. Comparison of the distribution of neuropeptides in the mammalian amygdala

4.1.1. Leu-enk

Previous work carried out on rats concerning the distribution of Leu-enk [35] described the presence of Leu-enk-positive cell bodies and fibers in the central nucleus of the amygdala, as was observed here in the cat. In the cat, however, cell bodies containing Leu-enk were observed only in the lateral part of the central nucleus, pointing to a more restricted distribution than in rats. By contrast, the distribution of Leu-enk-like-ir fibers is more widespread in the cat than in rats, because the medial nucleus of the cat amygdala, unlike that of the rat, did have these fibers.

4.1.2. Met-enk

On comparing our results with those obtained in rats using antibodies against Met-enk [22], the neuronal distribution was more widespread in the rat than in the cat: no cell bodies containing Met-enk were observed in the cat, whereas the rat amygdala does have these perikarya in the central nucleus. Concerning immunoreactive fibers, their distribution is more widespread in cats than in rats. Fibers were detected, at a low density, in the cortical and the medial nuclei of both species. However, in the cat we observed Met-enk-like-ir fibers in the anterior amygdaloid area and the basolateral nucleus, in which no immunoreactive structures containing Met-enk have been found in the rat [22].

4.1.3. NPY

The cat amygdala was found to have large amounts of both NPY-like-ir fibers and cell bodies. This neuropeptide shows a widespread distribution in the amygdaloid complex of the rat [1,8], monkey [23], and humans [34]. However, the distribution of immunoreactive structures differs among these species: some amygdaloid nuclei are devoid of fibers and/or cell bodies containing NPY in rats, monkeys and humans, but this is not the case for the cat, in which NPY-like-ir cell bodies and fibers were found in all the amygdaloid nuclei.

In monkeys, NPY-like-ir cell bodies have been detected in the basolateral, the lateral, and the cortical nuclei, as well as in both subdivisions of the central nucleus [23]. This distribution is restricted in comparison with that found in cats. The same phenomenon has been observed in humans, in whom the medial part of the central nucleus and the anterior amygdaloid area are devoid of cell bodies containing NPY [34]. In addition, fiber distribution in the human amygdala is more restricted than that of NPY-positive perikarya.

A similar distribution pattern was observed in the amygdaloid complex of both cats and rats, because both species showed NPY-like-ir cell bodies and fibers throughout all the amygdaloid complex [10]. However, the density of these immunoreactive structures is different between these species in several amygdaloid nuclei. On comparing our results with those obtained by Gustafson et al. [10], the density of NPY-positive fibers was higher in the cat than in the rat amygdala except in the medial nucleus, where the highest densities of NPY-containing fibers were found. In this nucleus, Gustafson et al. [10] also detected the most important amounts of NPY-positive cell bodies, whereas in the same nucleus of the cat amygdala only moderate densities were observed and, at the caudalmost levels of the cat amygdaloid complex, no NPY-like-ir neurons were detected in the medial nucleus. On the contrary, high densities of NPY-positive perikarya and fibers were observed in the cat, but not in the rat [10], in the medial part of the central nucleus, the anterior division of the basolateral nucleus and the basomedial nucleus. Concerning NPY-like-ir cell bodies, the lateral nucleus showed at caudal levels higher densities in the cat than in the rat, whereas in the rat the rostral regions of this nucleus displayed higher densities of NPY-positive neurons than in the cat [10].

In addition, several differences on the shape of neurons can be observed for the NPY-containing profiles between cats and rats. The most abundant type of NPY-like-ir neuron in the cat amygdala was medium-sized, round or elongated perikarya, although large multipolar neurons were detected in the basomedial, basolateral and cortical nuclei. The rat amygdala displayed in general small, bipolar NPY-positive cell bodies, and some large neurons were only observed in and at the base of the stria terminalis [10]. The morphologic characteristics of NPY-like-ir fibers was similar in both cats and rats, because varicose fibers were observed in both species. However, two types of NPY-positive nerve profiles not described in the rat [10] were detected in the cat amygdala: thick puncta (observed in the anterior amygdaloid area, posterior division of the basolateral nucleus and medial part of the central nucleus), and unbranched fibers, that were present throughout all the amygdaloid complex of the cat except in the medial nucleus.

4.1.4. GRP

On comparing the distribution of GRP-like-ir cell bodies in the amygdaloid complex of cats and rats, a different pattern was observed: the rat amygdala does not show cell bodies containing GRP [26], whereas the medial nucleus of the cat amygdala had a moderate density of GRP-like-ir perikarya. Regarding immunoreactive fibers, the opposite phenomenon was observed. All the amygdaloid nuclei in the rat show GRP-positive fibers [26], whereas in the cat these fibers were only observed in the cortical and medial nuclei.

4.1.5. CGRP

In the rat, Haring et al. [11] observed fibers containing CGRP in the basomedial, central, cortical and medial nuclei. Immunoreactive fibers were also detected in the basolateral nucleus of the rat by Skoifitsch and Jacobowitz [32]. Also in the rat, these authors described CGRP-like-ir cell
bodies in the medial nucleus, whereas Haring et al. [11] observed perikarya containing CGRP in the lateral part of the central nucleus, the medial nucleus, and the anterior and posterior cortical nuclei of the rat.

The results obtained in the cat are partially in agreement with the above reports. We also detected CGRP-positive cell bodies in the medial nucleus and the cortical nuclei of the cat amygdala, but the distribution pattern of CGRP-like-ir fibers is different in cats and rats. We did not find positive processes in the anterior and posterior cortical nuclei, as described for the rat [11,32]. By contrast, in the cat, the lateral nucleus of the amygdala showed scattered CGRP-like-ir fibers, never described hitherto in rats [11,32].

4.1.6. MSH

There are no data available on the immunohistochemical distribution of MSH-like-ir structures in the amygdaloid complex of mammals. However, a previous paper showing the distribution of POMC-derived peptides [13] reported that the rat amygdala is devoid of cells containing POMC, whereas all the amygdaloid nuclei displayed POMC-like-ir fibers. We did not observe any MSH-like-ir elements in the cat amygdala.

4.1.7. Dyn

Comparing our results with those obtained using antibodies against dynorphin A [1–17] and dynorphin A [1–13] in rats [14], the distribution pattern is widespread in rats, because we did not detect DYN-positive structures in the amygdaloid complex of the cat. However, these negative results should be interpreted with caution, because in the brain dynorphin A is subject to complex proteolytic processing pathways [see 14]. In most brain regions, dynorphin A is converted exclusively into dynorphin [1–8], a fragment not recognized by the antibody used in the present work.

4.1.8. Gal

The distribution of Gal binding sites has been studied in the brain of both monkeys and humans [15]. In both cases, high concentrations were detected in the amygdaloid complex. Results obtained using immunohistochemical methods have shown that the rat amygdala [24] contains Gal-like-ir fibers. In the cat, the amygdala was devoid of Gal-like-ir profiles. However, as in the case of Dyn, these negative results should be interpreted with caution. The possibility cannot be excluded that perikarya might synthesize galanin at a rate too low to allow a sufficient buildup for immunohistochemical detection. These observations have already been taken into account in the case of rats [24].

In general, the distribution of immunoreactive cell bodies for the different neuropeptides examined in this study is more widespread in the rat than in the cat amygdala. Thus, Met-enk [22], Leu-enk [35], CGRP [11,32], Dyn [14], and Gal [24] have been observed in perikarya located in several nuclei of the rat amygdala that were devoid of positive cell bodies in the cat. However, GRP [26] was detected in neurons of the cat amygdaloid nuclei, whereas in the rat these are free of labeled perikarya. Concerning immunoreactive fibers, both cats and rats show a similar distribution pattern in the case of CGRP [11,32], whereas the cat amygdala displays a more widespread distribution of Met-enk- and Leu-enk-containing fibers than those observed in rats [22,35]. NPY was the only neuropeptide studied in the present work that showed a similar distribution pattern of both cell bodies and fibers in the amygdaloid complex of cats and rats [10], although several differences concerning the density and the morphology of NPY-like-ir nerve elements were observed between these two species (see NPY section). GRP [26], Dyn [14], and Gal [24] showed a more restricted distribution of immunoreactive fibers in the amygdaloid complex of the cat than in that of the rat. The discrepancies observed could be due to species differences, because both animals -rat and cat- underwent the same treatment (injections of colchicine, immunohistochemistry, etc.). Moreover, in both monkey [23] and man [34] the distribution pattern of NPY-like-ir structures is restricted compared to that observed in the cat. These discrepancies could also be due to species differences. For example, NPY-like-ir cell bodies were observed in all the nuclei of the cat amygdala, even in animals without colchicine treatment, whereas in both the monkey [23] (the study was only carried out in animals not treated with colchicine) and man [34] several amygdaloid nuclei are devoid of NPY-like-ir cell bodies.

4.2. Possible neuropeptide-containing pathways in the cat amygdala

We lack sufficient information to decide whether the immunoreactive cell bodies observed in the cat amygdaloid complex are local or projecting neurons. Also, the origin of the fibers immunoreactive for the different neuropeptides examined in the present work remains to be elucidated. However, according to the present morphologic data, the central and the medial nuclei of the amygdala could receive afferents containing Met-enk, Leu-enk, and/or CGRP, because in these nuclei immunoreactive fibers -but very scarce cell bodies, or none- were detected for the above-mentioned peptides. Additionally, the cortical nuclei could receive afferents containing GRP and/or Met-enk, and the basolateral nucleus Met-enk- and/or CGRP-like-ir afferents. Moreover, fibers containing Met-enk may reach the anterior amygdaloid area, and CGRP could be present in afferents to the lateral, the basomedial, and the posterior cortical nuclei.

Multiple anatomic connections from different brain regions to the central nucleus of the amygdala have already been described in the cat [33]. Comparing the results obtained in the present and in previous papers [2–4,25,36], we suggest a peptidergic content for some of these cerebral pathways. For example, Leu-enk-like-ir fibers detected in the central nucleus could arise from the nucleus raphe dor-
salis in the brainstem, where a high density of Leu-enkephalin-like cell bodies, but not fibers, was observed in the cat [25]. For the same reason (presence of a high density of immunoreactive cell bodies but very few or no positive fibers), the caudal periaqueudal gray matter could be the origin of fibers containing CGRP observed in the cat central nucleus of the amygdala [2]. Other nuclei of the brainstem (lateral reticular, ambiguus, and parabrachial medialis) and the thalamus of the cat (parafascicularis and lateralis posterior) could be the source of NPY-fibers detected in the central nucleus of the amygdala [3,4]. However, Gustafson et al. [10] suggested that much of the NPY-like-ir plexus observed in the rat amygdala does not arise from brainstem sources, at least those in which NPY and noradrenaline are co-localized, and that the brainstem catecholamine neurons that contain NPY have only a minor projection to the rat amygdala. It is also possible that the NPY-positive neurons of the rat amygdaloid complex are local circuit neurons whose terminal fields lie within the amygdala [10]. Their lesion studies carried out in the rat are consistent with these hypothesis, although they remain to be elucidated in the cat.

The GRP-like-ir neurons detected in the medial nucleus could be projecting neurons, because such cell bodies were found at a moderate density but the number of fibers containing GRP was low, decreasing rostrally to scarce. Two projections from the medial nucleus have been described in cats: one to the medial hypothalamus, containing substance P [30], and another to the tuberomamillary hypothalamic nucleus [36]. No mapping of the distribution of GRP-like-ir structures in the cat diencephalon is available in the literature, and thus we cannot state that the GRP-containing cell bodies observed in the medial nucleus do project to the above hypothalamic nuclei.

4.3. Possible physiological functions of the cat amygdala neuropeptides studied

The co-localization of several neuropeptides in the same amygdaloid nucleus suggests that these neuropeptides have intricate interactions among one another and possibly exert a complicated control of different physiological functions in which the amygdala has been implicated, ranging from emotional states and visceral reactions to associative memory and learning [7]. The localization of the neuropeptides studied in the cat amygdala suggests that they could play important roles in the regulation of such mechanisms.

Several emotional mechanisms, such as defensive rage behavior, have recently been studied in the cat [29,31]. Some regions in the amygdala have been identified as powerful modulators of this behavior: the medial nucleus and the basal complex facilitate defensive rage, and the central nucleus suppresses this behavior [29]. These nuclei showed the widest diversity of neuropeptide distribution studied in the present work. Auditory fear conditioning is a mechanism also related to the amygdaloid complex. In cats, the lateral nucleus is the input station of the amygdala for auditory conditioned stimuli, and the medial part of the central nucleus is the output for conditioned fear responses. The basomedial and basolateral nuclei—the main intra-amygdaloid targets of the lateral nucleus—project to the medial part of the central nucleus, and they represent likely candidates for the transmission of auditory conditioned stimuli to the medial part of the central nucleus in auditory fear conditioning [27]. It is likely that the neuropeptides detected in such regions in the present study could have a modulatory role in such intra-amygdaloid connections, and would somehow influence this complex regulation.

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