LIGHT MICROSCOPIC IMMUNOCYTOCHEMICAL IDENTIFICATION OF LEUCINE ENKEPHALIN IN HUMAN CLAUSTRUM

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ABSTRACT

Leucine-enkephalin is a potent and naturally-occurring opioid peptide which serves to inhibit other neurotransmitters involved with pain perception, thereby reducing its emotional and physical impact. Nevertheless, there is little data in the literature concerning leucine-enkephalin-immunoreactivity (Leu-enk-ir) in the human claustrum. The objectives of this study were to confirm the existence of leucine-enkephalin immunoreactive neurons and fibers in the human claustrum. Light microscopy was used to describe their morphology and distribution. Samples of claustrum were obtained from the brains of two females (39 and 48 years of age) and two males (27 and 42 years of age). The brains did not show any overt signs of pathology or trauma. Immunoreactivity to Leu-enk was assessed via the Avidin-Biotin Complex Method.

Light-microscopic analysis confirmed the presence of Leu-enk-ir neurons and fibres in all areas of the human claustrum. The cell bodies varied in shape and size, and were divided into three groups: small, medium and large. The density of immunostaining varied both within and between the cell types, with some neurons, staining more darkly or lightly than others. The large and medium sized cells most likely correspond to claustrocortical projection neurons while the small-sized cells appear to be inhibitory interneurons. It is our hope that these results will be contributed to a better understanding the functions of claustrum, in both health and disease, given its relationship with the development of autism, schizophrenia, Alzheimer disease, Parkinson disease and Huntington disease.

Key words: *leucine-enkephalin, human claustrum, immunocytochemistry*

INTRODUCTION

The claustrum is a small telencephalic structure which size and form varies greatly throughout the mammalian phylogenetic scale. In primates it is a thin gray slab, bounded laterally by the extreme capsule and medially by the external capsule (5,12,16).

The objectives of this study were to confirm the existence of leucine-enkephalin immunoreactive (Leu-enk-ir) neurons and fibers in the human calustrum, employ light microscopy to describe their morphology and distribution.

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MATERIAL AND METHODS

Brains from 2 males and 2 females (between 27 and 48 years of age) without data for neurogical disorders were obtained at autopsy. The brains were cut into block thick 1-2 cm in frontal plane and were fixed for two days in 4% paraformaldehyde.

Serial coronal sections of 40 μm were cut on a freezing microtome and collected in the same phosphate buffer. All slices, prepared as described above, were treated with sodium borohydride for 45 min followed by three consecutive rinses in 0.01 M PBS, each for 2 min. Incubation for 30 min in a solution of 1% bovine serum albumin (BSA) was followed by incubation overnight in a solution of a polyclonal anti-Leu-enk antibody (*Sigma, St. Louis, MO, USA*), in a dilution of 1:1000. Afterwards three consecutive rinses in 0.01 M PBS (2 min each) were performed and sections were incubated for 20 min in 1% BSA in PBS, followed by incubation for 2 h in biotinylated anti-mouse IgG (Vector, Burlingame, CA, USA) in a dilution of 1:500. After three consecutive rinses in 0.01 M PBS (2 min each), sections were incubated in a solution of avidin–biotin–peroxidase complex (Vector, Burlingame, CA, USA) for 1 h. All incubations were carried out on a shaker at room temperature and preceded visualization of peroxidase activity with H_2O_2 and 3,3'-diaminobenzidine as substrates. Afterwards, the sections were rinsed for 5 min, 3 times in the same phosphate buffer and mounted on gelatin-coated glass slides. The slides were air dried for 24 hours, then washed in distilled water also for 5 min, 3 times, air dried again and coverslipped with Entellan (Merck, Germany) and exmined using a light microscope (Olympus,Tokio, Japan).

RESULTS

Light-microscopic analysis confirmed the distribution of (Leu-enk-ir) neurons in all areas of the human claustrum (Fig. 1). The cell bodies varied in shape (oval, fusiform, triangular, elongated, multipolar and irregularly shaped). Leu-enk-ir neurons were different in size and were divided in three groups: large - 25-40 μm in diameter, medium 19-25 μm in diameter and small - 13-17 μm in diameter.

We found large Leu-enk-ir neurons with multipolar cell body (Fig. 2), with an irregular flatened cell body (Fig. 3), with an oval cell body

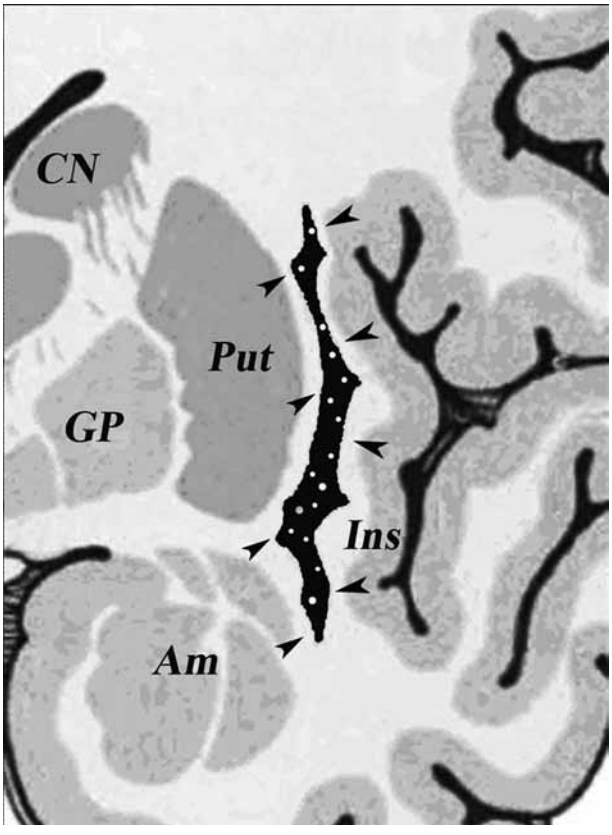

Fig. 1. Schematic drawing through coronal section of human brain amygdaloid body, Am-amygdaloid body, CN-caudate nucleus, Pu-putamen, Ins-insular cortex, GP-globus pallidum. The arrow heads indicate claustrum into which with white dots is shown the topographical distribution of Enk-immunopositive neurons

(Fig. 4) and with an elliptical cell body (Fig. 5). The immunoproduct was visible in the cell cytoplasm and processes, while the cell nucleus remained free. We observed the existence of a great amount of Leu-enk-immunopositive puncta, most probably terminal synaptic boutons. The medium-sized aspiny Leu-enk-ir neurons were with irregular and oval cell bodies (Fig. 6, 7).

Many puncta and fibres were observed in the neuropil. The small-sized Leu-enk-ir neurons were usually with oval cell bodies (Fig. 8, 9, 10). In some of Leu-enk-ir neurons the dendrites were situated parallelly to the white fibres of capsula externa, but others were crossing the white matter of capsula externa (Fig. 11). Immunopositive neurons were found also near to and inside the external and extreme capsule. The density of immunostaining varied both within and between the cell types, with some neurons, staining more darkly or lightly than others.

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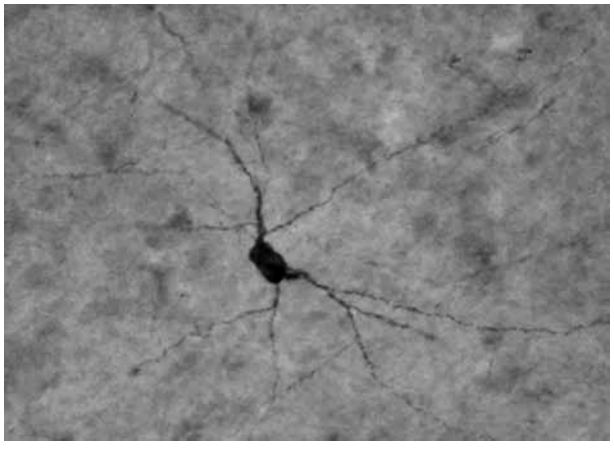

Fig. 2. Large spiny Leu-enk-ir neuron with multipolar cell body from which are arising several spiny dendrites bifurcating and passing in all directions (x400)

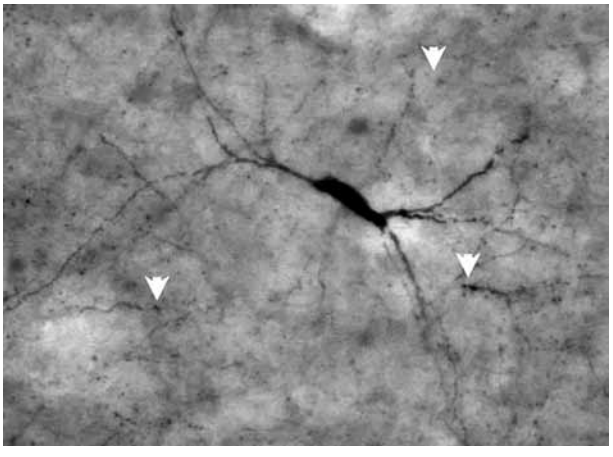

Fig. 3. Large spiny Leu-enk-ir neuron with an irregular flatened cell body,from the poles are emerging thick dendritic trunks. Note the existence of a great amount of Enk-immunopositive puncta, most probably terminal synaptic boutons (white head arrows) (x400)

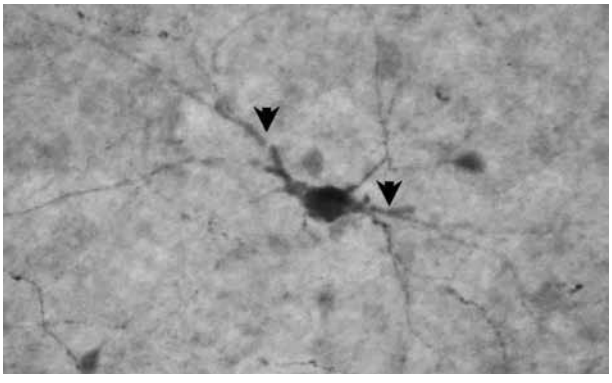

Fig. 4. Large aspiny Leu-enk-ir neuron with an oval cell body from which arise aspiny bulbous dendrites (black head arrows) (x400)

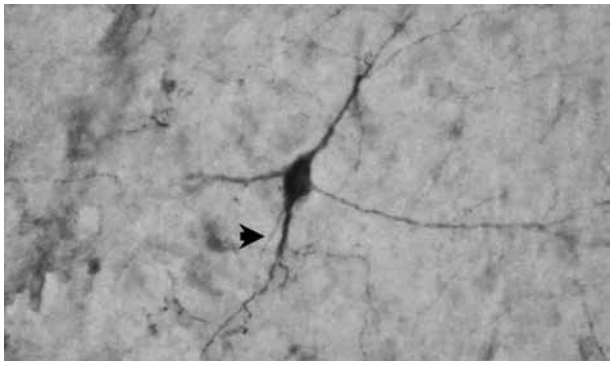

Fig. 5. Large aspiny Leu-enk-ir neuron with an elliptical cell body, from the poles are emerging thick dendritic trunks. From one of the poles is emerging the axon, the initial segment is well visible (black head arrow) (x400).

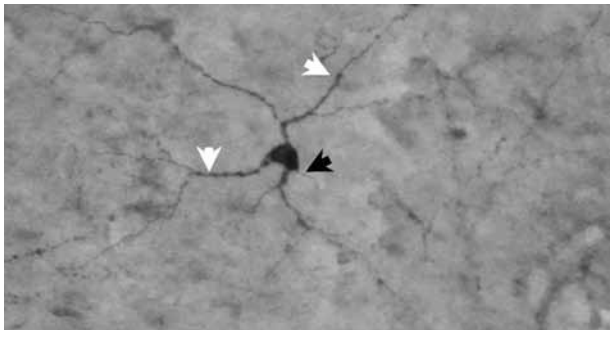

Fig. 6. Medium-sized aspiny Leu-enk-ir neuron with an irregular cell body. From the cell body arise aspiny bulbous dendrites (white head arrows). The axon arises from the cell body (black head arrow) (x400)

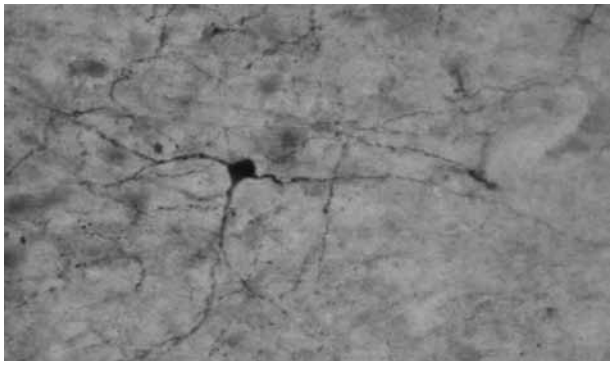

Fig. 7. Medium-sized aspiny Leu-enk-ir neuron with an oval cell body from which are emerging thin aspiny dendrites, branching into secondary bulbous dendrites. Many puncta and fibres are observed in the neuropil (x400)

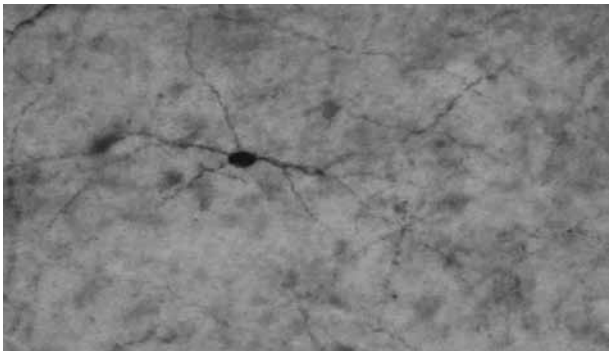

Fig. 8. Small-sized spiny Leu-enk-ir neuron with an oval cell body from which are emerging thin spiny dendrites, branching into secondary dendrites on a short distance (x400)

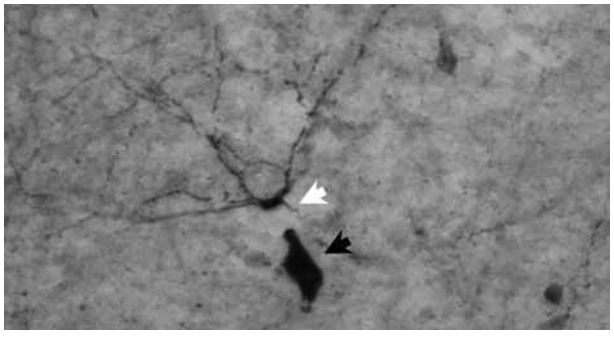

Fig. 9. Small aspiny Leu-enk-ir neuron, from the small cell body are emerging thick and long bulbous dendrites. Directly from the cell body arises the axon with well visible initial segment (white head arrow). In this field is visible the body of large Enk-immunopositive neuron (black head arrow) (x400)

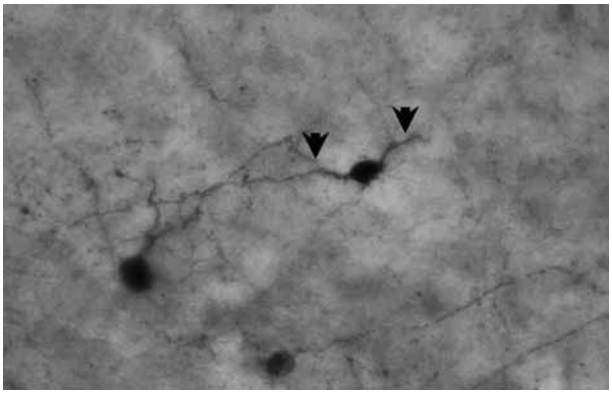

Fig. 10. Small spiny Leu-enk-ir neuron with an elliptical cell body from which are emerging short spiny dendrites (black head arrows) (x400)

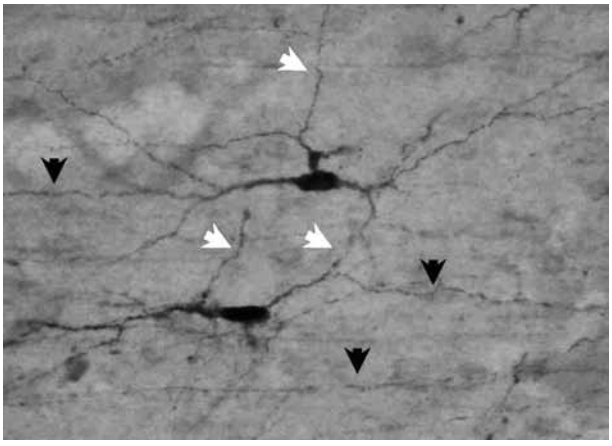

Fig. 11. Three Leu-enk-ir neurons. Some of the dendrites are running parallelly to the white fibres of capsula externa (black head arrows), but others are crossing the white matter of capsula externa (white head arrows) (x400)

DISCUSSION

Leucine-enkephalin is an opioid peptide, which is present in many brain regions. Leu-enk-ir neurons have a wide distribution throughout the central nervous system. They are present in brain regions implicated in autonomic and neuroendocrine regulation as well as in diverse behavioral functions. Numerous studies have extended the knowledge of the specific sites of enkephalin localization in the human brain (3,4,17), but only few works mentioned the presence of Leu-enk-ir neurons in human claustrum in details (11). Moreover, the methodology for demonstration of Leu-enk-ir in previous studies differs significantly from the methodology used in our present study.

One of the aims of the present study was to verify whether Leu-enk-ir is present in distinct subpopulations of claustral neurons. As we showed above, the observed population of Leu-enk-ir neurons was not homogeneous. It consisted of small, medium-sized and large neurons. Light-microscopic characteristics of the large Leu-enk-ir neurons show that these neurons are projective. This suggestion was proved by our previous works in cat (10) and in human (11).

The fact that Leu-enk-immunoreactivity was observed in neurons of varying shapes and sizes

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suggest that these cells play different roles in the context of claustral function (12).

Another aim of this study was to verify whether Leu-enk-ir neurons have a well-defined topographical distribution throughout the human claustrum. In fact, such an organization was not found. The enkephalins have been implicated in such central nervous system regulatory functions as nociception (2), neuroendocrine control (14), attention (13) and possible role of Leu-enk in Parkinson's disease (9), Alzheimer's disease (1) and epilepsia (15). On the other hand our previous studies demonstrated the presence of neuronal nitric oxide syntase, canabinoid 1 receptor-immunoreactivity and nicotinamide adenine dinucleotide phosphate diaphorase reactivity neurons and fibers in all areas of the human claustrum (6,7,8). All these facts and our present results contribute to a better understanding of the claustrum's function in both health and disease, given its relationship with the development.

CONCLUSION

In conclusion, this light microscopic study of Leu-enk-ir neurons and fibers in the human claustrum could expand the data analysis and it could contribute to a better understanding of the function of the claustrum, given its relationship with the development of autism, schizophrenia, Alzheimer disease, Parkinson disease and Huntington disease.

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