# LIGHT AND ELECTRON MICROSCOPIC STUDY ON TWO OF THE AFFERENT PROJECTIONS TO THE AMYGDALA

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## INTRODUCTION

Several studies were made on the level of light microscope to examine the various afferents which terminate in the amygdala. Studies with electron microscopic degeneration methods, however, were very limited in number.

Hall (1972) in her review article on the structural organization of the amygdala described preliminary results dealing with degenerated terminal boutons in the amygdala after lesions involving the pre-optic area and the temporal cortex.

Recently, Wakefield and Hall (1974) demonstrated degenerated terminal boutons containing flattened vesicles in the central, medial and basolateral nuclei after lesions in the lateral pre-optic area.

The present work is a combined light and electron microscopic investigation to localize the site and mode of termination of the olfactory fibres from the olfactory bulb, and of the neocortical afferents from the inferior temporal area to the various amygdaloid nuclei.

# MATERIALS AND METHODS

The experiments were carried out on cats where two types of operations were made. In the first type the olfactory bulb was destroyed through a trephine hole in the medial wall of the frontal air sinus. In the second type coagulation of the temporal cortex through a trephine hole in the temporal fossa was carried out.

Half of the operated cats was perfused with Karnowsky Fixative and prepared for electron microscopical examination. The other half was perfused after five days survival with 10% formalin, stained with the Fink-Heimer method (1967) and used for light microscopic study.

#### RESULTS

## Results of the olfactory bulb lesions :

In all the successfuly operated

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cases the left olfactory bulb is completely damaged with some occasional slight injury to the right bulb.

The sections stained with Fink-Heimer method, show that the degenerated fibres enter the anterior part of the amygdala through the lateral olfactory tract (Fig. 1).

The coarse preterminal fibres of this tract are distributed through the nucleus of the lateral olfactory tract (Fig. 2-A) and reach the lower part of the anterior amygdaloid area where terminal and preterminal fragments can be observed (Fig. 1-B). At a more posterior level the fine terminal degenerated fibres are observed in the superficial part of the cortical nucleus (Fig. 2-B) especially in its lateral part. The degenerated terminal fragments can be followed in the neighbouring piriform cortex until the rhinal sulcus. No clear terminal degenerations are observed in other amygdaloid nuclei. In the contralateral side some degenerating preterminal fibres are observed in the nucleus of the lateral olfactory tract.

The presence of degenerated axons and terminal endings in the amygdala following lesion of the olfactory bulb is verified with electron microscope. A few electron dense boutons are observed in the nucleus of the lateral olfactory tract (Fig. 3-D), in the medial nucleus (Fig. 3-A & C) and cortical nucleus (Fig. 3-B) as well as in the anterior amygdaloid area (Fig. 3-E). None are seen in the other amygdaloid nuclei. In almost every instance when the vesicle population of the degenerated boutons can still be distinguished the round type can be recognized.

The synaptic contacts of such post-synaptic profile is usually the shaft of a larger dendrite. This type boutons are asymmetrical and the of synaptic terminal corresponds to bouton type I described by Tömböl and Kamal (1975).

## Results of the temporal cortex lesions :

In these operations the lesions involve the posteurior Sylvian gyrus. The anterior part of the posterior ectosylvian and the anterior Sylvian gyri are sligtly coagulated also. The lesion is localized in the cortical grey matter,. The subcortical white matter in all cases investigated is left intact.

The sections stained with Fnik-Heimer method show that the degenerated fibres spreading from the lesions can be traced as association, commissural and projective fibres (Fig. 4). The degenerated projective fibres pass medially, horizontally or ascend obliquely upwards penetrating through the external capsule, putamen, globus pallidus and the dorsal part of the amygdala.

In the amygdala the degenerated cortical fibres are observed only in the lateral (Fig. 5-A) and central (Fig. 5-B) nuclei.

In the lateral nucleus the degene-

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rated preterminal and terminal fibres are observed in the dorsal part and its lateral part just close to the external capsule.

In the central nucleus the preterminal and terminal degenerated fibres are localized in its lateral part. No degeration is observed in other amygdaloid nuclei.

The electron microscopic studies verified the previous light microscopical findings. Following a similar lesion, degenerated axons and terminal boutons are identified in the dorsal and lateral part of the lesions can be traced as association, lateral nucleus (Fig. 6 A, B & C) and in the lateral part of the central nucleus. (Fig. 6-D & E). The most favourable time to get marked degeneration of these boutons is six days. The degenerated profiles are frequently still in contact with their postsynaptic structures : exclusively dendritic spines or small dendrites. A distinct post-synaptic thickening as well as round shaped synaptic vesicles within the boutons are usually observed. Especially in early stage of degenerathe tion these vesicles appear to be varying in diameter. Therefore this type of synaptic terminal corresponds to bouton type IV described by Tömböl and Kamal (1975).

## DISCUSSION

The previous light and electron microscopic experiments settled that fibres from the lateral olfactory tract terminate in the nucleus of that tract, anterior amygdaloid area, cortical and medial amygdaloid nuclei. These results confirm those of Cragg (1961), Lohmann (1963), Lohmann and Lammers (1963), Powell et al. (1965), White (1965), Scalia (1966), Girgis and Goldby (1967) and Girgis (1970) Using the Nauta method, as well as the results of Winans and Scalia (1970) using the Fink-Heimer method.

The results of the present study are not in full agreement with those of Le Gros Clark and Meyer (1947), Meyer and Allison (1949), Allison (1963) and Johnson (1959), Who reported also some degenerated fibers in the central amygdaloid nucleus after lesion in the olfactory bulb. However, the findings of most of these authors based on Glees' technique might be due to certain msinterpretation of the pseudodegeneration observed by using this particular technique (Szentagothai, 1962 and Cowan et al., 1965).

The results of the present study showed that the direct olfactory fibers from the olfactory bulb terminate, via the lateral olfactory tract in a group of amygdaloid nuclei. These nuclei are the nucleus of the lateral alfactory tract, anterior amygdaloid area, medial and cortical amygdaloid muclei which form less than 20% of the total volume of the amygdaloid complex. These results confirm Lammer's opinion (1972) who mentioned that the direct olfactory projection from the olfactory bulb is a limited one in comparison with the indirect olfactory projection by way of the piriform cortex.

Concerning the temporo-amygdaloid connection, the results of the present study confirm the light microscopic studies of Whitlock and Nauta (1956) in the monkey and Lammers and Lohmann (1957), Druga (1969), Lescault (1969) and 1971) in the cat, which showed that fibres from the temporal cortex terminate in the lateral and central amygdaloid nuclei.

Regarding the functional significance of the temporoamygdaloid connection, it is assumed that this connection is the morphological basis for a neocortico-amygdaloid relation. By means of this connection the neocortex and especially the temporal cortex can influence the activity of the deep structures of the limbic system.

#### SUMMARY

In order to determine the origin of the various terminals observed in the ulterastructore of the amygdaloid complex, two series of opertions were made on cats.

In the first series the olfactory bulb was destroyed, while in the second, coagulation of the temporal cotex was carried out. The animals were sacrificed, half of them was prepared for E.M. examination while the other half was stained with Fink-Heimer method and used for E.M. study. In the brains of the first series, E. M. examination showed degenerated boutons containing round vesicles in the nucleus of the latefral olfactory tract, anteroir amygdaliod area, anterior part of the cortical nucleus and in the medial nucleus. The boutons usually articulated with the big dendrites. In the second series, degenerated boutons containing round vesicles were observed in the lateral and central amygdaloid nuclei. They preferably articulated with dendritic spines and small dendrites.

The site and density of the degenerated terminals observed by the E.M. confirmed the results of similar brains stained with the Fink-Heimer method.

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Fig. (1) Olfactory bulb lesion :

- (1) A diagram showing a lesion involving the olfactory bulb.
- (2), (3) and (4) are drawings of coronal serial sections through the anterior third of the amygdaloid complex showing the course and termination of the degenerated fibres of the lateral olfactory tract as they appear in sections stained with Fink-Heimer Method. The degenerated fibres (dotted lines) terminate in the nucleus of the lateral olfactory tract, anterior amygdaloid area, cortical amygdaloid nucleus as well as in the piriform cortex.
- Fig. (2) Photographs of the degenerated fibres of the lateral olfactory tract as they terminate in some of the amygdaloid nuclei. Fink-Heimer technique, x 800.
  - (A) The degenerated fibres as they are distributed in the nucleus of the lateral olfactory tract.
  - (B) Preterminal and terminal degenerated fibres in the cortical nucleus.
- Fig. (3) Degenerated boutons in the amygdaloid nuclei after lesion of the olfactory bulb.
  - (A) and (C) Degenerated boutons in the medial nucleus.
  - (B) Degenerated boutons in the cortical nucleus.
  - (D) Degenerated boton in the nucleus of the lateral olfactory tract.
  - (E) Degenerated bouton in the anterior amygdaloid area.
    All the boutons are in contact with large dendrites.
    The boutons in A, B, C and are

round, equal in size. Scale = 1 micron.

- Fig. (4) Temporal cortex lesion.
  - (1) cortical lesion involving mainly the the posterior sylvian gyrus. The lesion slightly encroaches upon the anterior sylvian and the posterior ectosylvian gyri.
  - (2), (3), (4), (5) and (6) are drawings of frontal serial sections through the middle and posterior third of the amygdaloid complex, showing the course and termination of the degenerated fibres (short lines) and terminals (dots) as they appear in sections stained with Fink-Heimer method. In the amygdala the degenrated fibres are located in the dorsolateral part of the lateral nucleus and the lateral part of the central nucleus.
- Fig. (5) Photographs of the degenerated cortical fibres as they terminate in some of the amygdaloid nuclei. Fink-Hemer technique, x 500.
  - (A) Horizontal degenerated fibres as they cross the dorsal part of the lateral nucleus.
  - (B) Degenerated preterminal and terminal fibres in the lateral part of the central nucleus.
- Fig. (6) Degenrated terminal boutons in the amygdaloid nuclei after lesion of the temporal cortex.
  - (A), (B) and (C) degenerated boutons in the dorsolateral part of the lateral nucleus.
  - (D) and (E) degenerated boutons in. the lateral part of the central nucleus. The boutons are in contact with the dendritic spines.
    - The synaptic vesicles in A, B and D appear to be varying in diameter. Scale = 1 micron.



Fig. (1)



Fig. (2. A)





Fig. (2. B)

Fig. (3)

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Fig. (5. A)

Fig. (4)





Fig. (5. B)

Fig. (6)