

CHAPTER 3

RESULT AND INTERPRETATION

3.1 Localization and extension of the injection sites

Using HRP method for studying neuronal connections in the CNS, one must ensure that the presence of HRP labeled neurons is produced by the retrograde transport of HRP taken up by axon-terminals of those neurons projecting to the injected site. This accurate interpretations could be obtained from careful evaluation of the localization and spreading of HRP fluid in the brain tissue (Nauta et al, 1974; Walberg et al, 1976) only. When the injected-HRP is limited in the area of interest that it can be concluded that those HRP-labeled neurons are the neurons whose axons terminate in the injected area.

In the present study the injection sites were carefully evaluated. Eventhough they were varical in sizes depending upon the tip dimension of the micropipette and the intensity of current used in electrophoretic ejection, they usually limited within the nucleus accumbens and located in different parts as summerized in Table I. In most cases, the injection sites contained small amount of HRP. Since the area of interest located adjacent to the caudatoputamen, spreading of the HRP beyond the nucleus accumbens migh lead to a false interpretation. This problem

could be overcome by limited the diameter of micropipette tip. In addition, prevention of HRP leaking into other brain tissues during the introduction or withdrawal of the micropipette could be done by holding the current as described in the microelectrophoresis. Examples of injection sites in various sections from cases number 22, 4, 2 and 30 were shown in Plate 1.

All repetition cases, HRP product appeared as solid dark color. In which was seen around the small hole produced by the tip of the micropipette (Figure 1). This area was surrounded by a more peripherally located area from the hole which appeared as light brown color, presumably containing lesser density of HRP products spreading from the inner zone (Figure 1). In practice it was presumed that these areas containing HRP product were the area in which HRP was taken up by axons and their terminals. The area containing high density of HRP product were indicated by solid-black (●), while the peripheral (spreading) zone marked by parallel lines (▨).

3.2 Labeled cells in the brain following injection of HRP into the nucleus accumbens

Comparable to characteristics of typical HRP labeled cells reported elsewhere (Lavial, 1975; Walberg et al, 1976; Olsson and Malmgren, 1978; Otterson, 1980). It appeared as if the cells were stippled by dots of HRP reacts product

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which were different from the surrounding unlabeled neurons stained by neutral red as shown in Plate 2-8. All HRP labeled cells in the present study showed outlined of the cell soma and one or more dendritic-like processes containing HRP granules (Plate 2-8). Labeled neurons containing high density of HRP granules in their perikarya and processes were classified as heavily labeled neurons (Plate 8) while those cells containing low density of HRP granules were classified as lightly labeled neurons. Furthermore, the number of labeled neurons in each area of the brain were defined as; "*a lot of*" "*some*" and "*few*" labeled cells by their number of labeled neurons as more than 10, 3-10 and less than 3 respectively.

3.3 Localization and distribution of HRP labeled cells in brain areas following HRP injection in the nucleus accumbens

This is the interesting part of the present study. Since there are many variations in the localization, distribution and the number of labeled cells in various cases with different injection sites, the experimental results will be presented and interpreted by selected sequence.

In the present study, the nucleus accumbens is arbitrarily divided into four parts e.i, rostral region, which is applied to the area situated dorsal to the anterior

commissure (level 1 a to 1 c in Figure 1), middle region which is used to the largest part of nucleus where it surrounds the anterior commissure (level 1 d to 1 f, Figure 1). The middle region is further subdivided into two parts: the medial part and the lateral part of the middle region. The caudal region of the nucleus accumbens composed of the cells forming a narrow rim around the anterior commissure (level 1 g to 1 h, Figure 1).

3.3.1 Distribution of HRP labeled cells following HRP injection into the rostral part of the nucleus accumbens

In rat No 1 (Plate 2) a large iontophoretic application of HRP was stained the rostral pole of nucleus accumbens, with the center of the injection site was in the rostral part of the nucleus and medial to anterior limb of the anterior commissure.

A lot of heavily labeled neurons were found ipsilaterally in ventral portion of subiculum (Plate 2) and in the parataenial nucleus (Plate 5B). Some heavily labeled neurons were seen in parafascicular nucleus and paraventricular nucleus. In the medial thalamic nuclei of both pars lateralis (Plate 7A and 7B) and pars medialis. a lot of lightly labeled neurons were also observed. In ipsilateral side of brainstem few of heavily labeled cells were observed in the dorsal raphe nucleus and in the interpeduncular nucleus while a lot of heavily labeled

neurons were also found distributing in the ventral tegmental area (Figure 48).

In other cases, where the injection site was confined in the rostral part of the nucleus accumbens, they were rat No. 15, 23, 25, 33. In the rat No. 15 the injection site was small and located in the medial part of the rostral region. HRP labeled cells in this case were found in the parataenial nucleus and the ventral tegmental nucleus. In the rat No. 23 and 33 the injection were larger than in rat No. 15 a lot of lightly labeled neurons were observed in the medialis thalamic nucleus, the parafascicular nucleus paraventricular nucleus, the reuniens nucleus, the interpeduncular nucleus and the ventral tegmental nucleus.

3.3.2 Distribution of HRP labeled cells following HRP injection into the middle part of nucleus accumbens.

The middle region of nucleus accumbens was divided into two parts; middle-medial and middle-lateral portions.

In the rat No. 7, 20 and 32 (Figure 3, Plate 1A) the HRP injection sites were very large and occupied the entire middle portion of the nucleus. The distribution of heavily and lightly labeled cells in these rats were similarly found in hippocampus field CA1, the entorhinal cortex. Some lightly labeled were also observed in the ipsilateral

pars lateralis (Plate 7D) and pars medialis of the nucleus amygdaleus basalis.

A lot of heavily labeled neurons were located ipsilaterally in the medialis thalamic nucleus, the parataenial nucleus and the ventral tegmental nucleus (Plate 3). Some of lightly labeled neurons were seen in the reuniens nucleus (Plate 6C), the dorsal raphe nucleus and the interpeduncular nucleus (Plate 8A). A few lightly labeled neurons were found in ipsilateral areas of the parafascicular nucleus (Plate 5D) paraventricular nucleus (Plate 8B).

In the rat No. 2 (Figure 4, Plate 1C), where the moderate injected HRP fluid were applied to the more lateral part of the middle region of nucleus accumbens; some lightly labeled cells were found ipsilaterally in the amygdala nucleus in both basolateral and basomedial part as well as in the entorhinal cortex. A lot of heavily labeled neurons were found in the ventral tegmental nucleus. Similar findings were also made in the rat No. 5, 6, 8, 13, 14, 16, 21, 22, 26, 31, 34 and 37 in which the injected sites locate in the middle-lateral part of the nucleus accumbens.

In rat No. 4 (Figure 5, Plate 1B), in which the moderate injection site was confined in the more medial part of the middle region of nucleus accumbens, some lightly labeled neurons were found in the amygdala nucleus in both basolateral and basomedial part and hippocampal

area field CA 1. A lot of heavily HRP-labeled neurons were found in the parataenial nucleus, the ventral tegmental nucleus and some HRP labeled cells were found in the parafasicular nucleus, the medialis thalamic nucleus, the periventricular nucleus, the reuniens nucleus, the interpeduncular nucleus and the dorsal raphe nucleus. Few heavily labeled cells were observed in the substantia nigra, pars compacta. In the rat No. 3, 11, 17, 18, 19 and 28 where the injected fluid deposited in the more rostral part of the middle-medial region of the nucleus accumbens, HRP-labeled neurons were similarly found in those areas indicated above. The rat No. 12, 24, 29 and 36 the injection sites were located in the more caudal part of the middle-medial region. In these rats, the heavily labeled cells were seen in parataenial and ventral tegmental nucleus while some of lightly labeled cells were observed in the interpeduncular nucleus.

3.3.3 Distribution of HRP labeled neurons following HRP injection into the caudal part of nucleus accumbens

In the rat No. 9, 10, 30 (Plate 5, Figure 1D) which the HRP injection fluid were stained in the caudal part of the nucleus accumbens, a lot of heavily labeled neurons were distributed in the ventral tegmental nucleus some heavily labeled cells were observed in the parataenial nucleus and a few of lightly labeled cells were seen in the ipsilateral interpeduncular nucleus.

3.4 Interpretation of the findings

The present study clearly demonstrated that HRP injections in different regions of the nucleus accumbens produced retrograde labeling of neurons in various regions of the brain. Since it has been demonstrated and generally accepted by most neurobiologist that the presence of these retrogradely HRP-labeled neurons can be use as anatomical evidence for the afferent projections of these neurons to the site of injection (for detail see Walberg et al 1976), the presence of labeled cells in the present study. Therefore, can be interpreted as evidence for the afferent projections from the neurons in those nuclei to the nucleus accumbens.

It has been awared among investigators, who apply HRP as tracer substance, that passing fibers when damaged may be able to take up the injected HRP molecules. Although it has been argued that not all system show evidence of HRP uptake by mechanically damaged passing fibers (Ottersen, 1982), it is advantageous to restrict the damage as less as possible. This has been acheived by limiting the tip dimension of the micropipette.

Comparison the number of labeled cells in different cases with different amount of HRP injections (measured by size of injection site) in the same or similar regions of the nucleus accumbens suggested that the amount of labeled cells may be proportional to the area of the nucleus

accumbens stained by HRP products. On the other hand, different amount of HRP labeled cells may indicate differences in the number of cells in these region which project to the nucleus accumbens. In addition, it has been awared that negative findings do not totally exclude the possibility of cells in other regions which may project to the nucleus accumbens but only the positive findings clearly demonstrate the presence of such projections (see Walberg et al, 1976).

Based on the finding obtained from the present study, evidence suggest that various parts of the brains projects to the nucleus accumbens in topograpical organized pattern. Figure 9 summeries the topographical projection from various areas to the nucleus accumbens.

Projections from nuclei in the limbic system were topographically organized i.e. neurons in basolateral and basomedial amygdala nucleus send their axons terminate in the middle region of the nucleus. Hippocampal projection terminates only in the medial part of middle region while axons from the ventral subicular neurons terminate mainly in the rostral part of the nucleus accumbens.

Projections from various thalamic nuclei to the nucleus accumbens also show topographical organization. Neurons in the parataenial nucleus project to the entire lenght of the nucleus accumbens and terminate mainly in the medial part while other thalamic nuclei including

parafasicular nucleus, paraventricular nucleus, reuniens nucleus and medialis thalamic nucleus both pars lateralis and pars medialis terminate mainly in the rostral and middle-medial region of the nucleus accumbens.

Neurons in many nuclei of the brainstem sent their axons to terminate in nucleus accumbens and showed less topographical organization. Neurons in ventral tegmental area projected heavily to the entire nucleus, no preferential termination was observed.

Interpeduncular neurons projected to most parts of nucleus accumbens except the middle-lateral part.

Neuron in the dorsal raphe nucleus appeared to project mainly to the rostral region of nucleus accumbens while some cells were shown to project to the middle-medial region.

Another source of projection to the nucleus accumbens was from the substantia nigra. pars compacta which projected to the middle-medial region of the nucleus.

Table I : Summary of localization and the extension of HRP injection site in cases included in the present study

Case No.	Localization and extension of HRP injection site
Rat No.1	large area in the rostral part.
Rat No.2	moderate area in the lateral part of middle region.
Rat No.3	small area in the medial part of middle region.
Rat No.4	moderate area in the more rostral medial part of middle region.
Rat No.5	small area in the lateral part of middle region.
Rat No.6	moderate area in the lateral part of middle region.
Rat No.7	large area occupied entire middle region.
Rat No.8	small area in the lateral part of middle region.
Rat No.9	moderate area in the caudal part.
Rat No.10	small area in the caudal part.
Rat No.11	moderate area in the medial part of middle region.
Rat No.12	small area in the medial part more caudal of middle region.

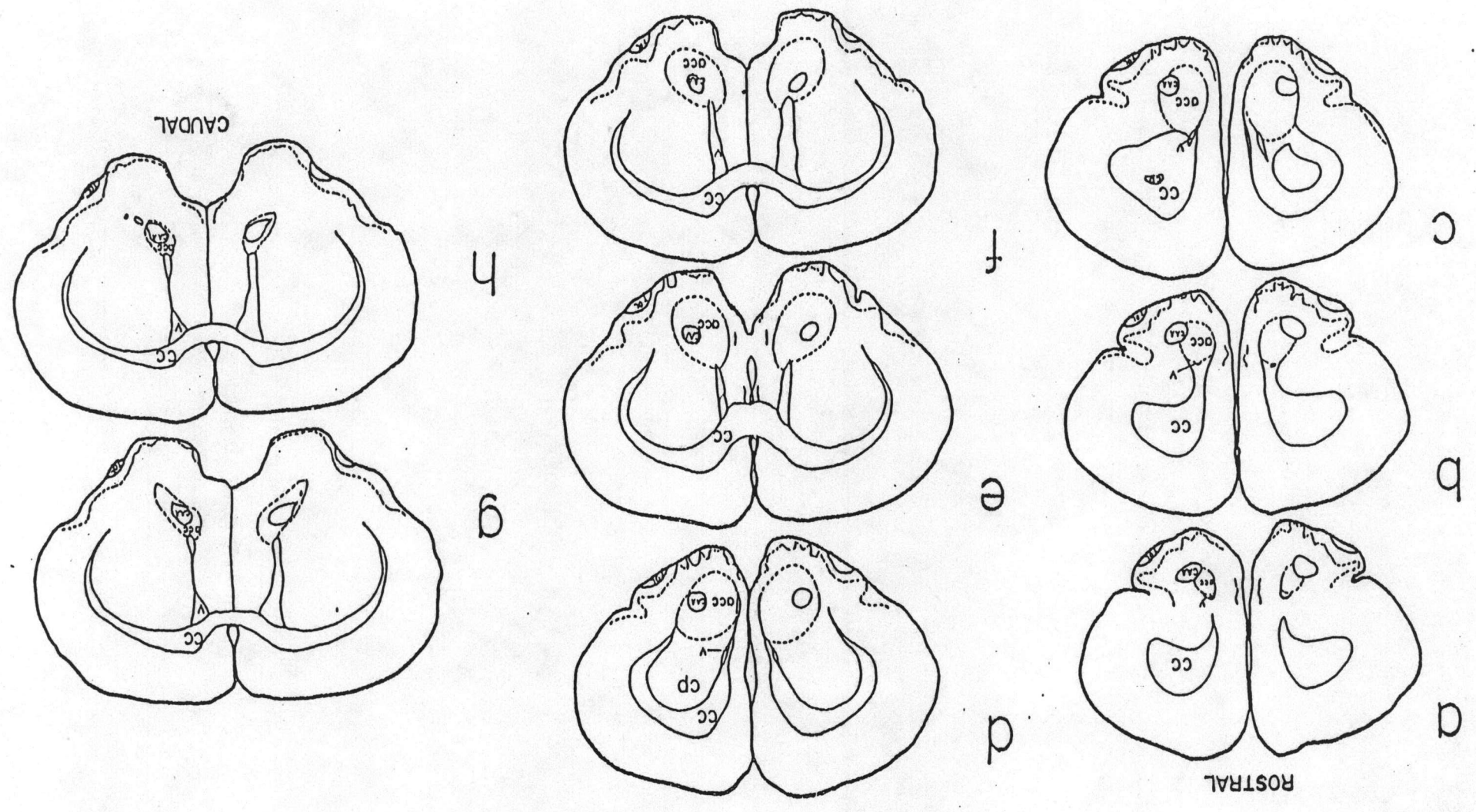
Case No.	Localization and extension of HRP injection site
Rat No. 13	small area in the lateral part of middle region.
Rat No. 14	small area in the lateral part of middle region.
Rat No. 15	small area in the rostral part more medial.
Rat No. 16	small area in the lateral part of middle region.
Rat No. 17	small area in the medial part of middle region.
Rat No. 18	moderate area in the medial part of middle region.
Rat No. 19	small area in the medial part of middle region.
Rat No. 20	large area occupied the whole middle region.
Rat No. 21	small area in the lateral part of middle region.
Rat No. 22	small area in the lateral part of middle region.
Rat No. 23	moderate area in the rostral part more middle region.
Rat No. 24	small area in the medial part of middle region.
Rat No. 25	small area in the rostral region.
Rat No. 26	small area in the lateral part of middle region.

Case No.	Localization and extension of HRP injection site
Rat No.27	large area in the caudal region.
Rat No.28	small area in the medial part of middle region.
Rat No.29	small area in the medial part of middle region.
Rat No.30	moderate area in the caudal region.
Rat No.31	small area in the lateral part of middle region.
Rat No.32	large area in the middle region.
Rat No.33	small area in the lower rostral part more lateral region.
Rat No.34	small area in the lateral part of middle region.
Rat No.35	moderate area in the caudal region.
Rat No.36	moderate area in the medial part of middle region.
Rat No.37	small area in the lateral part of middle region.

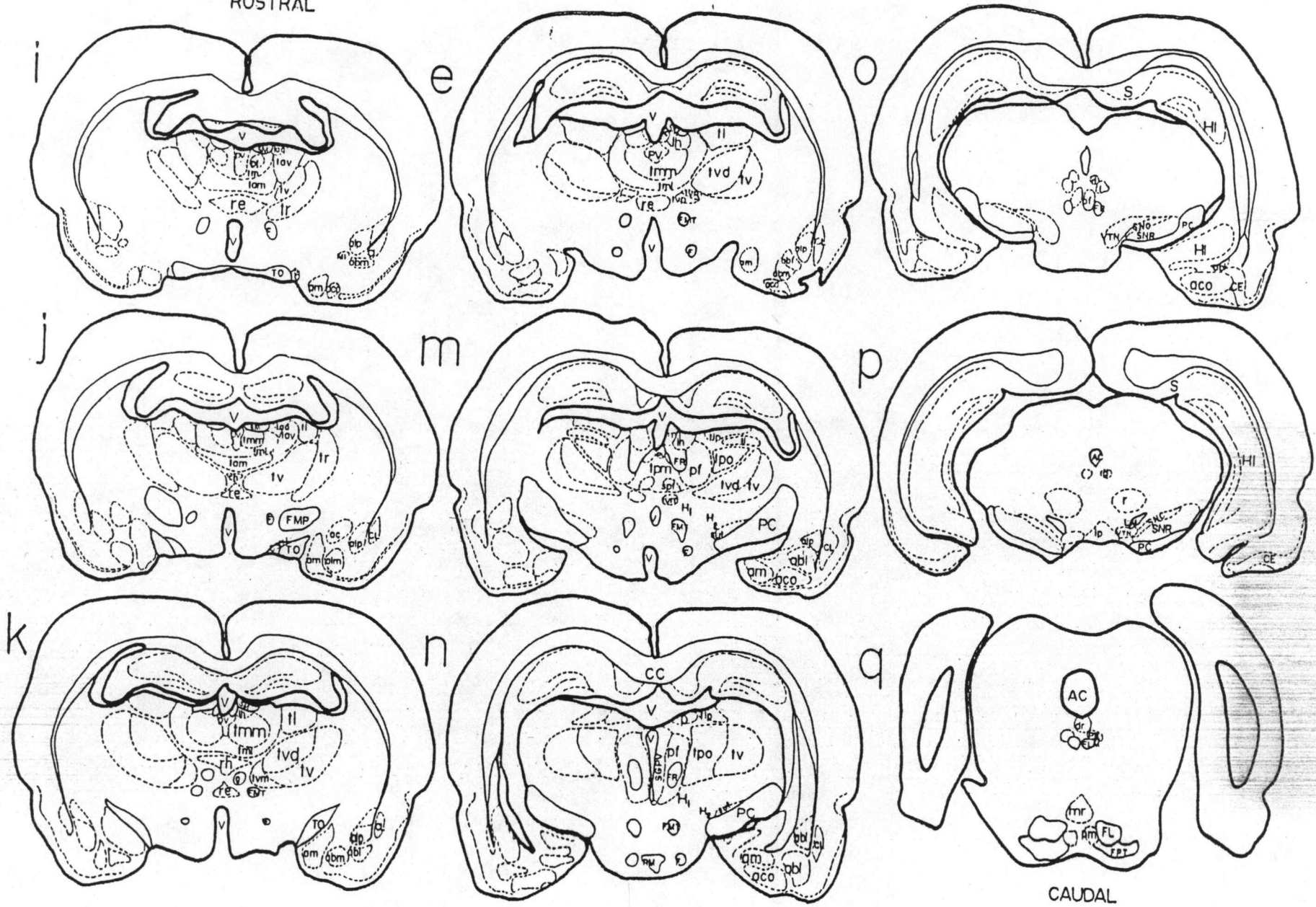


Figure 1 : A standard diagram composed of
a to h : coronal equally spaced serial
sections of the rat brain showing location
and topographic relation of various region
of the nucleus accumbens arbitrarily
devided in the present study as
a. to c.: rostral region
d. to f.: middle region
g. to h.: caudal region
i. to q.: coronal equally spaced
serial sections of the rat brain showing
various areas for the mapping of HRP-labeled
cells distribution. Abbreviations see
the list.

Figure 1



ROSTRAL



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Figure 2-6 : Diagrams of coronal equally spaced serial sections of the rat brain showing a. to h.: the extension of the injected HRP fluid in various cases

in the rostral region of the nucleus accumbens (Plate 2).

in the middle region of the nucleus accumbens (Plate 3).

in the lateral of middle region of the nucleus accumbens (Plate 4).

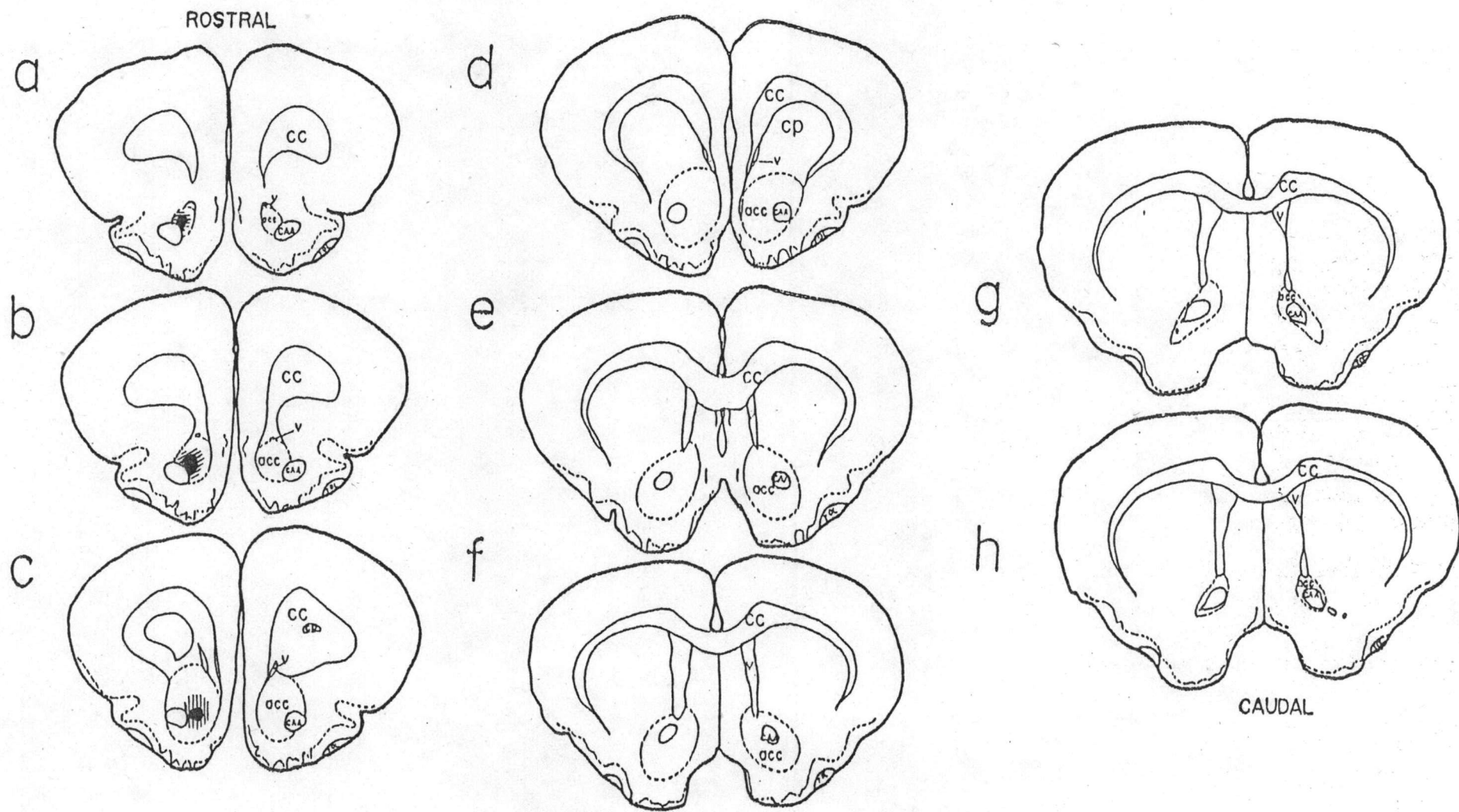
in the medial part of middle region in the nucleus accumbens (Plate 5).

in the caudal region of the nucleus accumbens (Plate 6).

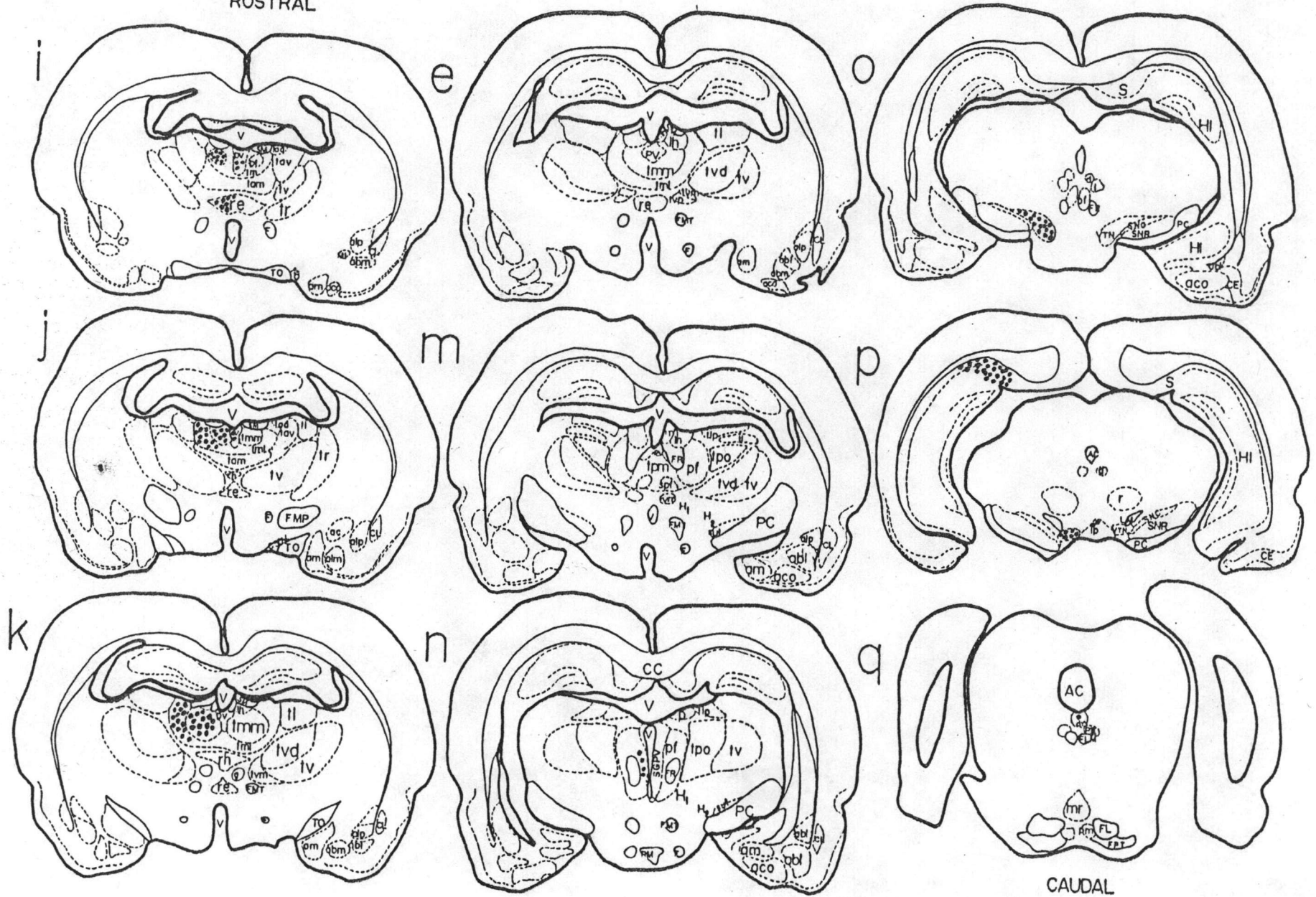
indicated by solid black areas are the injection sites and by dash lines are the spreading zone.

i. to q.: the distribution of HRP-labeled cells (represented by dots) in various cases (for details see text and list of abbreviations).

Figure 2 : Rat No 1

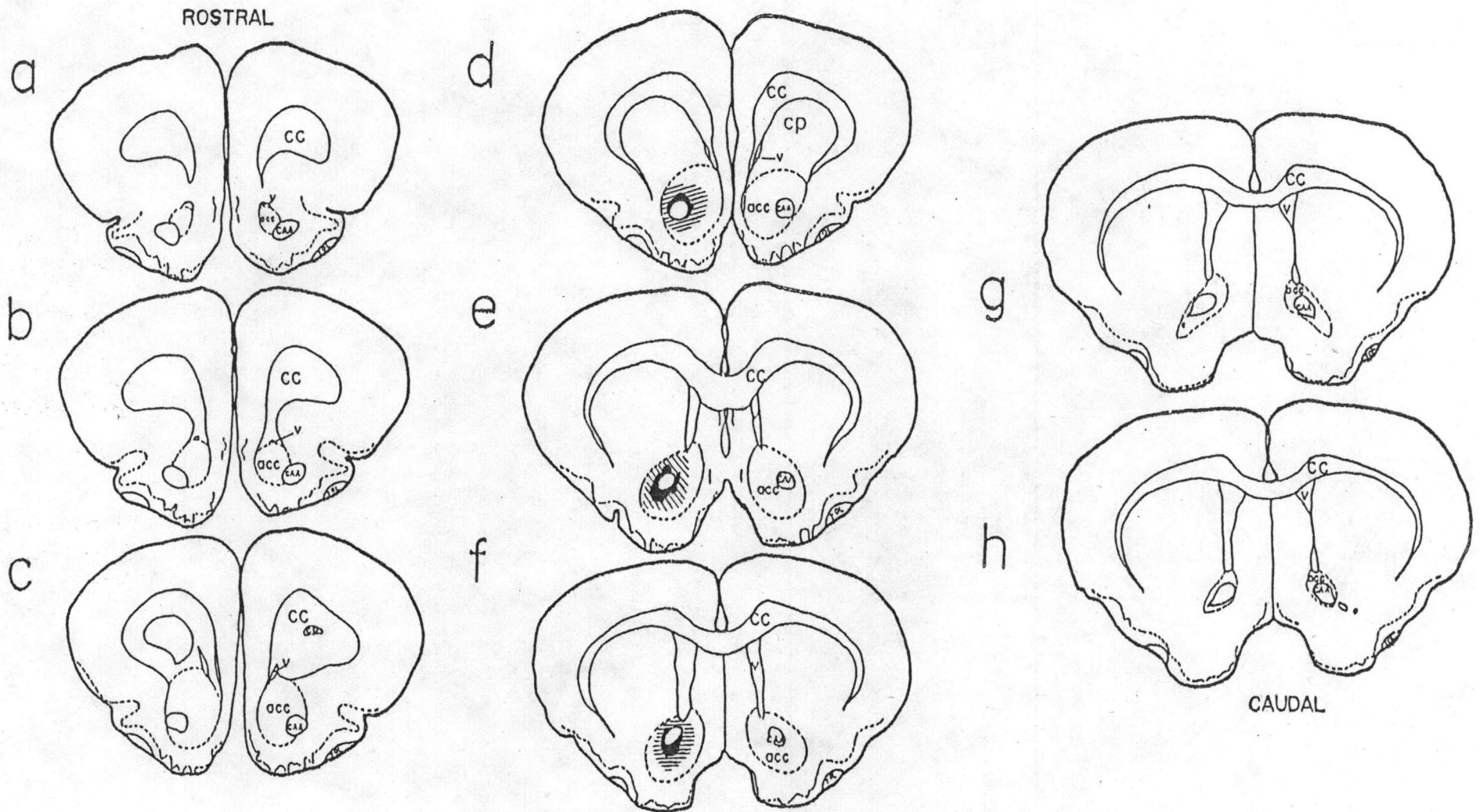


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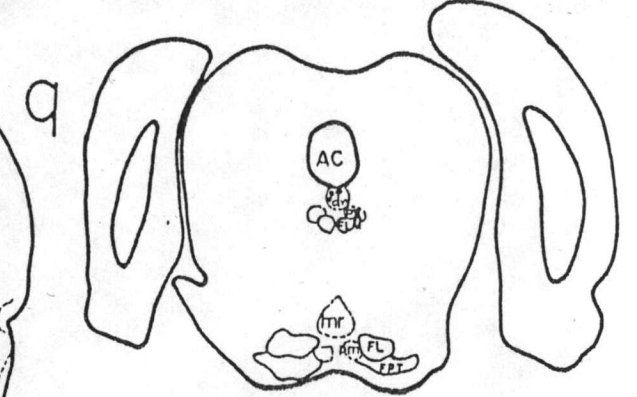
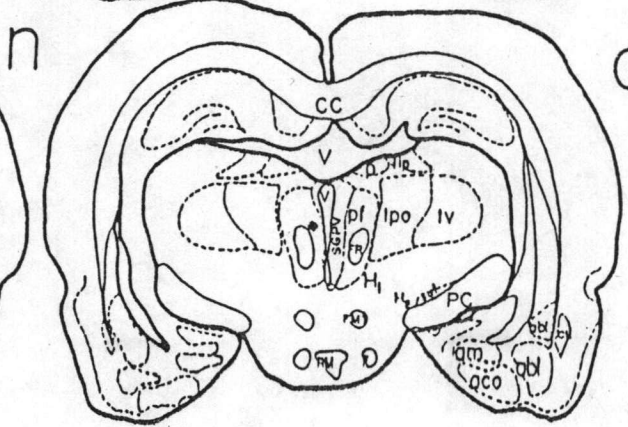
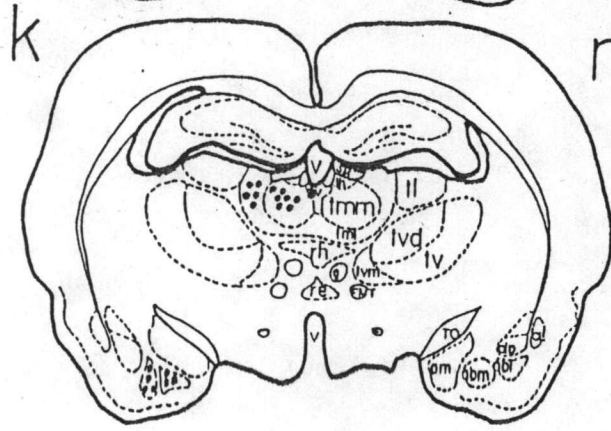
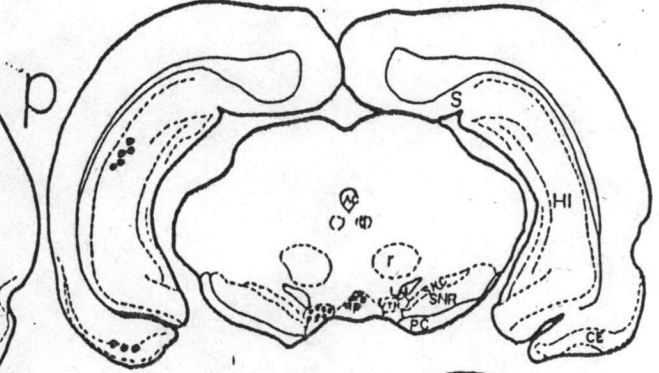
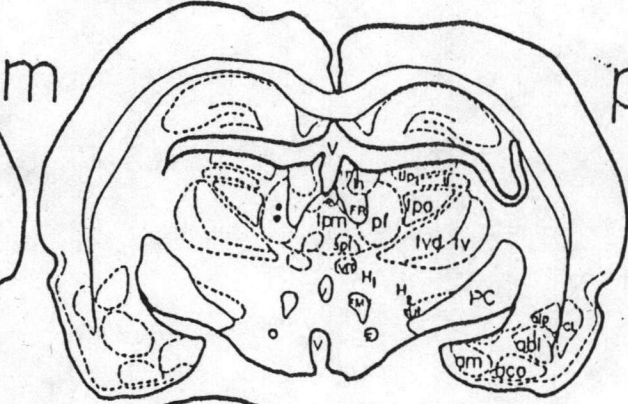
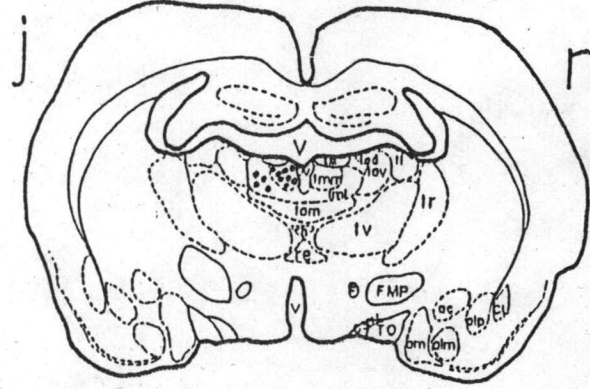
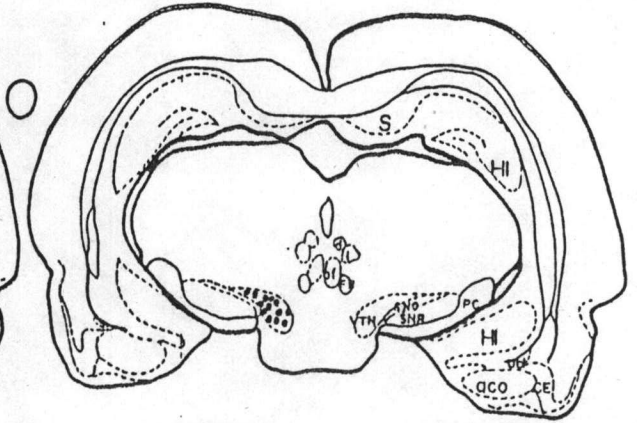
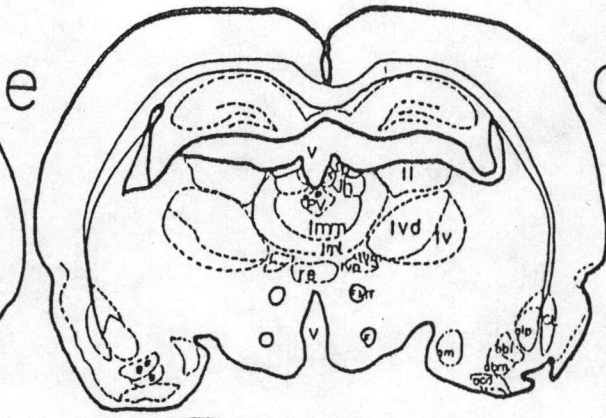
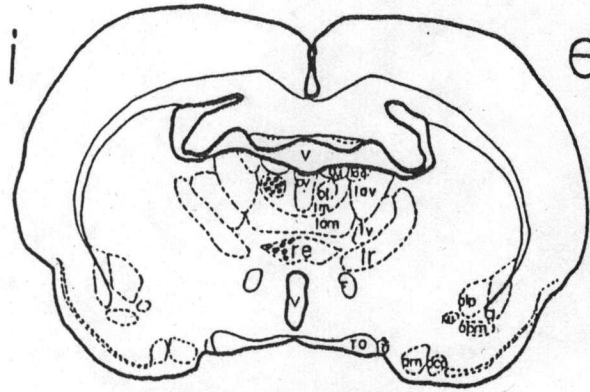


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Figure 3 : Rat No. 32



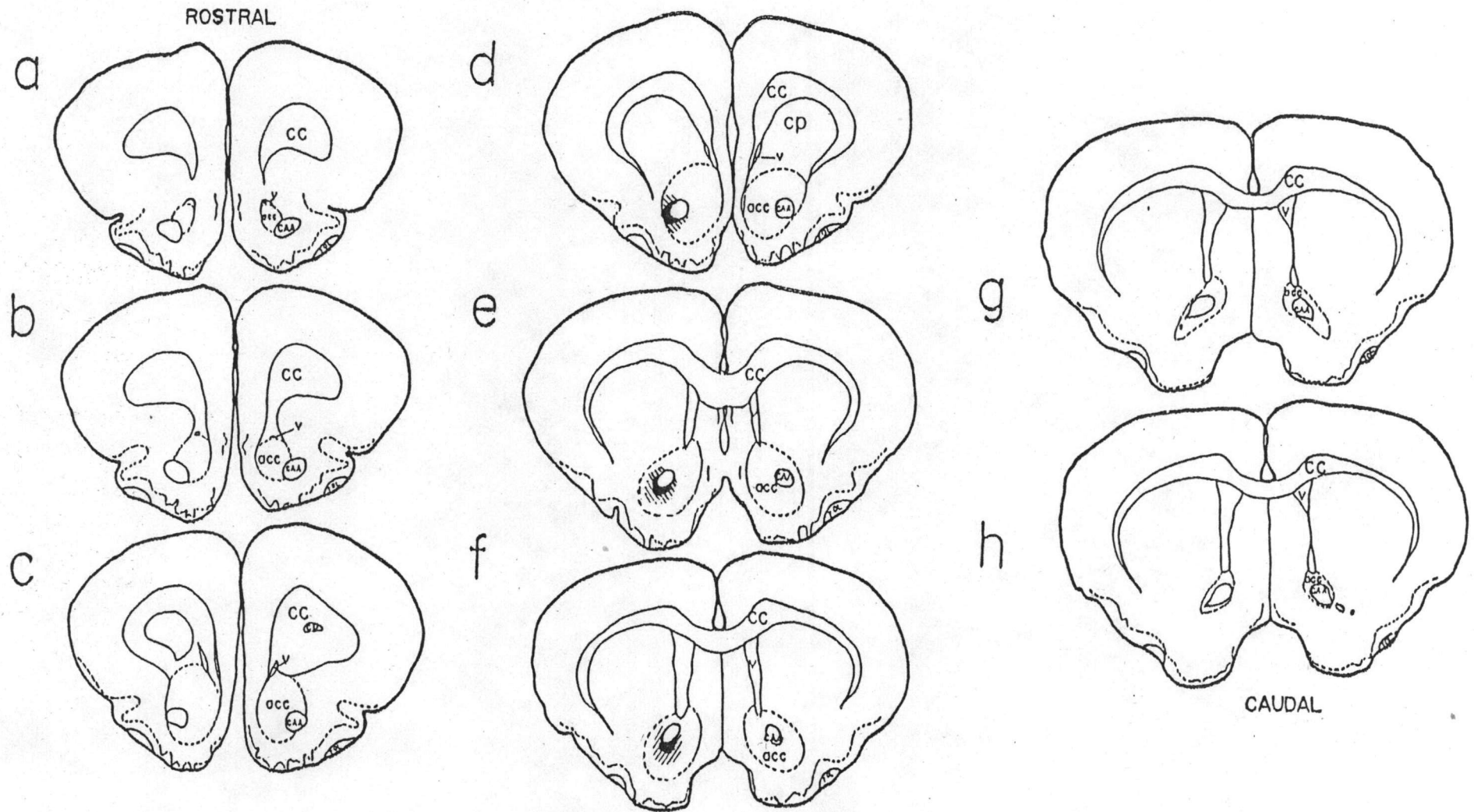
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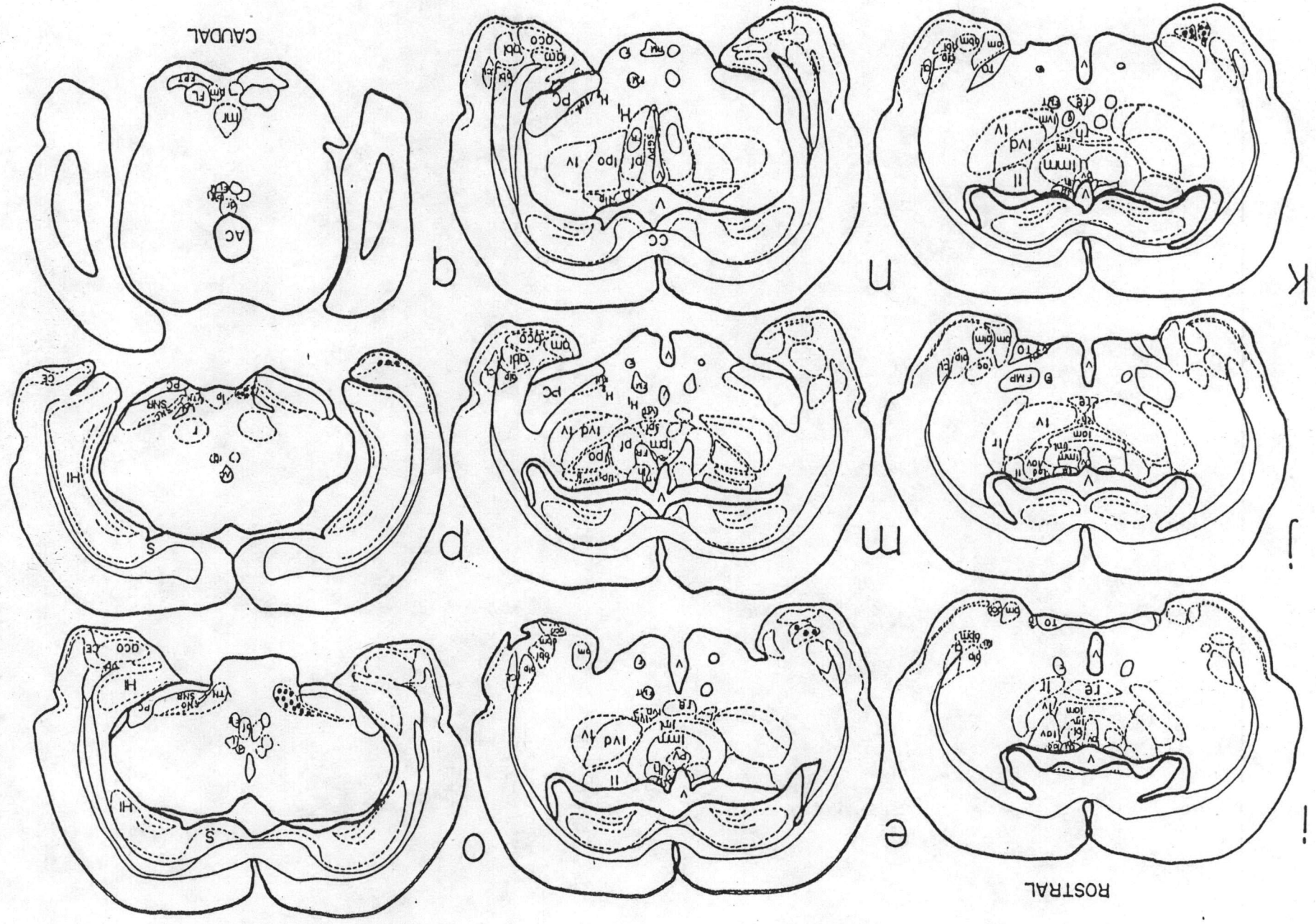


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Figure 4 : Rat No. 2



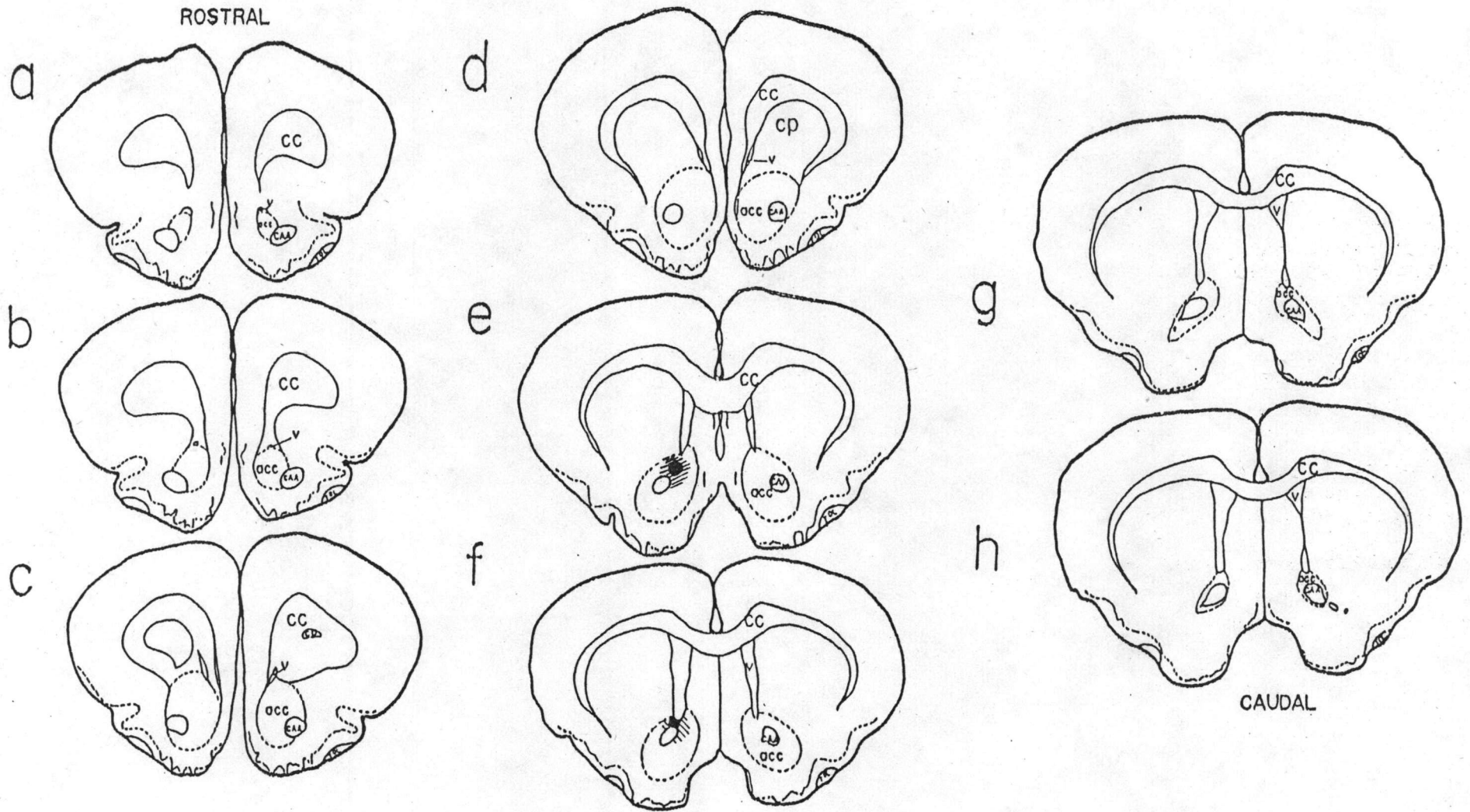


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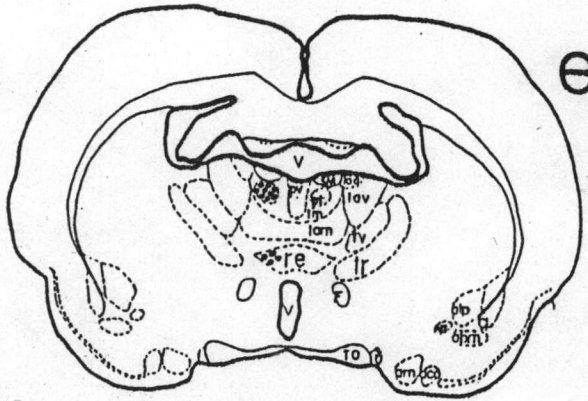
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Figure 5 : Rat No. 4

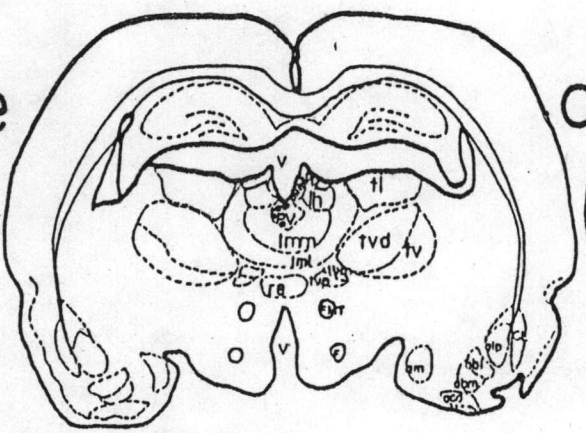


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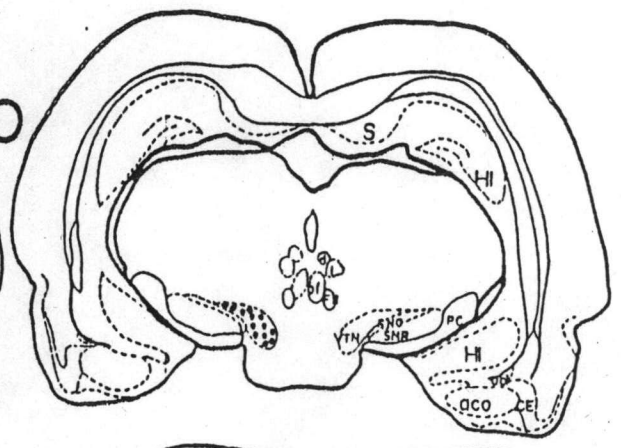
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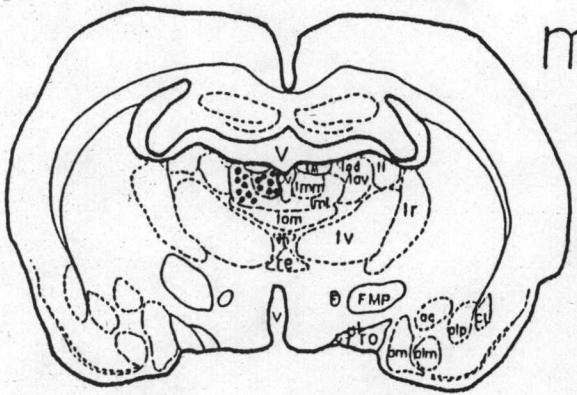
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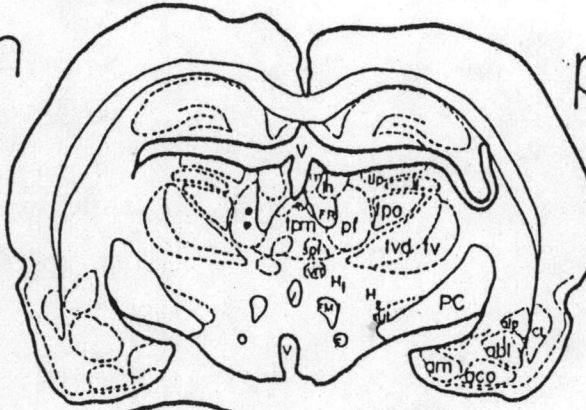
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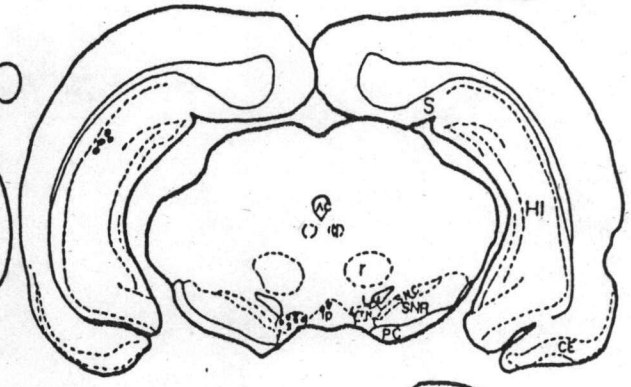
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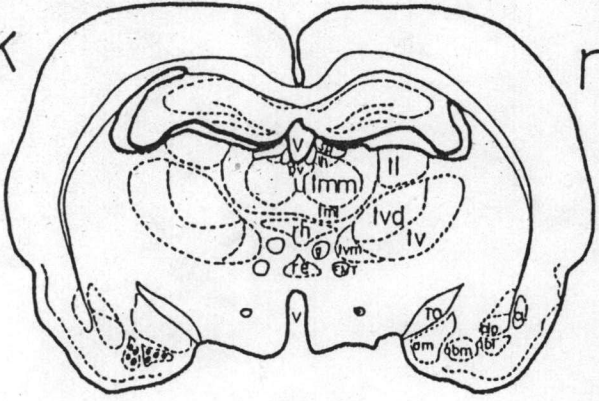
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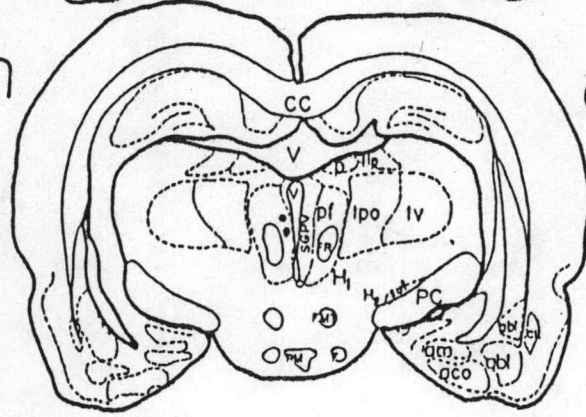
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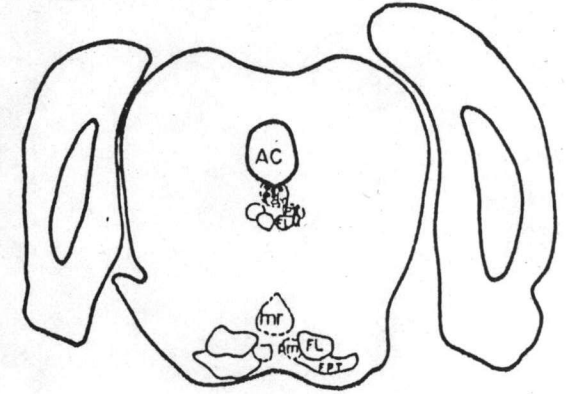
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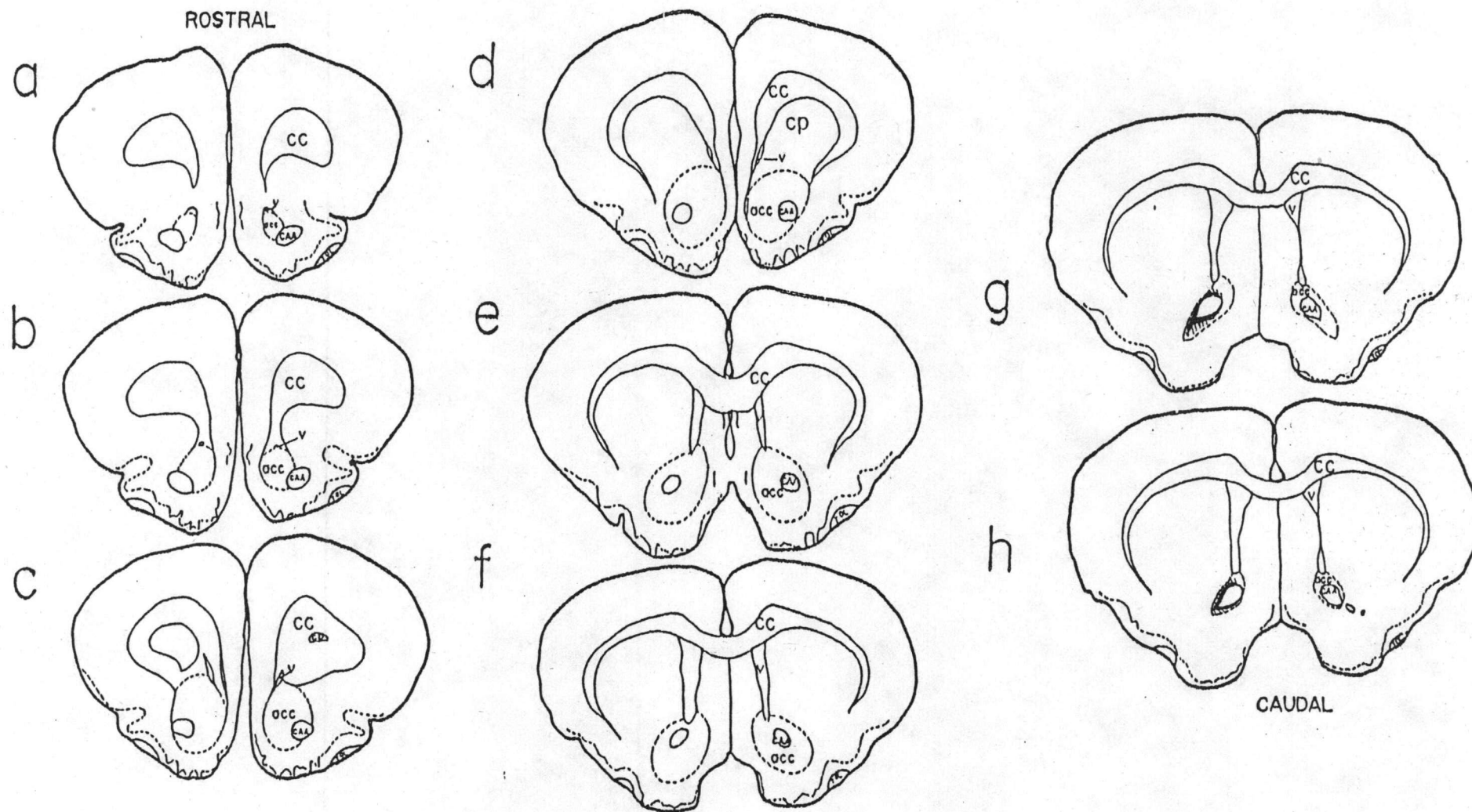


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Figure 6: Rat No. 30



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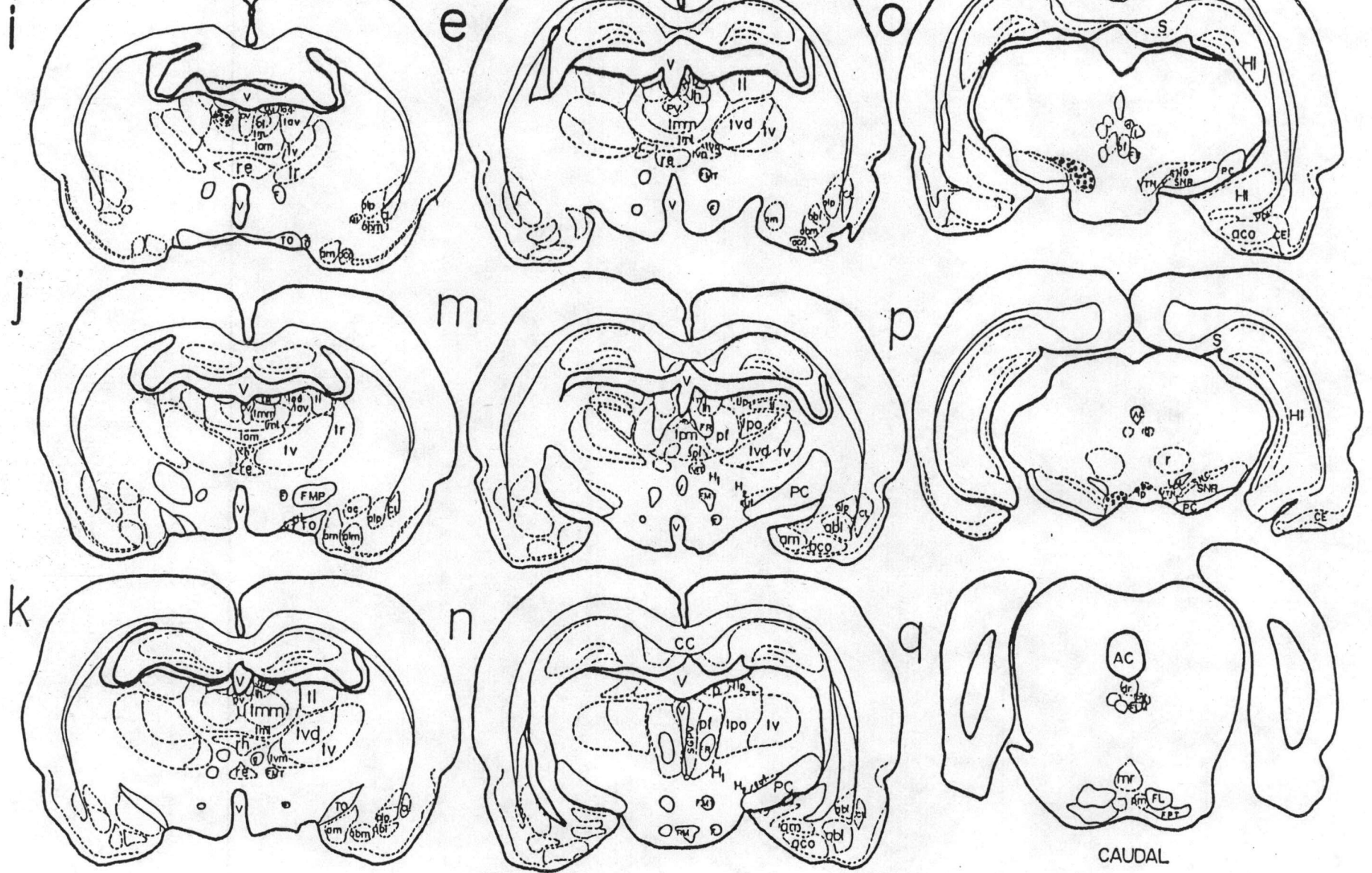




Plate 1 : Photomicrographs of coronal sections of the rat brain in the region of the nucleus accumbens showing the localization and extension of HRP injection site in various parts of the nucleus.

A. A large injection site involving the whole middle part of the nucleus of the rat No. 32.

B. A moderate injection site in the medial region of the middle part of the nucleus taken from the rat No. 4.

C. A moderate injection site in the lateral region of the middle part of the nucleus of the rat No. 2.

D. A small injection site in the caudal part of the nucleus of the rat No. 30.

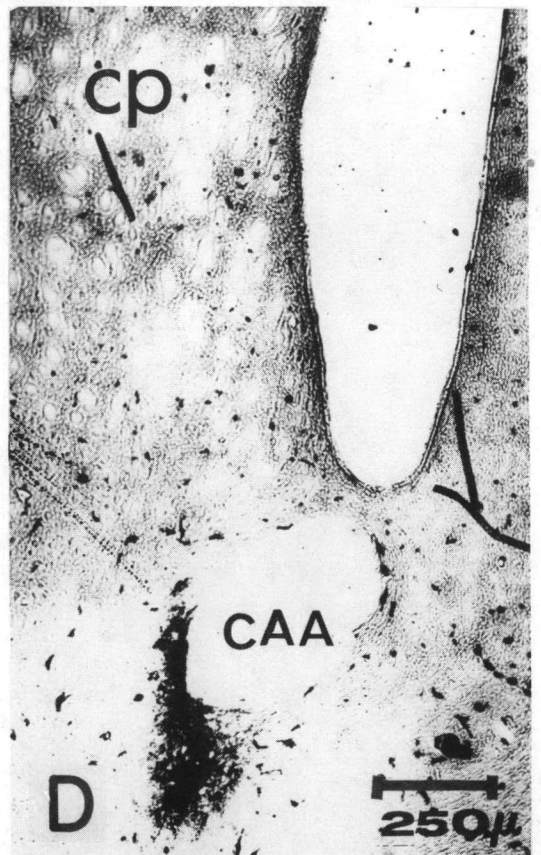
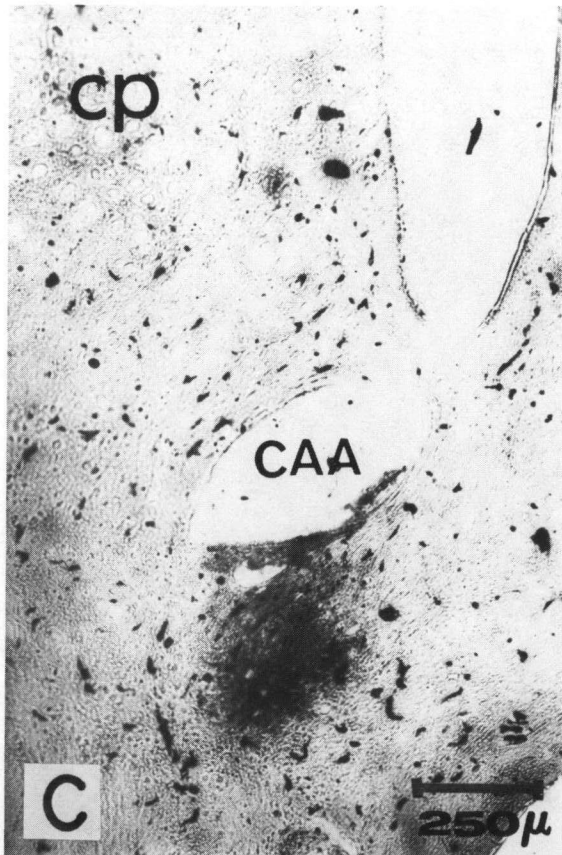
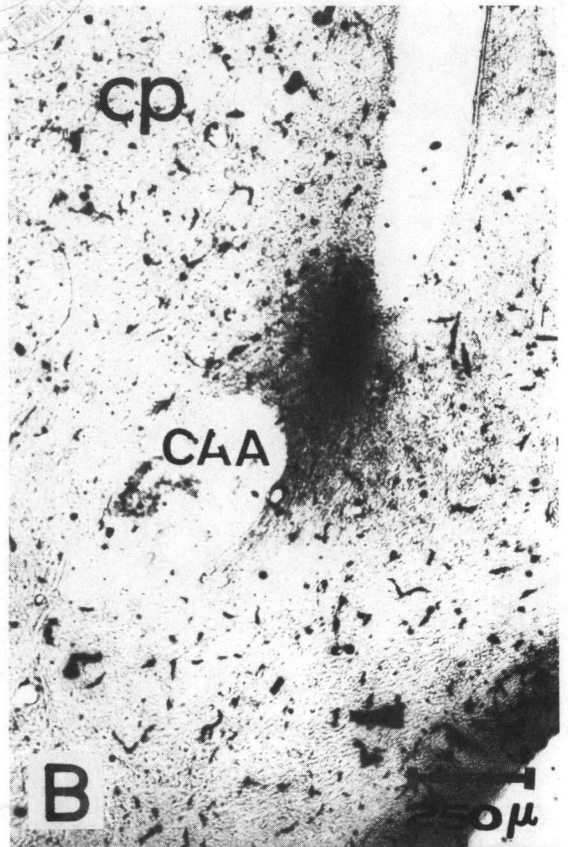
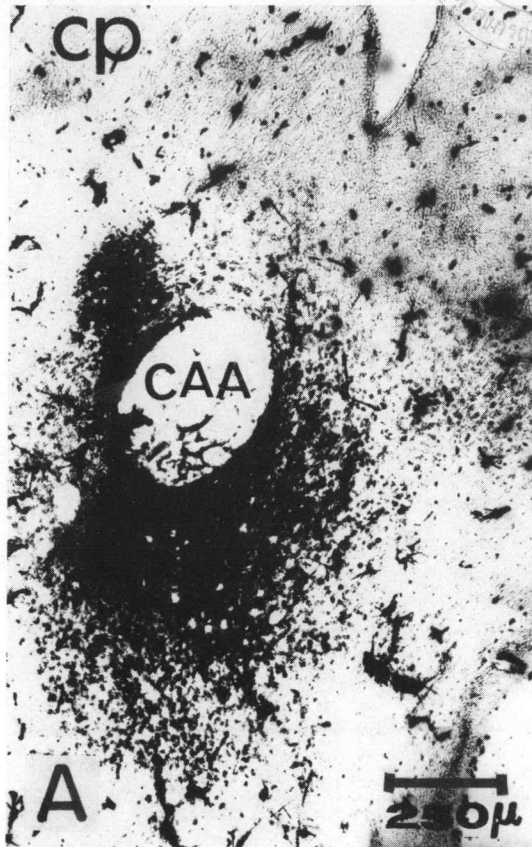


Plate 2 : Photomicrographs of HRP-labeled cells in the ventral part of subiculum in rat No. 1.

- A. At a low magnification showing location of subiculum. A groups of HRP-labeled cells is indicated in circle (○).
- B. The same groups of cells at a higher magnification.
- C. Highest magnification showing the appearance of HRP granules in the cells indicated in circle in B.

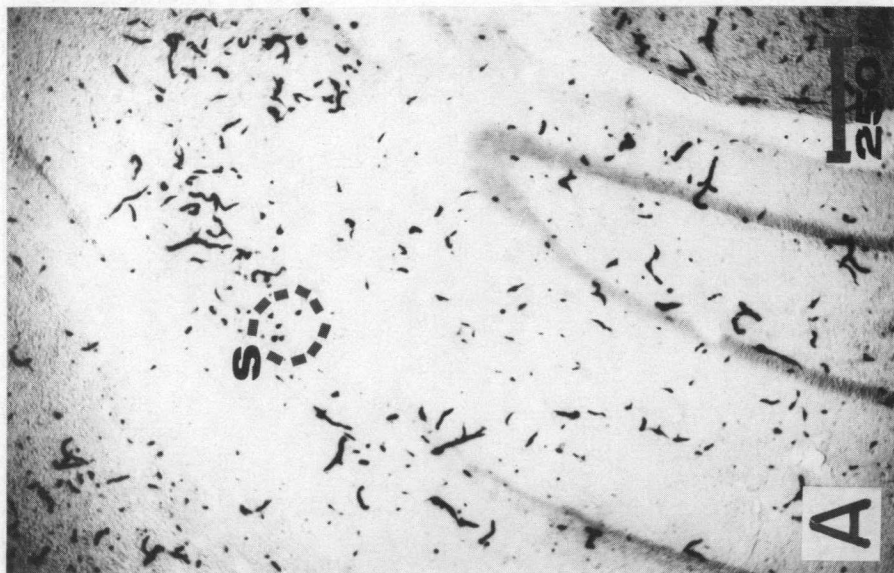
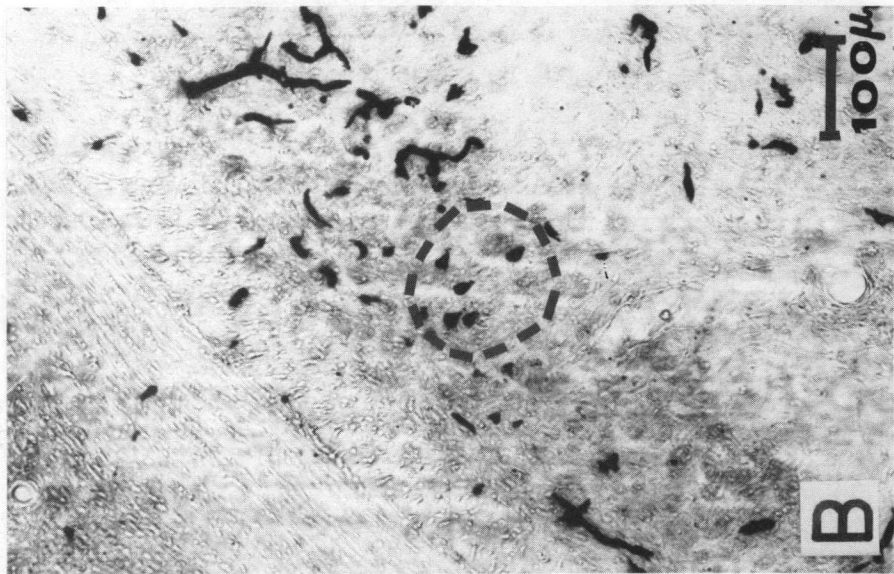
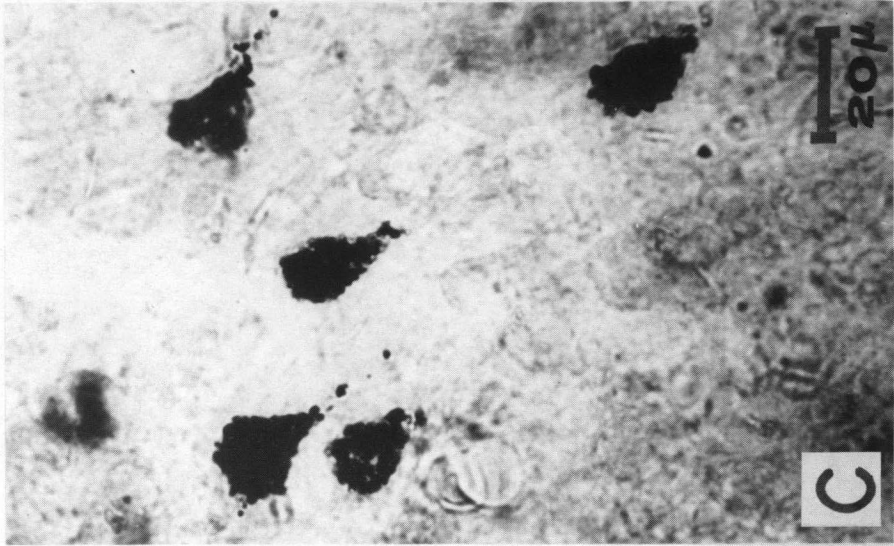


Plate 3 : Photomicrographs of HRP-labeled cells
in the ventral tegmental area taken from
rat No. 32.

A. At a low magnification showing
boundary of ventral tegmental area.

B. At higher magnification showing
HRP-labeled cells indicated with
circle in A.

C. Higher magnification of the cells
in B.

D. Highest magnification of 3 labeled
neurons with dark round and oval HRP
granules in the perikarya and
dendritic processes.

(Abbreviations see list)

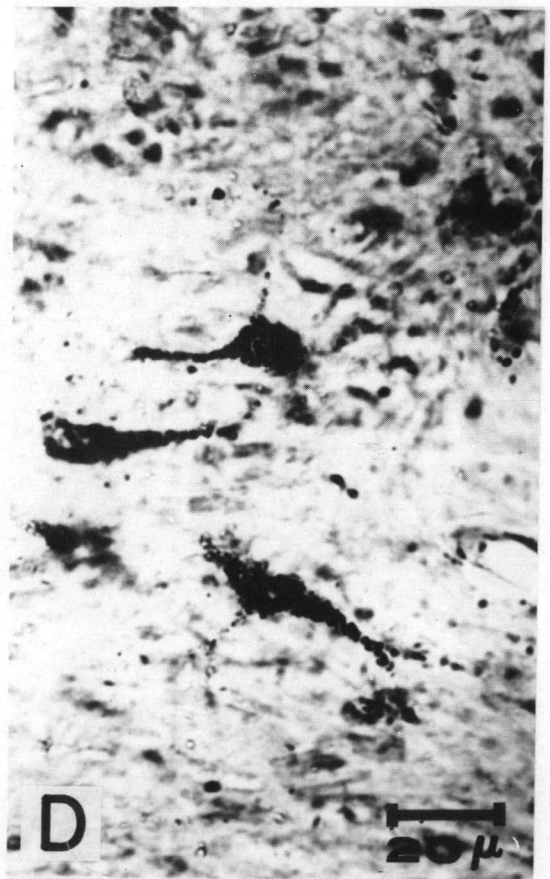
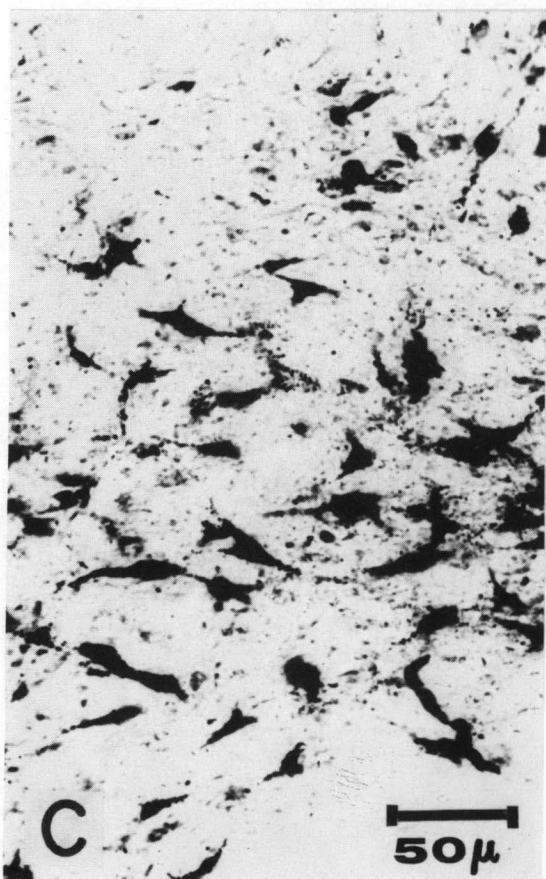
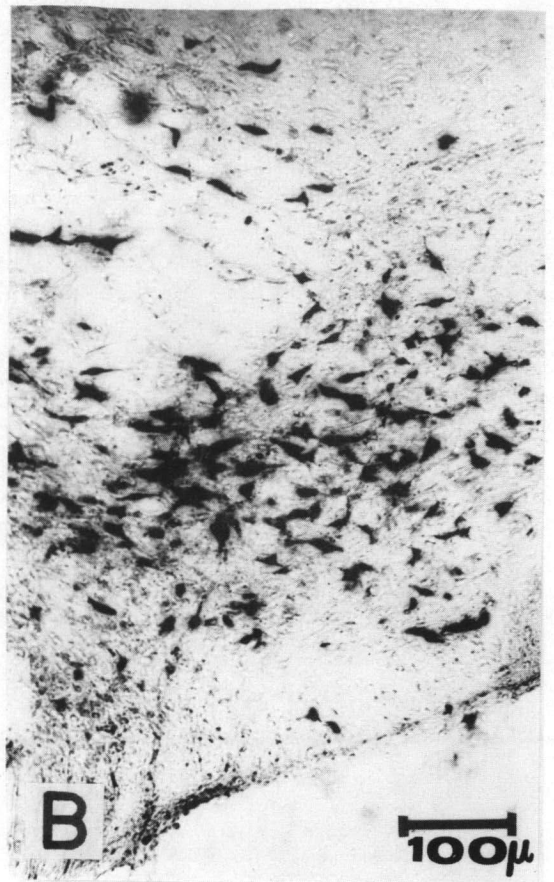


Plate 4 : Photomicrographs of labeled neurons
A and B in the dorsal raphe of the
rat No. 1.
A. At a low magnification showing
boundary of dorsal raphe.
B. At highest magnification of HRP-
labeled cells.
C and D in the paraventricular nucleus
of rat No. 23.
C. At low magnification of the
paraventricular nucleus HRP-labeled
cell indicated with circle in enlarger
in D.
(Abbreviations see list)

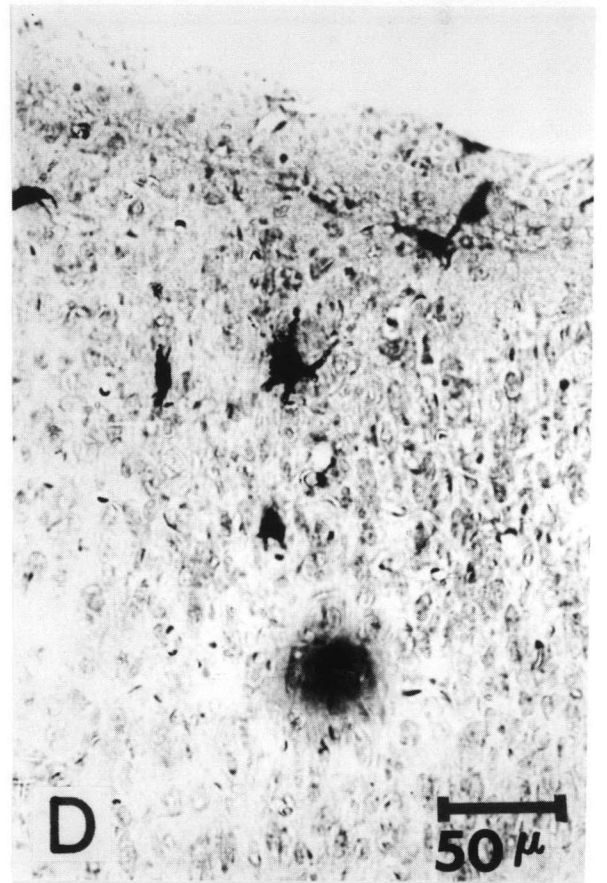
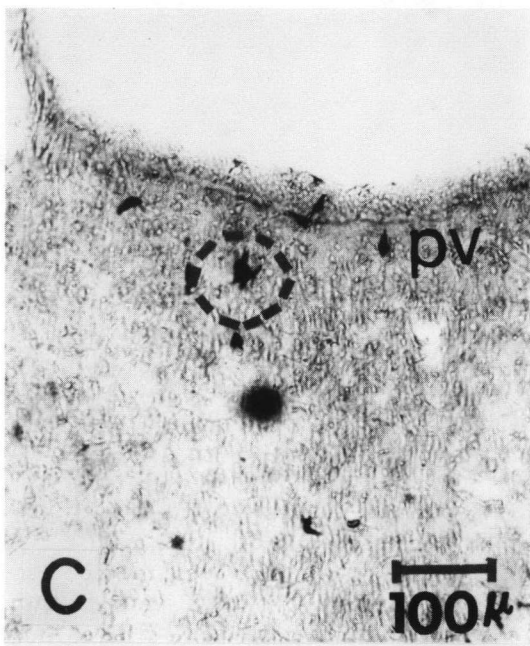
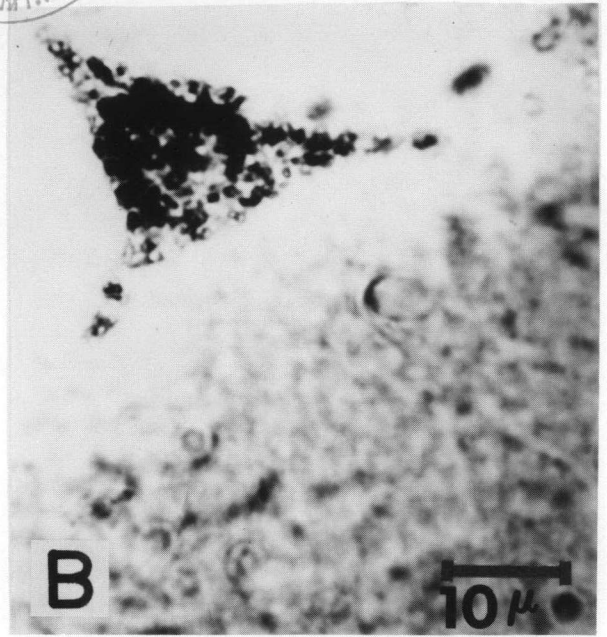
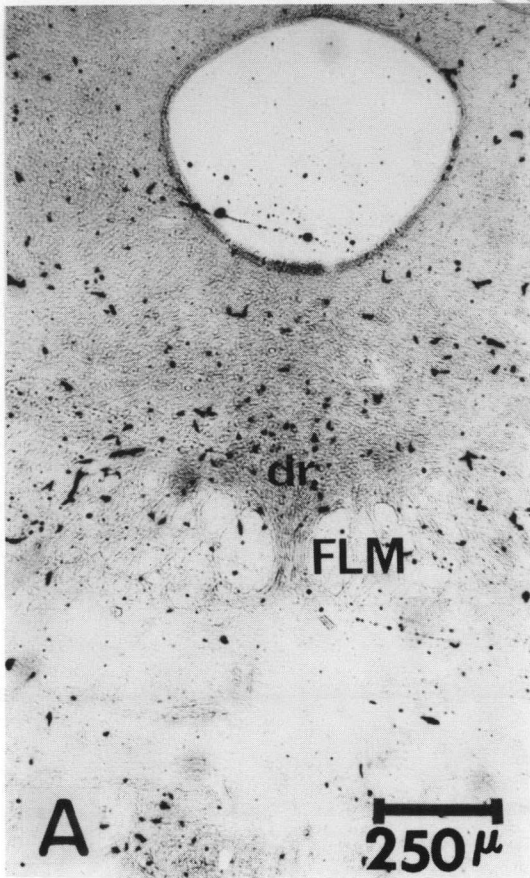


Plate 5 : Photomicrographs of HRP-labeled cells
A and B in the parataenial nucleus
taken from the rat No. 1.
A. At low magnification indicating
boundary of parataenial nucleus.
B. Higher magnification showing
HRP-labeled cell.
C and D in the parafasicular nucleus
taken from rat No. 7.
C. At low magnification showing
boundary of the parafasicular nucleus
HRP-labeled cell. and Higher magnifi-
cation showing HRP-labeled cell in D.
(Abbreviations see list)

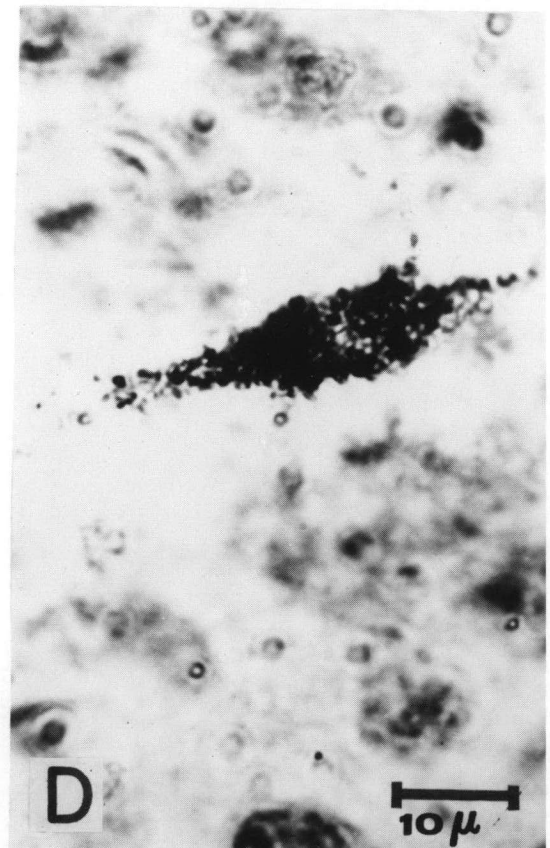
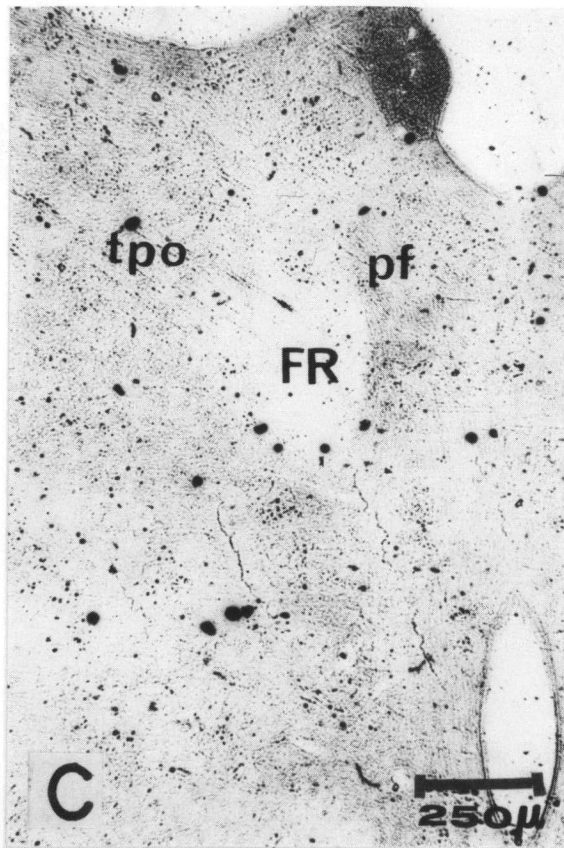
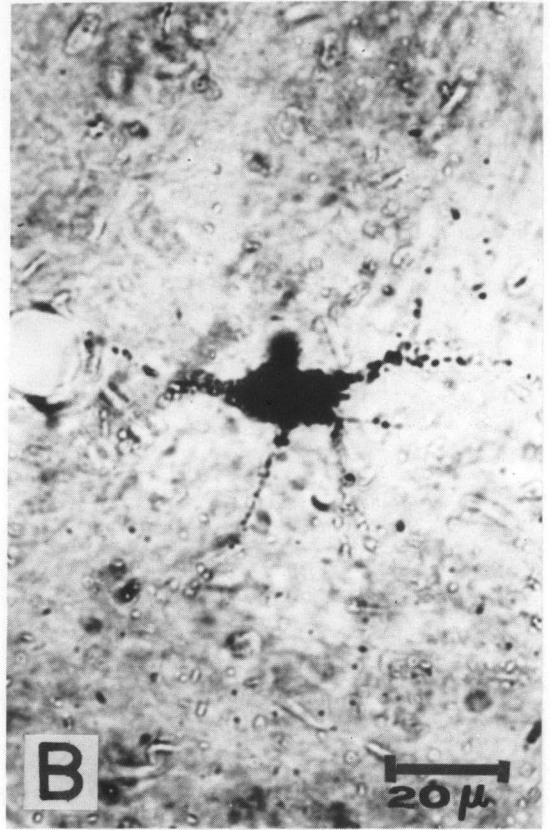
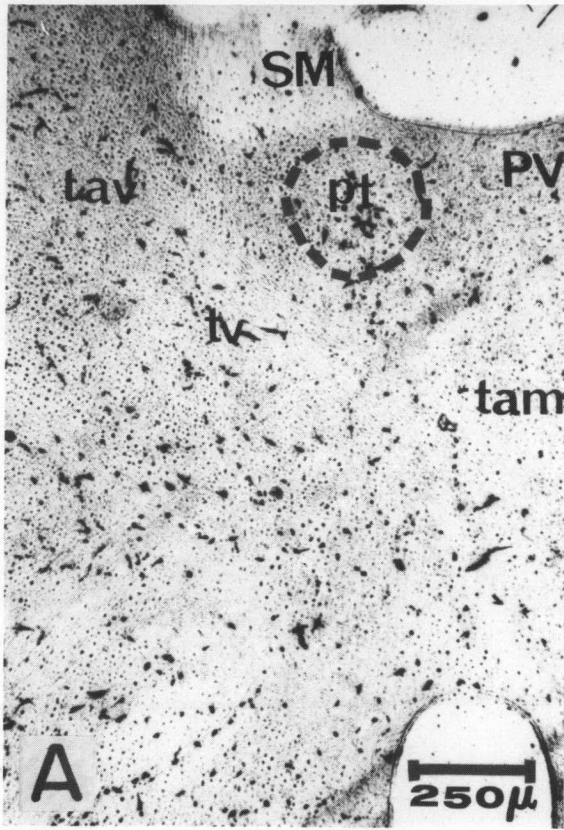


Plate 6 : Photomicrographs showing the characteristics of HRP-labeled cells with oval dark round granules in their perikarya and dendrites.

A. Two HRP-labeled cells in the parataenial nucleus of rat No. 7.

B. A HRP-labeled cell in the parataenial nucleus of rat No. 1.

C. HRP-labeled cell in the reuniens nucleus of the rat No. 7.

D. Another HRP-labeled cell in the reuniens nucleus of rat No. 1.

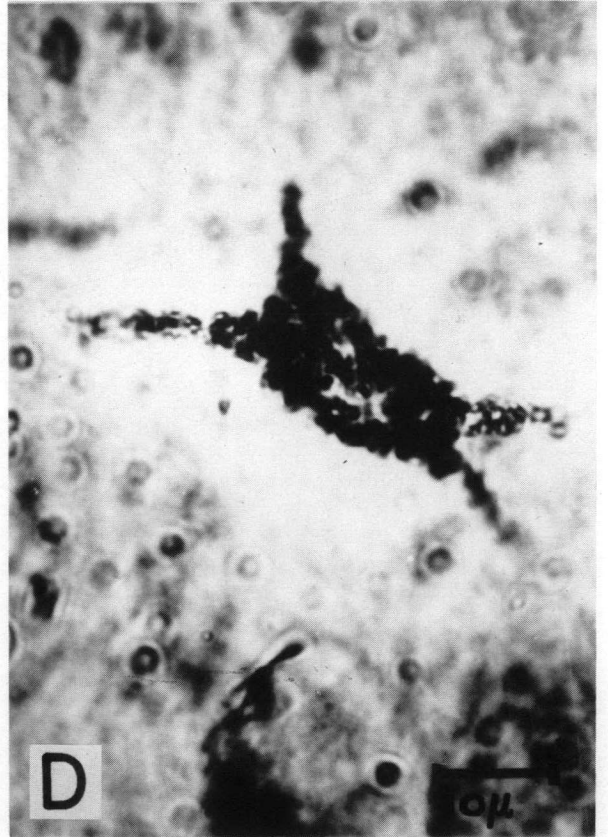
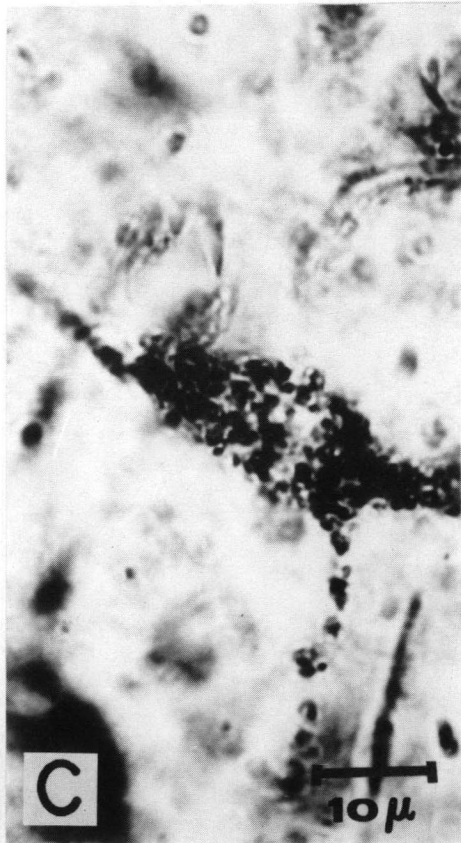
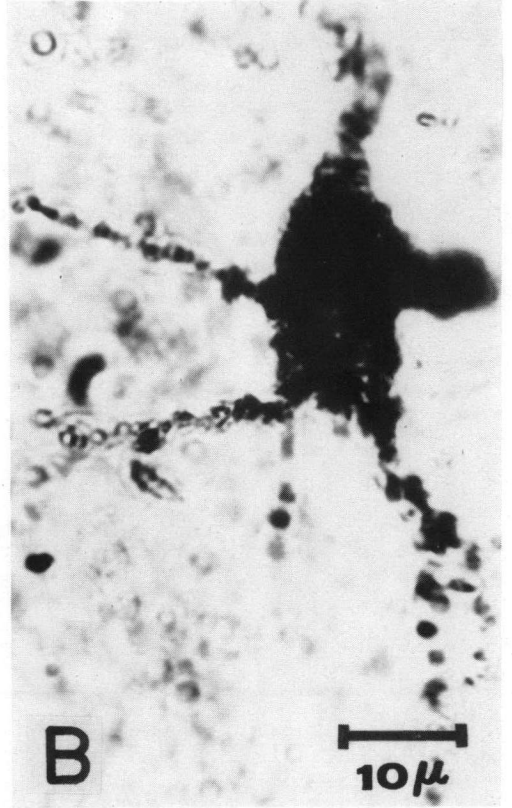
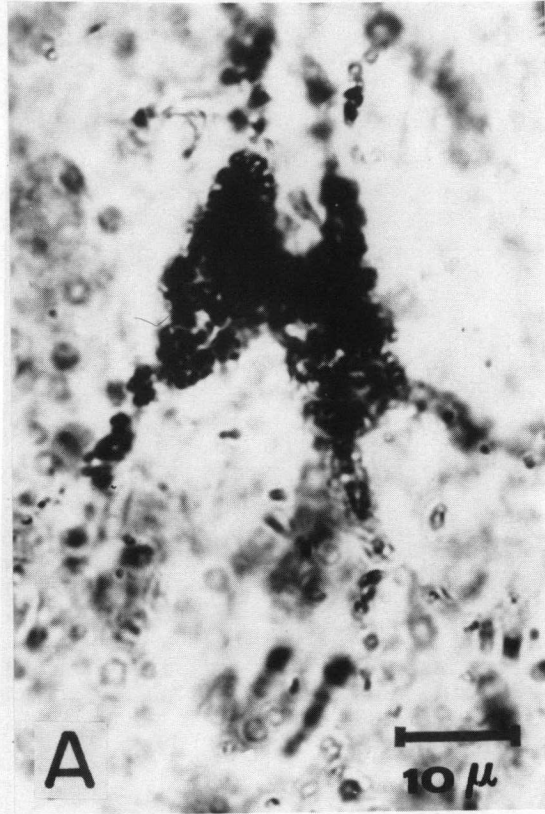
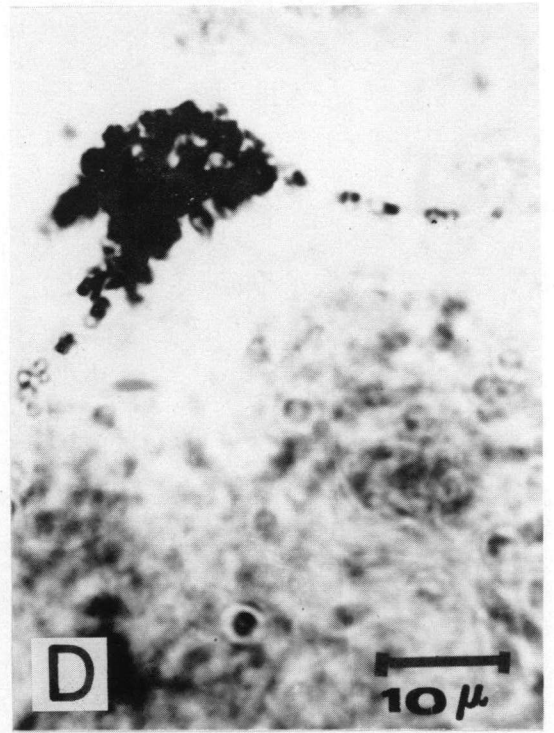
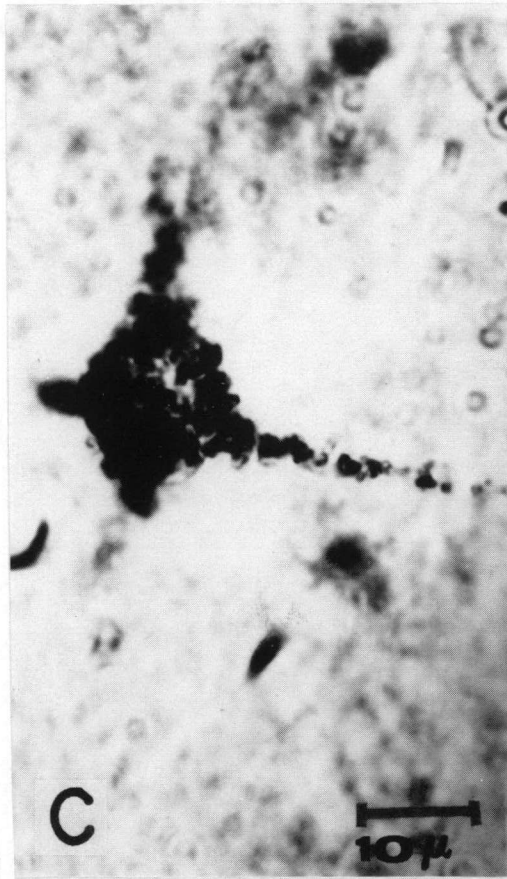
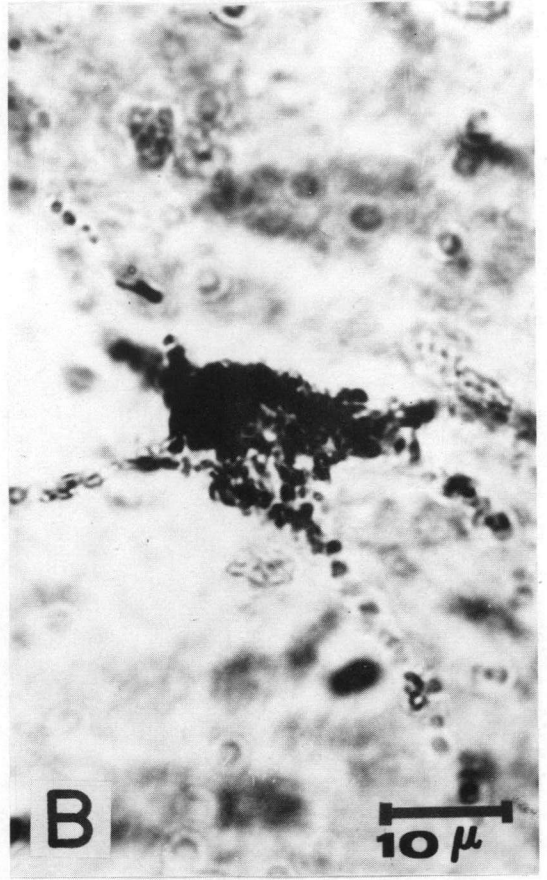
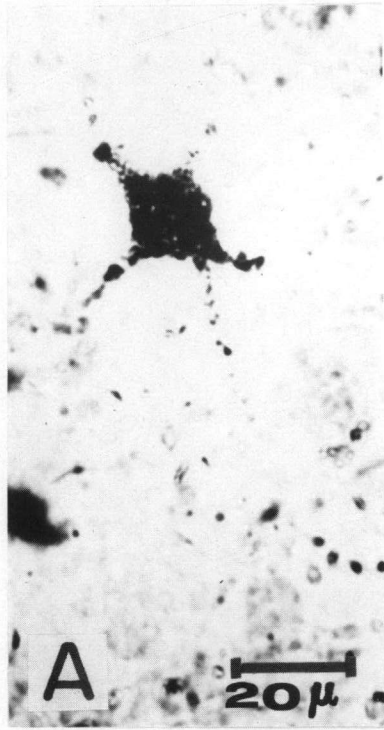


Plate 7 : Photomicrographs of HRP-labeled cells
A and B in the medial thalamic nucleus
pars lateralis of rat No. 1.
C. In the medial thalamic nucleus, pars
medialis of rat No. 1.
D. In the nucleus amygdaleus basalis,
pars lateralis of rat No. 32.



- Plate 8 : Photomicrographs of the HRP-labeled cells.
- A. in the interpeduncular nucleus of rat No. 20.
 - B. in the paraventricular nucleus of rat No. 32.
 - C. in the substantia nigra, pars compacta in the
rat No. 4.

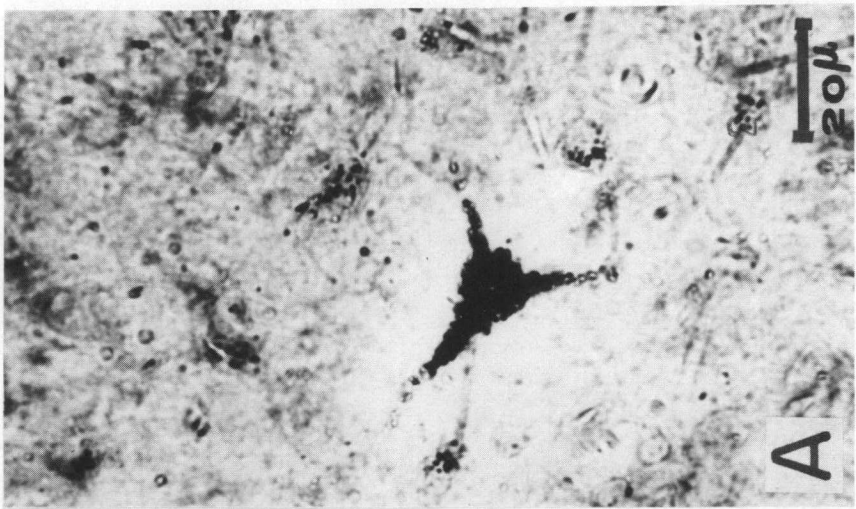
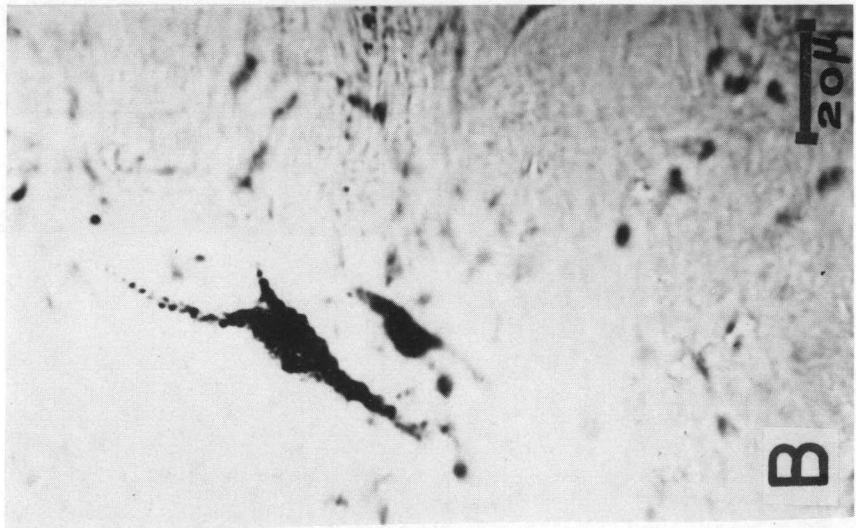
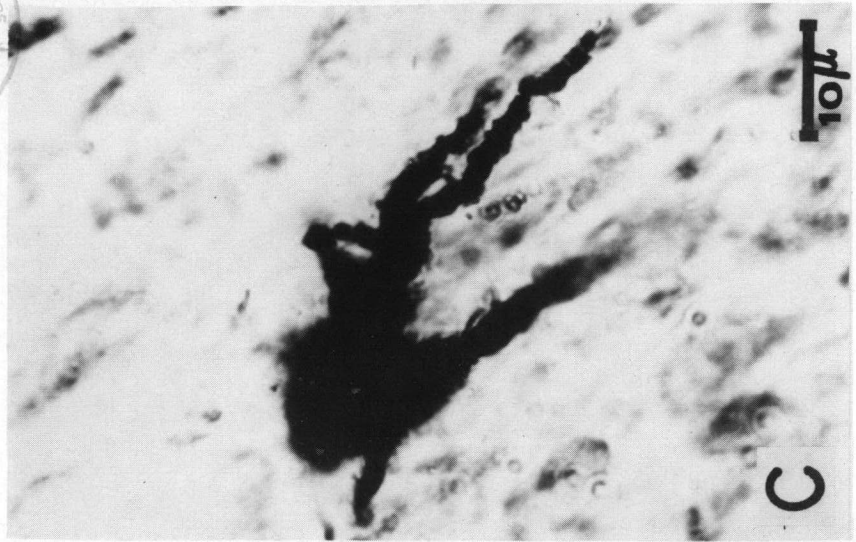


Plate 9 : A summaried diagram of the present finding
on the afferent projections to the nucleus
accumbens.

DIAGRAM OF THE AFFERENT CONNECTIONS TO THE NUCLEUS ACCUMBENS

