RELATIONSHIPS OF THE TUFTED CELLS OF THE OLFACTORY BULB TO THE LATERAL OLFACTORY TRACT

Dissertation for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
ROBERT C. SWITZER III
1973

This is to certify that the

thesis entitled

RELATIONSHIPS OF THE TUFTED CELLS OF THE OLFACTORY
BULB TO THE LATERAL OLFACTORY TRACT

presented by

Robert C. Switzer III

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Biophysics

John I. Johnson Jr.

Major professor

Date November 5, 1973

O-7639

ABSTRACT

RELATIONSHIPS OF THE TUFTED CELLS OF THE OLFACTORY
BULB TO THE LATERAL OLFACTORY TRACT

By

Robert C. Switzer III

The mitral and tufted cells are the two primary neurons of the mammalian olfactory bulb. The mitral cells, whose perikarya are exhibited in a narrow band, have long been recognized as the source of output from the olfactory bulb. On the other hand the tufted cells, whose perikarya are distributed in concentric bands external to the layer of mitral cells, have long been a subject of controversy. The controversies have been directed at the destination of neuraxes projected from the perikarya of tufted cells and have questioned the basis for considering the tufted cells as unique neuronal elements.

This study is directed at determining the electrophysiological manifestations of the presence of an accentuated population of middle tufted cells in opossum. By the coupling of data from quantitative anatomical methods with that from recording electrical activity with micropipette electrodes, evidence is presented which indicates that the population of tufted cells with large perikarya send neuraxes through the lateral olfactory tract along with the neuraxes of the mitral cells. The anatomical study revealed that the perikarya of tufted cells in the external plexiform layer are not uniformly distributed but preferentially occupy the outer half of the external plexiform layer in the olfactory bulbs of rat, rabbit and

opossum. The size of the perikarya of tufted cells increases with proximity to the mitral cell layer as was first reported by Cajal ('11, '55).

The correspondence between the position of the narrow mitral cell layer and the inflection point of phase inversion of the field potential "seen" by a recording micropipette electrode was precisely established by marking the site of phase inversion with a dye (Pontamine sky blue) iontophoretically driven from the tip of the electrode. With the mitral cell layer as a precise reference in the electrophysiological picture, unit recordings obtained by antidromic stimulation of the lateral olfactory tract could be accurately correlated with the anatomical structure. Units were deemed antidromically driven if they were able to follow a stimulus rate of 100Hz. The profile of the histogram of units *vs.* position matches closely that of the histogram of relative density of perikarya of tufted cells in the external plexiform layer. The two histograms are the same under the Kolomogorov-Smirnov test ($p=0.05$). It also appears that an even better correlation exists between the unit histogram and the histogram of the density of large perikarya. The physiological density corresponds to anatomical density but an even better correspondence is obtained by excluding the smallest perikarya. Possibly all or certainly a constant proportion of the larger perikarya of tufted cells contribute neuraxes to the lateral olfactory tract.

RELATIONSHIPS OF THE TUFTED CELLS OF THE OLFACTORY BULB TO THE LATERAL OLFACTORY TRACT

By

Robert C. Switzer III

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Biophysics Department

Dedicated to my family and friends who have been the mortar for the blocks which I have fashioned in building this far.

ACKNOWLEDGEMENTS

It is with a feeling of gratitude and appreciation that I thank the following people for their efforts and talents which made this study possible and bearable when "the going got rough": Grace Kim for her beautiful histology; Cynthia Keller for her encouragement and the many hours of planimetry; Dr. Victor Chen and Norm St. Pierre for their contributions to the electronic technique of this study;and Herm Rummelt for lending a critical ear.

With a note of affection, deep respect and gratitude I thank my professor, Dr. J. I. Johnson for his understanding and support; I am sure that at this juncture I could not possibly appreciate the extent to which he has formed and influenced my outlook and approach to science.

I am indebted to Dr. Jean Toth-Allen who introduced me to Biophysics and to Dr. Barnett Rosenberg who started me in Biophysics.

To my wife, Julie, I cannot give enough thanks for her understanding and support during our graduate school years; to my Father and Mother I give my love and thanks for helping me achieve this milestone with their physical and spiritual support.

With deep reverence I acknowledge my God.

This research was supported by NIH training grant GM 1422, NIH Research Grant NS05982 and NSF Research Grants GB13854 and GB30783.

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

There has been much controversy over the role of the tufted cells of the olfactory bulb and especially over where their neuraxes project. Cajal ('11, '55) thought that the tufted cells communicated with the contralateral olfactory bulb by a direct projection of their neuraxes through the anterior limb of the anterior commissure (AAC). This has since been shown to not be the case from lesion studies (Lohman and Mentink '69) and electrophysiological evidence (Nicoll '70). Valverde ('65) considered the tufted cells to be inwardly displaced periglomerular cells; he never was able to trace tufted cell axons beyond the bulb in newborn rats. This notion was contrary to what Crosby et al in many publications (eg. '39) considered the tufted cells to be: outwardly wandered mitral cells. Lohman and Mentink ('69) realized that some of the tufted cells projected neuraxes to the lateral olfactory tract (LOT) along with the neuraxes of the mitral cells. Nicoll ('70) electrophysiologically identified tufted cells which projected their neuraxes to the LOT by antidromic stimulation of the LOT while recording from the olfactory bulb. Nicoll's findings reveal in rabbit that most of the tufted cells with neuraxes in the LOT were nearest the mitral cell layer (MCL); only a few were found more externally. See Figures 1 and 2 for fiber tracts.

Cajal observed that there were three classes of tufted cells based on size and position. Those tufted cells nestled closely to the MCL are

FIGURE 1. Opossum Brain Profile. In this figure the placement of recording and stimulating electrodes are schematically shown in relation to prominent, topographic landmarks of the opossum brain.
LOT: lateral olfactory tract; NC: neocortex; OB: olfactory bulb; OT: olfactory tubercle; PC: paleocortex

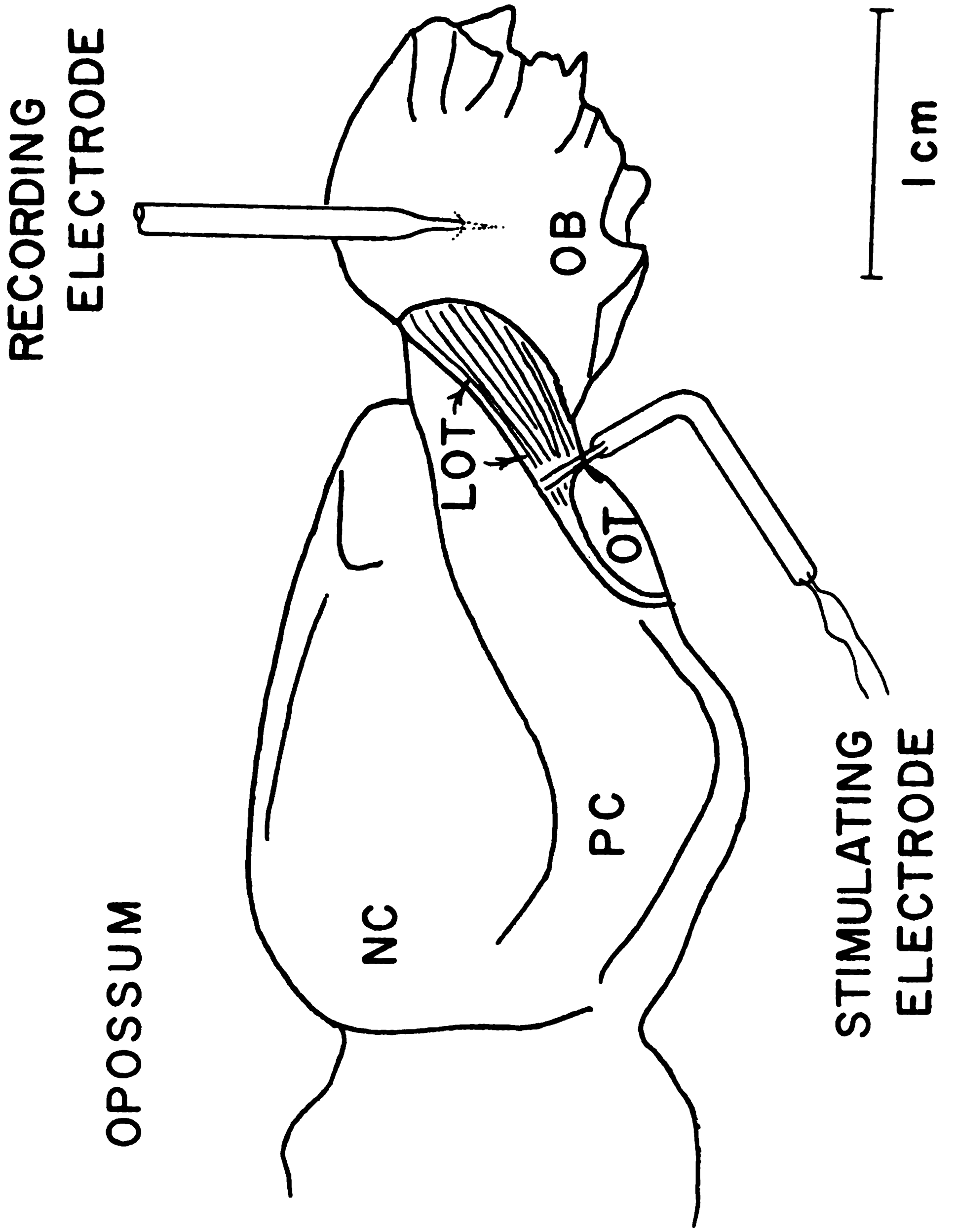

RECORDING ELECTRODE
OB
1 cm
LOT
OT
PC
NC
OPOSSUM
STIMULATING ELECTRODE

FIGURE 1.

FIGURE 2. Fiber Tracts of the Olfactory Bulb. This photomicrograph of a section stained by the Heidenhain-Sanides method for myelin shows the fiber tracts of the olfactory bulb.
LOT: lateral olfactory tract; AAC: anterior limb of the anterior commissure (AC); OB: olfactory bulb.
This horizontal section is from the marsupial, Trichosurus vulpecula or brush tailed possum.

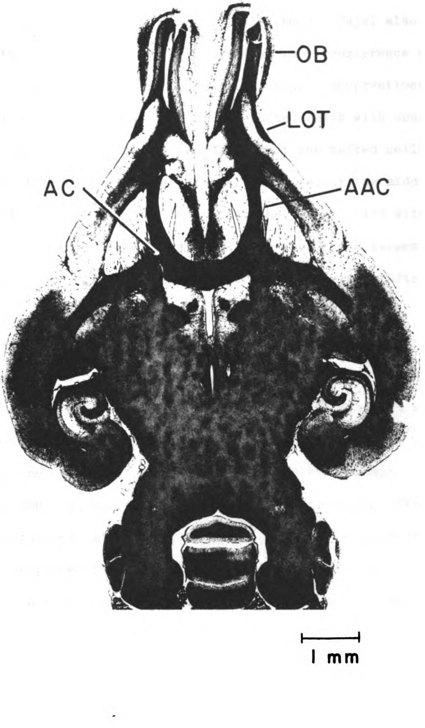


FIGURE 2

the internal tufted cells (see Appendix II, Figure A2); those tufted cells in the external aspect of the external plexiform layer (EPL) are the middle tufted cells; the external tufted cells occupy the zone about the glomeruli--the external granule layer (EGL). Cajal also reported that the tufted cell perikarya diminish in circumference as one progresses outward from the MCL. Coupling Cajal's observations with the findings of Nicoll--ie. that most of the tufted cells with neuraxes in the LOT are nearest the MCL--one could say that the tufted cells with neuraxes in the LOT are of the internal and possibly the middle tufted cell variety. One could also say that the tufted cells with large perikarya project their neuraxes to the LOT. In rabbit the largest tufted cells are found almost exclusively among the internal tufted cell population.

In an anatomical study of some marsupial olfactory bulbs Switzer ('72) found the carnivorous family, the Dasyuridae, to have very accentuated populations of middle tufted cells in terms of number and size. So accentuated is this cell population that a well defined layer of tufted cells in the outer half of the EPL is distinctly evident. Some placentals, eg. mongoose and shrew, also show an accentuated tufted cell population. North America's only marsupial, opossum, is counted with the Dasyuridae in possessing accentuated tufted cell populations in the olfactory bulb. Not only are these tufted cells in greater number than commonly observed in other animals but the larger tufted cell perikarya appear to be equal to the mitral cells in size.

Since Nicoll did his experiments in rabbit, an animal not having a tufted cell population comparable to that seen in opossum, and since there is some criticism (Shepherd '72) of Nicoll's transecting the LOT weeks prior to his recording, it is not possible to conclude which tufted cells project their neuraxes to the LOT. Shepherd suggested in his criticism of Nicoll that the long and varied latencies of presumed tufted cells could be due to the effects which lead to chromatolysis following the transection of the LOT several weeks before the recording experiment. Nicoll also failed to take into account that the probing electrode might favor recording from larger cells which seem to lie predominantly (in rabbit) close to the layer of mitral cells.

At least three possible situations could exist based on present knowledge of the projection of the neuraxes of the tufted cells as to the LOT:

1. Only the tufted cells of the internal type project neuraxes to the LOT.

2. The tufted cells of the internal type and those middle tufted cells with large perikarya project neuraxes to the LOT.

3. Some or all of the tufted cells from the middle and internal types project neuraxes to the LOT.

This study seeks evidence which might strengthen one of the above possibilities. It couples both electrophysiological techniques and quantitative anatomical techniques in order to correlate electrical activity of the neural tissue and its structure.

Overview

The

the spati

anatomica

mitral ce

shrinkage

histologi

driven u

tufted c

Preparat

Opo

greater

large o

1kg; hi

Ra

(15cc/k

Tr

caused

eyes we

6 month

facial

METHODS

Overview

The experimental design was to: (1) quantitatively ascertain the spatial organization of perikarya; (2) correlate a distinct anatomical feature with a distinct electrophysiological feature (the mitral cell layer and the field potential inversion); (3) determine the shrinkage factor of the olfactory bulb tissue having been through the histological process; and (4) correlate the position of antidromically driven units relative to the mitral cell layer with the occurrence of tufted cell perikarya relative to the mitral cell layer.

Preparation

Opossums, Didelphis marsupialis, of ages ranging from 4 months to greater than 1 year were anesthetized with Dialurethane (2cc/kg for large opossums greater than 1kg and 2.75cc/kg for opossums less than 1kg; higher dosages were found necessary for younger animals.

Rats weighing 500-600 grams were anesthetized with 10% urethane (15cc/kg).

Tracheotomies were performed to reduce airway resistance which caused excessive pulsation of the brain. In rats and young opossums eyes were removed along with associated muscle and fat. In opossums 6 months and older, removal of the eye was not necessary since the facial bones had advanced the eye far enough anteriorly so that the olfactor

dorsal a

the olfa

reflecte

Electroni

Micr

were held

fluid wit

tive capa

the high

essence o

A high im

directly

deliveri

electrode

could be

such an

and its

Iontopho

To

by apply

experime

Co., Chi

by Hello

olfactory bulb was not occluded by it. The skull was exposed on the dorsal and lateral surface. Holes were made through the bone over the olfactory bulb and lateral olfactory tract and the dura mater was reflected. The exposed brain was covered with warm mineral oil.

Electronics

Micropipette electrodes (manufactured as described in Appendix I) were held in WPI micropipette holders which make contact between the fluid within the micropipette and a disc of sintered $Ag/AgCl_2$. A negative capacitance amplifier was constructed using a FET transistor for the high input impedance stage (see Appendix III, Figure A3a). The essence of such an amplifier is treated well in the book by Geddes, 1972. A high impedance, magnetic switch (Hamlin, #MRH-15-185) was connected directly to the point of input to the FET transistor as a means of delivering a current to the electrode for iontophoresis of dye filled electrodes. A magnet, mechanically separate from the amplifier chassis, could be swung into position to make the switch contact to "ON". With such an arrangement there is no mechanical disturbance to the electrode and its placement when a dye mark was made.

Iontophoresis

To deliver dye out of the electrode a direct current was passed by applying 10 volts (negative to the electrode). The dye used in these experiments was Pontamine sky blue 6BX (Keystone Aniline and Chemical Co., Chicago, Illinois) applied according to the procedure described by Hellon ('71). Dye was successively driven from the electrode tip by allowing a 10 volt potential to be applied for 20 minutes; negative side to the electrode in the case of Pontamine sky blue which in solution is a (-4) charged ion.

Resistance of Electrodes

Electrode resistance was measured by use of a circuit described by Bureš ('67) or Geddes ('71) (see Appendix III, Figure A3b).

A number of electrodes could routinely be checked rather quickly--generally there was surprising uniformity in the resistances. Electrodes with resistances of 20-30 megohms were found to be most suitable.

Output of the negative capacitance preamplifier was fed directly into a Tektronix FM122 low level preamplifier. The output from the FM122 was divided to go to the oscilloscope and an audio monitor.

Monophasic, positive pulses were applied using a Grass S-8. Stimuli to the LOT were of suprathreshold values of 12v and 0.1msec duration. A Grass isolation unit mediated the output of the stimulator and the stimulating electrodes.

Stimulus electrodes were bipolar tungsten wire (0.13mm) protruding from sealed parallel glass tubes. Tips of the electrode were 0.5-1mm apart. The LOT stimulus electrode was placed visually on the LOT after removal of the dura mater (see Figure 1). The electrodes were aligned perpendicular to the LOT. The end of each tip was in an "L" configuration so as to touch as much of the LOT as possible.

Anatomy: Quantitative

Sections of olfactory bulbs stained with thionin were projected through a Zeiss microscope (equipped with a photo changer, projection tube and deflection prism) onto paper where cell perikarya were traced. Magnification was established by projecting the image of a stage micrometer. The boundary of the mitral cell layer, the inner boundary of the external granule cell layer and the inner boundary of the middle tufted cell layer were drawn (see Appendix II, Figure A2). The cell perikarya were then traced such that the outline followed the perimeter of each perikaryon as revealed by the Nissl substance which had been stained by thionin. Perikarya of cells were drawn in place so that relative position with respect to the mitral cell layer could be established.

Perikaryon area was measured with a compensating polar planimeter. The true cell area cross section (uncorrected for shrinkage due to histological processing) was obtained by dividing the measured area by the square of the linear magnification.

Since the distance between the mitral cell layer and external granule layer varied within a given animal and certainly among animals, the position of cellular perikarya could not be noted merely in micrometers. Instead they were related in terms of percentage units of the distance between the EGL and MCL. From this data the sizes and densities of cellular perikarya in any given position with the EPL can be determined and compared with animals with different magnitudes of olfactory bulb dimensions. Regions of the olfactory bulb were sampled which approximated the zones through which electrode penetrations were made. Consequently, the medial side of the olfactory bulb is represented more than any other region of the olfactory bulb.

Histology: Tissue Bearing Electrode Dye Marks

Following removal of a formalinized brain, the brain was washed for several hours in running tap water. The brain was then penetrated with a 50% solution of Farrant's medium (Humason '62) for 24 hours while on a tilted, rotating platform for stirring. Frozen sections were then taken and mounted on gelatinized slides, drained and firmly blotted. The slides were then put into 50% ethanol before going to a neutral red staining bath (Humason '62). After staining for 10 minutes the sections were differentiated through a dehydrating series of ethanol. The intensity of the stained sections diminished quickly in 95% and 100% ethanol. Consequently, the sections were exposed for less than one minute to each of these concentrations of ethanol. The slides were then put through 3 xylene changes and covered by coverslips with Permount.

Before staining, wet, mounted sections were scanned with a dissecting scope to locate dye marks. The blue of Pontamine sky blue stood out brilliantly--the location of each mark was noted so that subsequent location, after staining, would be facilitated. As there was some loss of dye in the staining procedure, the scanning before staining was necessary. After staining with neutral red the blue marks became somewhat purple.

Shrinkage

Determination of the shrinkage of tissue after it has been prepared for sectioning, staining and mounting must be established in order to correlate the position of the electrode with structural features. Although nervous tissue probably does not shrink uniformly it was assumed that it does. Two known points were established in the living tissue and compared later in a finished section of that tissue to provide a sample estimate.

Tw

1.

formali

were ma

Th

blue.

made th

The loc

"major"

measure

Th

celloid

lying t

section

thionin

Ag

between

factor

cedure

2.

bulb, r

tion of

the fie

positio

obtaine

region

distance

Two different approaches were employed:

1. An olfactory bulb from a brain which had been perfused with formalin was cut approximately through the zone from which recordings were made.

The piece of olfactory bulb was surface stained with methylene blue. Once the MCL was visible all the way around, measurements were made through a dissecting scope fitted with a movable micrometer eyepiece. The locus of the MCL in coronal sections is crudely like an ellipse. The "major" and "minor" axes of the elliptical locus made by the MCL were measured.

The end of the bulb was put through the sequence necessary for celloidin embedding. Once embedding was complete the celloidin overlying the cut face of the bulb was sectioned away and a few thick sections (ca. 80um) were taken from the bulb. They were stained in thionin and mounted.

Again the "major" and "minor" axes were measured. The ratio between these and the first measurements yielded a linear shrinkage factor with respect to formalin fixed tissue for this particular procedure in this tissue.

2. During the recording of electrical activity in the olfactory bulb, note had frequently been made within any given electrode penetration of the position of the MCL as indicated by the phase inversion of the field potential. The difference between the depths of these two positions yielded an inter-MCL distance. Inter-MCL distances were also obtained from measurements made in sections from approximately the same region where electrode penetrations were made. The average inter-MCL distance in living tissue, divided by the average inter-MCL distance in

secti

as a

right

compo

of la

Varia

nesse

depth

with

The f

readi

just

spina

conti

this

sections, yielded a linear shrinkage factor. This ratio also served as a measure of any error in Part 1 due to swelling or shrinkage which might occur subsequent to formalin fixation.

To obtain data for variable shrinkage within lamina of different composition (mostly perikarya or mostly fibers) the relative thicknesses of layers could be compared before and after histological processing. Variable shrinkage would be revealed if the proportions of layer thicknesses were different.

It is not satisfactory to have dye marks made at a particular depth during recording and then compare that depth reading in a section with the distance of the mark from the penetrated surface of the bulb. The flaw with this approach is in accurately knowing that the "zero" reading of the electrode was really at the surface of the bulb and not just in electrical contact above the surface by way of the cerebrospinal fluid. The cerebrospinal fluid was not at a constant level but continually was welling up beneath the covering of mineral oil. Also this exposed surface was subject to damage.

RESULTS

Anatomical

Compilation of the data of size and position of the perikarya of tufted cells and mitral cells are shown in Figures 3 to 5. One rather striking feature about the frequency of different size classes of perikarya shown in Figure 3 is the sameness of the frequency profile in rat and rabbit while that of opossum is quite different.

Cajal ('11, '55) noted that the size of perikarya of tufted cells increased as the MCL was approached. This is quantitatively portrayed in Figure 4. While the profile of median perikaryon size for each percentage zone of the EPL is very nearly the same for rat and rabbit the profile found for opossum reveals a population of tufted cells with much larger perikarya; the largest one very nearly the same as those of mitral cells (indicated in the 0-10% zone). In fact, the median size of mitral cell perikarya is almost twice that found for rat and rabbit.

Previously (Shepherd '63, Pinching '72, Nicoll '70) the perikarya of tufted cells of the EPL have been described as "scattered throughout the EPL", a rather subjective description. Figure 5 illustrates the subjectiveness of such a description. Quite noticeably in all three types of animals the relative densities of perikarya are highest in the approximate outer half of the EPL. Valverde ('65) noted this and actually called this zone the tufted cell layer. Note that in opossum this zone of higher density extends further inward toward the MCL than

FIGURE 3. Histograms of Size of Perikarya of Tufted Cells. This figure shows histograms of size of perikarya of tufted cells occupying different radial zones in rabbit, rat and opossum. These data reveal that rabbit and rat have nearly identical profiles of frequencies of perikaryon size. The histogram of cell sizes of perikarya in opossum reveal a dramatically different profile than that found for rat or rabbit. The perikarya of highest frequency in opossum are more than twice as large as the perikarya with highest frequency in rat and rabbit.
N: number of cells sampled.
In this figure and in any subsequent figures containing a histogram the number appearing within the profile of the histogram is the number of elements (N) comprising the histogram.

HISTOGRAMS OF SIZE OF PERIKARYA OF TUFTED CELLS IN THE EPL

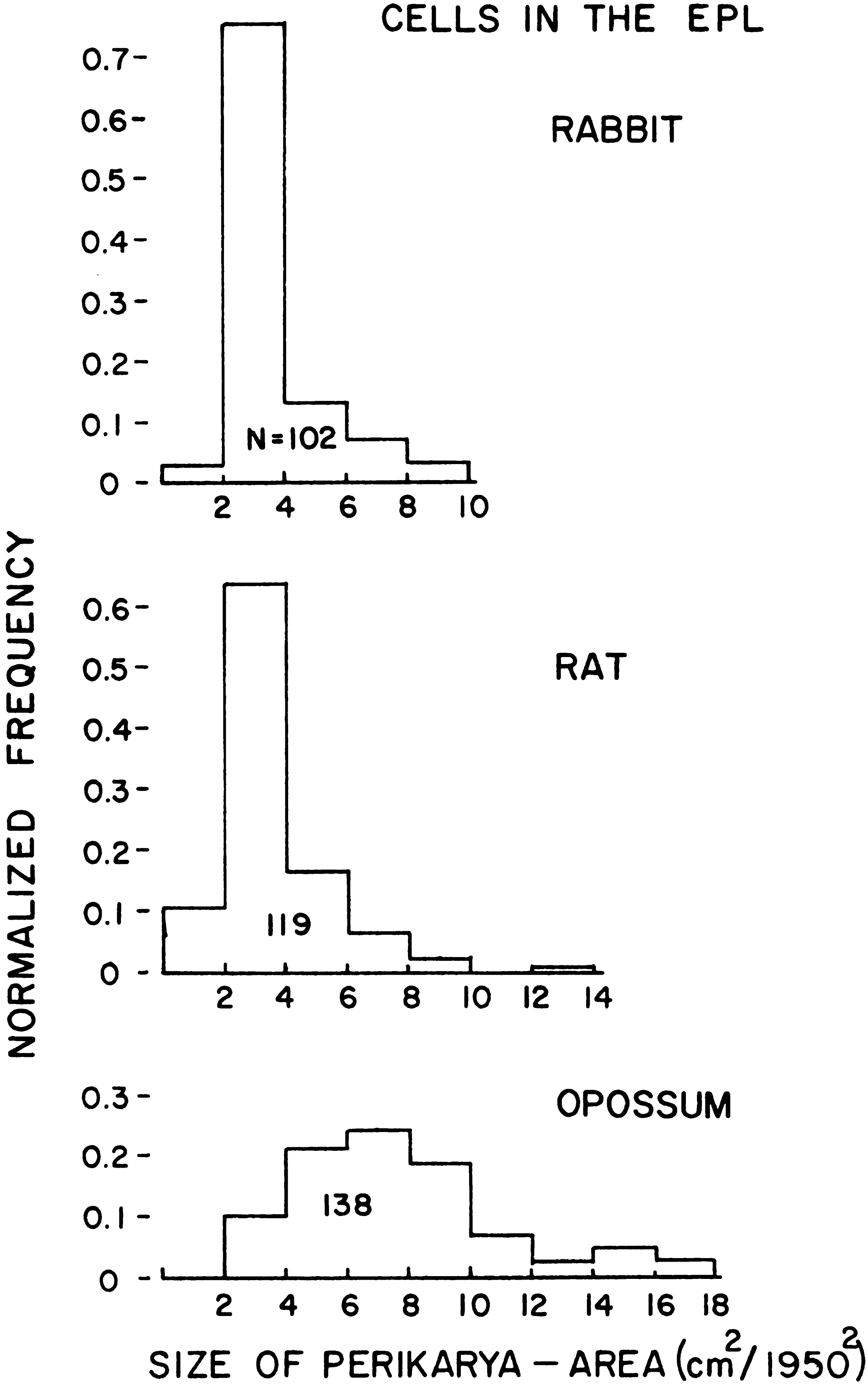


FIGURE 3

FIGURE 4. Median Size of Perikarya of Tufted and Mitral Cells. The median size of perikarya of tufted and mitral cells in radial zones of the external plexiform layer are shown in this figure. The distance between the inner edge of the external granule layer (EGL) and the mitral cell layer (MCL) was divided into percentage zones. This was done for purposes of comparing olfactory bulbs of different animals which have greatly different olfactory bulb dimensions. As can be seen the zones with the largest median size of perikarya in the opossum are roughly twice that found in either rat or rabbit. Mitral cell perikarya are considered to be large perikarya in the 0-10% zone although tufted cell perikarya which may occur there are indistinguishable from those of mitral cells. In these spectra the 20-40 or 50% zones were left vacant for lack of enough cells to determine a meaningful median. These histograms quantify the subjective observation that the perikarya of tufted cells increase in size with proximity to the mitral cell layer. Note that the scale of the ordinate for opossum is twice that for rabbit and rat.

MEDIAN SIZE OF TUFTED & MITRAL CELL PERIKARYA

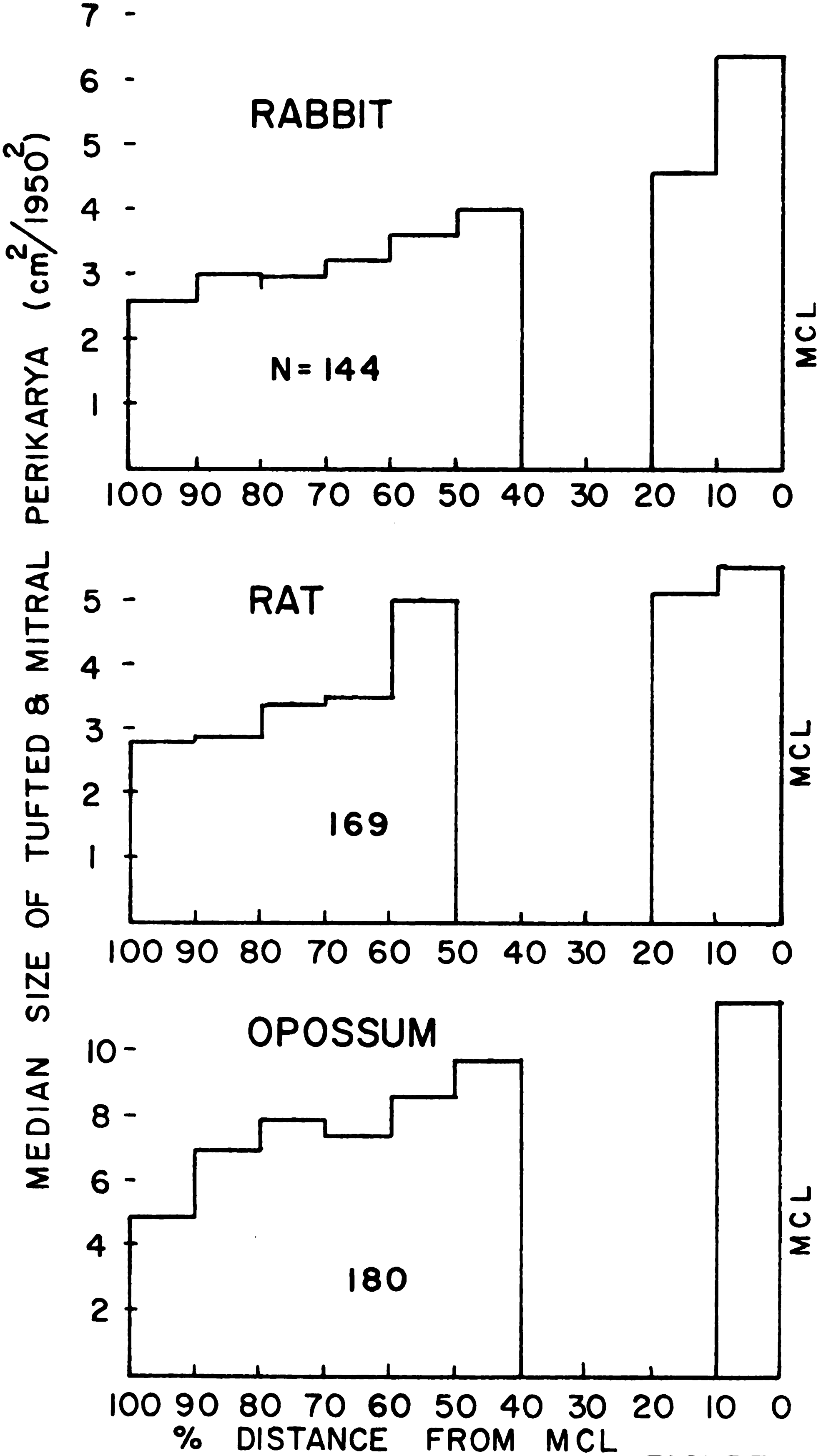


FIGURE 4.

FIGURE 5. Histograms of Densities of Perikarya. This figure presents the spectra of relative densities of perikarya within the external plexiform layer (EPL) in the olfactory bulbs of rabbit, rat and opossum. As is clearly evident, there is not a uniform distribution of the perikarya of tufted cells within the EPL for any of these three types of animals. The rather sharp decrease in density which occurs at the 60% mark in rabbit, 50-60% in rat, and 40% in opossum, marks the inner boundary of the zone of the middle tufted cells.
TCB: middle tufted cell boundary; N: number of cells sampled.

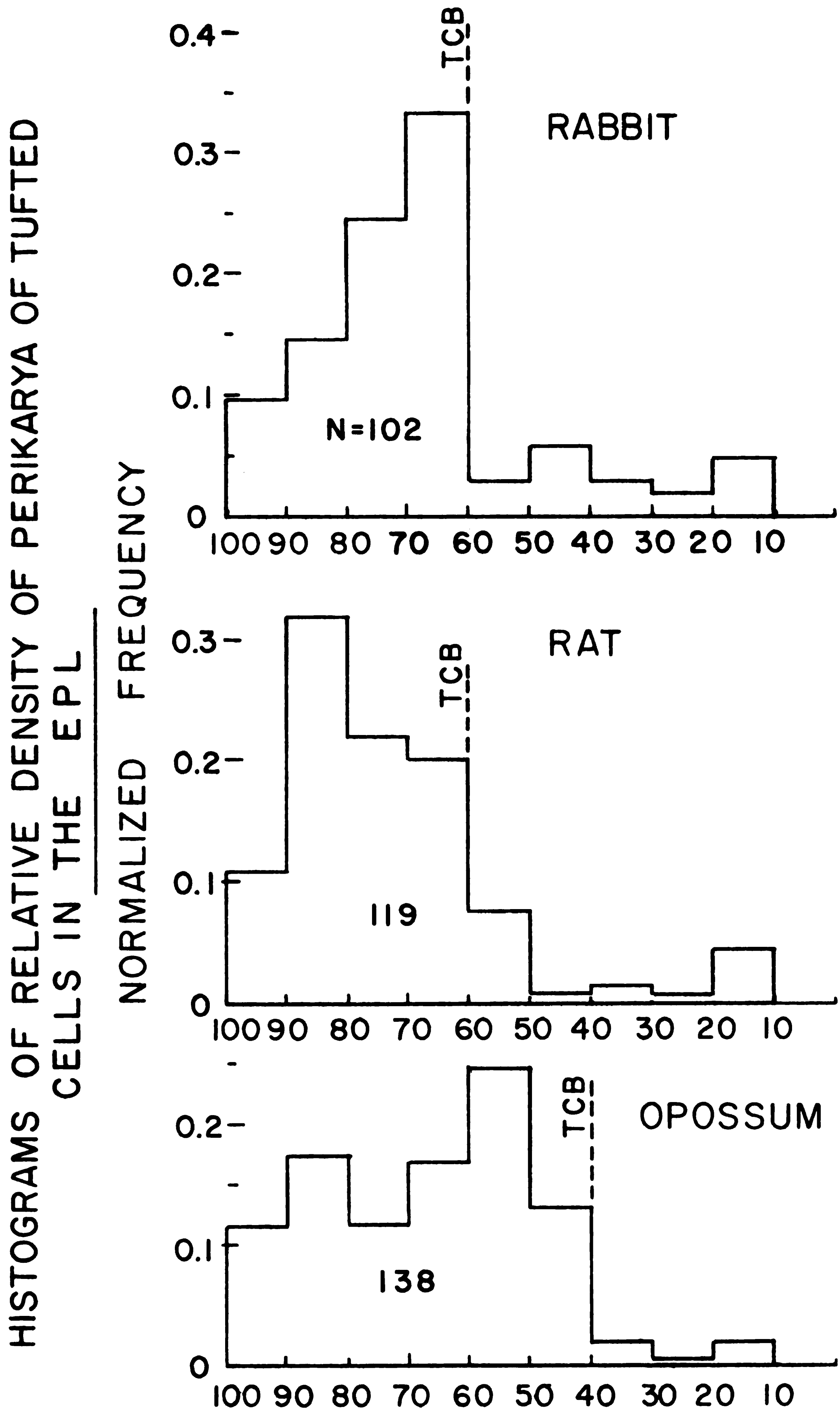


FIGURE 5. % of DISTANCE FROM MCL to EPL

it does in either rat or rabbit. These density spectra do not reflect the relative density of perikarya of mitral cells with respect to the tufted cells.

Electrophysiological

Advancing a micropipette electrode through the lamina of the olfactory bulb while stimulating the LOT one observes field potentials with varying conformations depending on the depth of the electrode. These field potentials have been thoroughly examined by Rall and Shepherd ('68). Rall and Shepherd, as others before them, Philips et al ('63) and Ochi ('63), described the correlation of each particular wave form of the field potential with the lamina of the olfactory bulb. Field potentials are the summed activity of a multitude of units activated synchronously by stimulation to the LOT. Even when particular neural elements are not being recorded, the currents associated with the synchronous activation of neural elements permeate the whole of the tissue. For this study the most important aspect of the field potential was the phase inversion which occurs when the electrode tip was at the MCL. Figure 6Aa shows the appearance of the field potential just before the MCL while the tip of the electrode is still in the EPL; Figure 6Ac shows the field potential just after the MCL in the internal plexiform layer (IPL). This is referred to as the field potential phase inversion.

That the field potential inversion occurs precisely at the row of perikarya of mitral cells which mark the MCL is shown in Figure 6A.

A micropipette electrode filled with Pontamine sky blue 6XB (see Methods) was used to place the dye marks indicated in Figures 7A and 7B,

FIGURE 6A. Field Potentials and Layers of the Olfactory Bulb. The field potentials which arise in the different lamina of the olfactory bulb of opossum following antidromic stimulation of the lateral olfactory tract are shown here. Illustrated here is that part of the field potential [arrow in (a)] which undergoes sign or phase inversion when the electrode tip traverses the mitral cell layer (MCL). Note that the gradient of change is very steep--the inversion takes place over a distance of only 50um. The field potential shown in (d) illustrates the great magnitude of the positive phase when the electrode tip is deep in the internal granule cell layer (IGL).

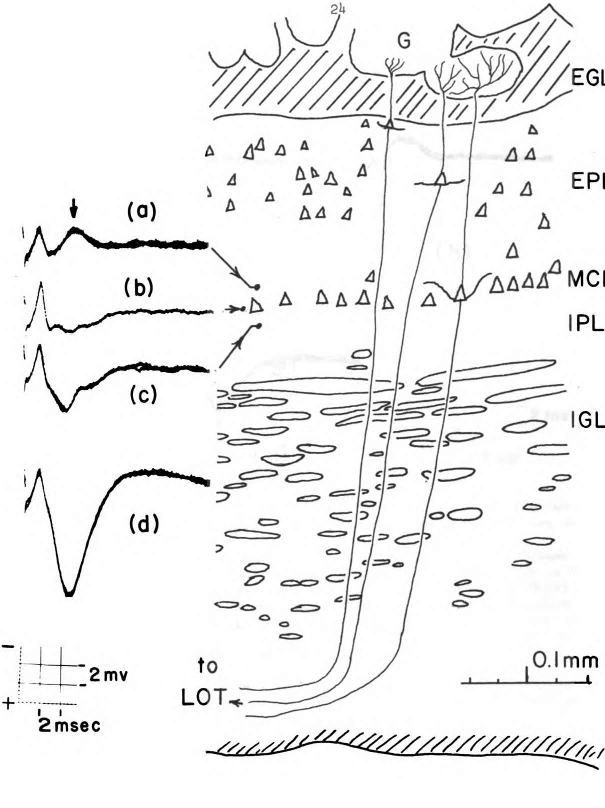


FIGURE 6A

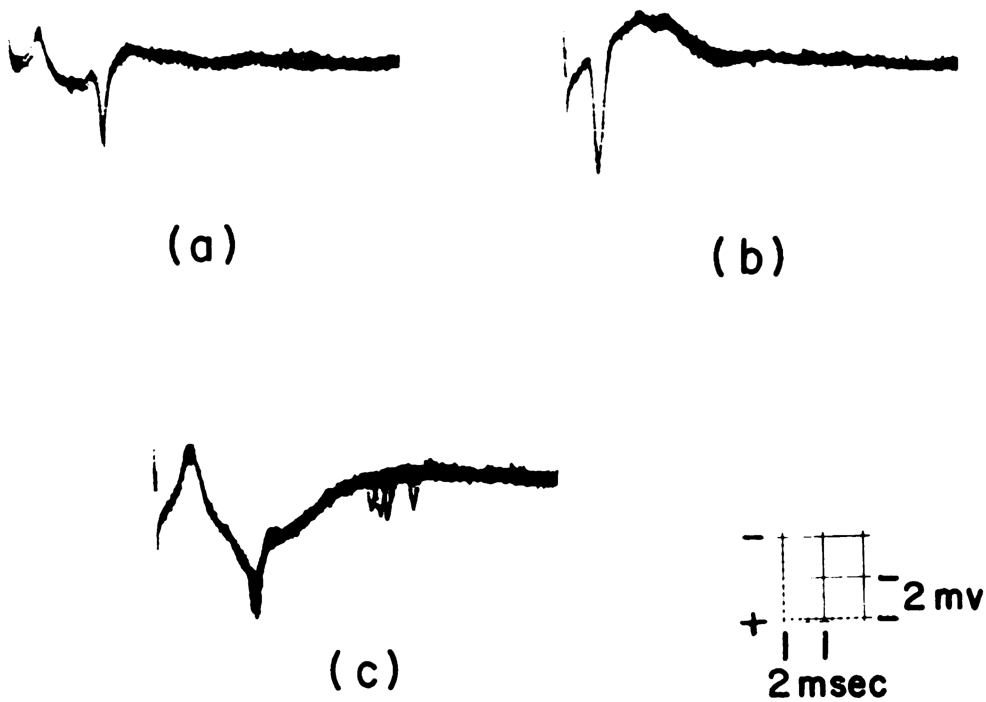


FIGURE 6B. Units and Layers of the Olfactory Bulb. Units activated antidromically by stimulation of the lateral olfactory tract are shown here:

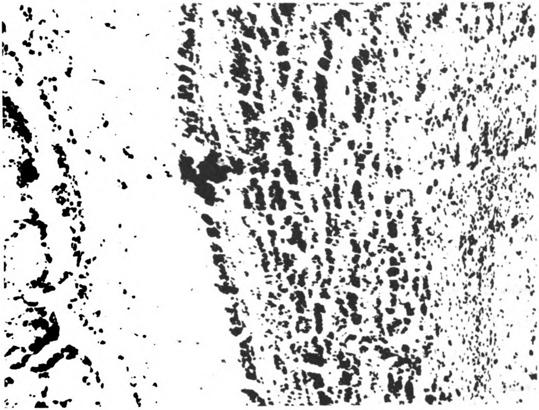
(a) A unit obtained in the external plexiform layer, presumably a tufted cell, this unit was directly driven by antidromic stimulation. This was ascertained by the maintenance of the unit while stimulating a frequency up to 100Hz. A unit which failed to follow the stimulus up to 100Hz was considered to be synaptically activated. That this unit is in the EPL can be seen by the field potential upon which the unit response is superimposed (see Figure 6A).

(b) This unit is from a cell in the mitral cell layer responding to antidromic stimulation of the LOT. The flatness of the field potential reveals that this is the response of a cell in the mitral cell layer.

(c) The multiple units in this response are typical of unit activity found in the internal granule cell layer (IGL). That the electrode was well within the IGL to record this response is indicated by the wave form of the field potential. These units were synaptically activated by antdromic stimulation of the LOT since the units were able to follow only to 20-40Hz before they would not "fire" with every stimulus.

FIGURE 7. Dye Marks in the Mitral Cell Layer. The photomicrographs shown in this figure are of sections from the olfactory bulbs of rat and opossum. Particular note is to be made of the dark marks coincident with the mitral cell layer (MCL) in each photomicrograph. These marks were made by dye (Pontamine sky blue) iontophoretically deposited by a micropipette electrode. The dye was deposited at the depth which is the locus of the phase inversion, that is, the field potential becomes inverted when crossing this locus. Clearly the mitral cell layer is the locus of the phase inversion of the field potential. Two of two marks placed in a rat were recovered and 6 of 7 marks placed at the inflection of the inversion of the field potential in a opossum were recovered.

(a) RAT



(b) OPOSSUM

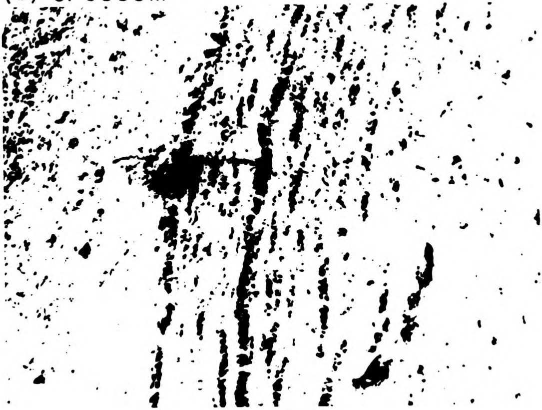


FIGURE 7.

rat and opossum respectively. (Two of two were recovered in rat and six of seven were recovered in opossum). With this distinct correlation between the phase inversion of the field potentials and the location of the mitral cell layer the field potential phase inversion is established as a well defined reference point.

The position of all units encountered can then be related to the position of the mitral cell layer within any given penetration of the electrode.

Position of Antidromically Activated Units

Units are antidromically activated by stimulation of the LOT when the antidromic impulse invades the perikaryon directly and not synaptically. Units were defined as antidromically activated only if the recorded unit impulse was of constant latency from the time of stimulation and the units followed stimulus frequencies of up to 100Hz. Units which were synaptically activated and even had constant latencies would fail to follow stimulus frequencies of 10-40Hz. The positions, with respect to the MCL, of the collection of units which met these criteria are shown in Figure 8(a) for both opossum and rat. The data for rat is intended only as an indicative comparison with what Nicoll ('70) found for rabbits in a similar experiment.

Correlation of Unit Positions and Cytoarchitecture

Since the anatomical measurements were made from sections of olfactory bulb which had been histologically processed the dimensions between structural elements is different than that which exists in living tissue. The shrinkage factor for celloidin embedded material

FIGURE 8. Units and Latencies. Part (a) of this figure shows the histograms of antidromically driven units in rat and opossum as a function of position within the external plexiform layer (EPL). Only those units which followed stimulus rates up to 100Hz were compiled here.

Part (b) shows the histograms of latencies for units recorded in the EPL of rat, rabbit (from Nicoll '70) and opossum regardless of their following rates. Note that in the data from Nicoll the large latencies encountered as compared to those from rat or opossum. Possibly this is due to effects following transection of the neuraxes of the lateral olfactory tract which Nicoll performed on the rabbits used in his recordings.

UNITS WHICH FOLLOWED STIMULI FREQUENCIES UP TO 100 Hz *vs* POSITION IN THE EPL

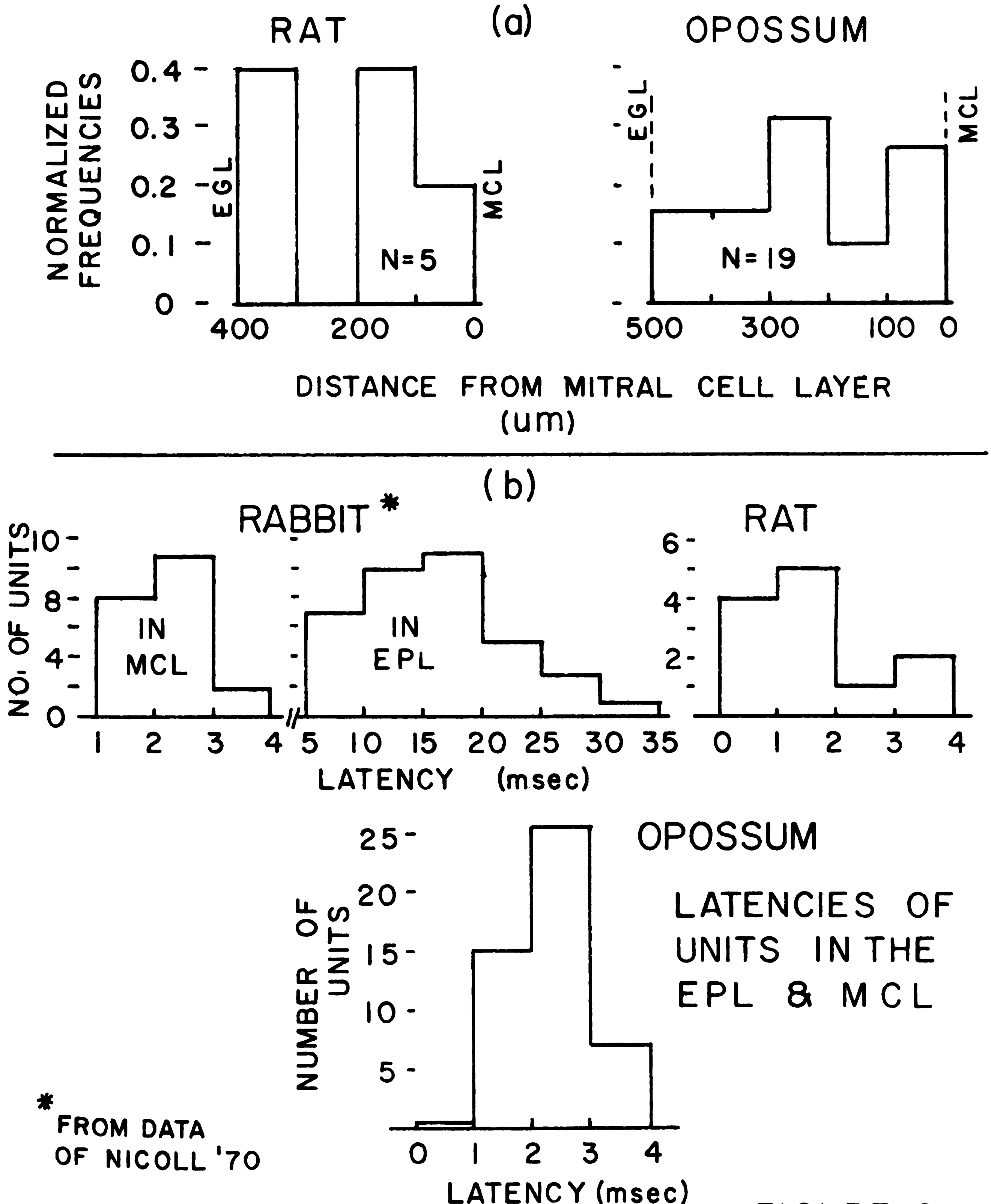


FIGURE 8.

was found to be 1.36; that is, the anatomical measurements made from sections must be multiplied by 1.36 to approximate measured dimensions found for _in vivo_ dimensions. The dimensions _in vivo_ are the same as in formalin fixed tissue. Any change in dimensions due to formalin was found to be negligible compared to the changes due to dehydration by alcohol and embedding in celloidin.

Figure 9 shows the correlated spectra of density of perikarya, recorded units, and density of perikarya of specific size classes. The region of the olfactory bulb most often recorded from was used to determine an average dimension of the EPL. For opossum it was nearly 500um and for rat and rabbit nearly 400um between the inner boundary of the EGL and the MCL. The spectra in row A of Figure 9 show the same data as found in Figures 5 and 6 except that the percentage zones have been combined to span 20% units. In this way in opossum 20%=100um while in rabbit 25%=100um.

Figures 10 and 11 illustrate the density spectra of different size classes of perikarya of tufted cells. Note that in opossum the perikarya of the size class 6-20 ($cm^2/1950^2$) have a frequency distribution almost identical to the frequency distribution of recorded units. The unit histogram for rat seemed to follow the profile of the histogram of density of all sizes of perikarya while the histograms of units as reported by Nicoll ('70) for rabbit (Figure 9B) does not bear any resemblance to the density of perikarya histogram.

FIGURE 9. Correlation of Anatomy with Electrophysiology. This figure relates the peaks and troughs of particular perikarya density histograms with the corresponding zone within the external plexiform layer of the olfactory bulbs of rat, rabbit and opossum. The rabbit data have been extracted from Nicoll ('70). The first row (A) of histograms shows the relative density of cells within the EPL. The camera lucida drawings above Row A are meant to schematically show the correspondence between olfactory bulb layers and the histogram; thus the reader should not expect to extract measurements from the camera lucida drawings. Note the greater breadth of high density of perikarya of the middle tufted cells in the EPL and in all three animals the very low relative density of cells in the 100-200um adjacent to the mitral cell layer.

Row B shows the histograms of the relative occurrence of recorded units within the EPL. In these histograms only those which followed a stimulus to the lateral olfactory tract of up to 100Hz are shown. Note that some units were recorded in zones of the EPL which are relatively sparse of perikarya. It is also noteworthy that the maximum occurrence of units in opossum corresponds to the maximum density of perikarys in the EPL. Units are also frequently found near the MCL but between 100 and 200um units are scarce as are cells. The data for rat, far from enough to be conclusive, indicate that antidromically driven units seem to be relatively frequent near the outer zone of the EPL. This is contrary to Nicoll's data from rabbits.

The histograms of relative density of perikarya in Row C show, in comparison with the histograms of Row A, those perikarya near the mitral cell layer which are out of that layer by 4% or more of the distance from the MCL to the external granule cell layer. For opossum the histogram shows the frequency of perikarya of the size class greater than 154 $(um)^2$ in area. Note the similarity of this profile and that for the recorded units in opossum. For rat and rabbit no size class of the population of tufted cell perikarya could be found which matched the unit histogram profile.

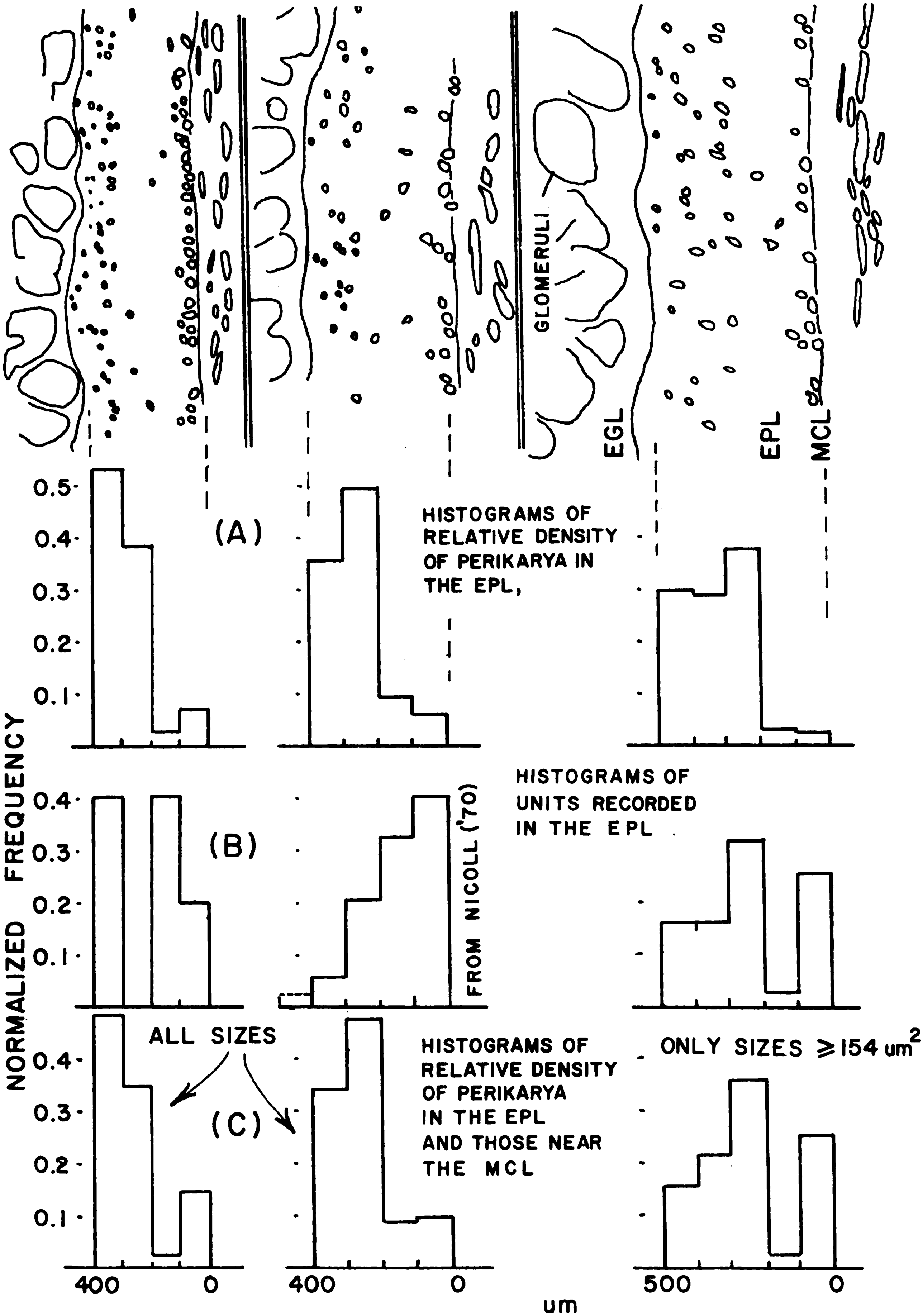

GLOMERULI
EGL
EPL
MCL
NORMALIZED FREQUENCY
0.5
0.4
0.3
0.2
0.1
(A)
HISTOGRAMS OF RELATIVE DENSITY OF PERIKARYA IN THE EPL,
(B)
FROM NICOLL ('70)
HISTOGRAMS OF UNITS RECORDED IN THE EPL
ALL SIZES
(C)
HISTOGRAMS OF RELATIVE DENSITY OF PERIKARYA IN THE EPL AND THOSE NEAR THE MCL
ONLY SIZES ≥154 um²
400
0
400
0
500
0
um

FIGURE 9.

FIGURE 10. Histograms of Different Size Classes of Perikarya in Opossum. This figure illustrates how the cell perikarya of various size classes are disposed within the external plexiform layer (EPL) in the olfactory bulb of opossum. Perikaryon size is presented in the units by which their traced outlines were measured (cm^2). Actual size, uncorrected for shrinkage is obtained by dividing by the square of the magnification, ie. $(1950)^2$. As can be seen the largest cells ($12\text{-}20cm^2$) are most frequent near the inner boundary of the middle tufted cells (TCB) in the EPL, 40-60%. Proceeding outward toward the glomeruli the perikarya of smaller size become more frequent and the large, less.

HISTOGRAMS OF DENSITIES OF TUFTED CELL PERIKARYA

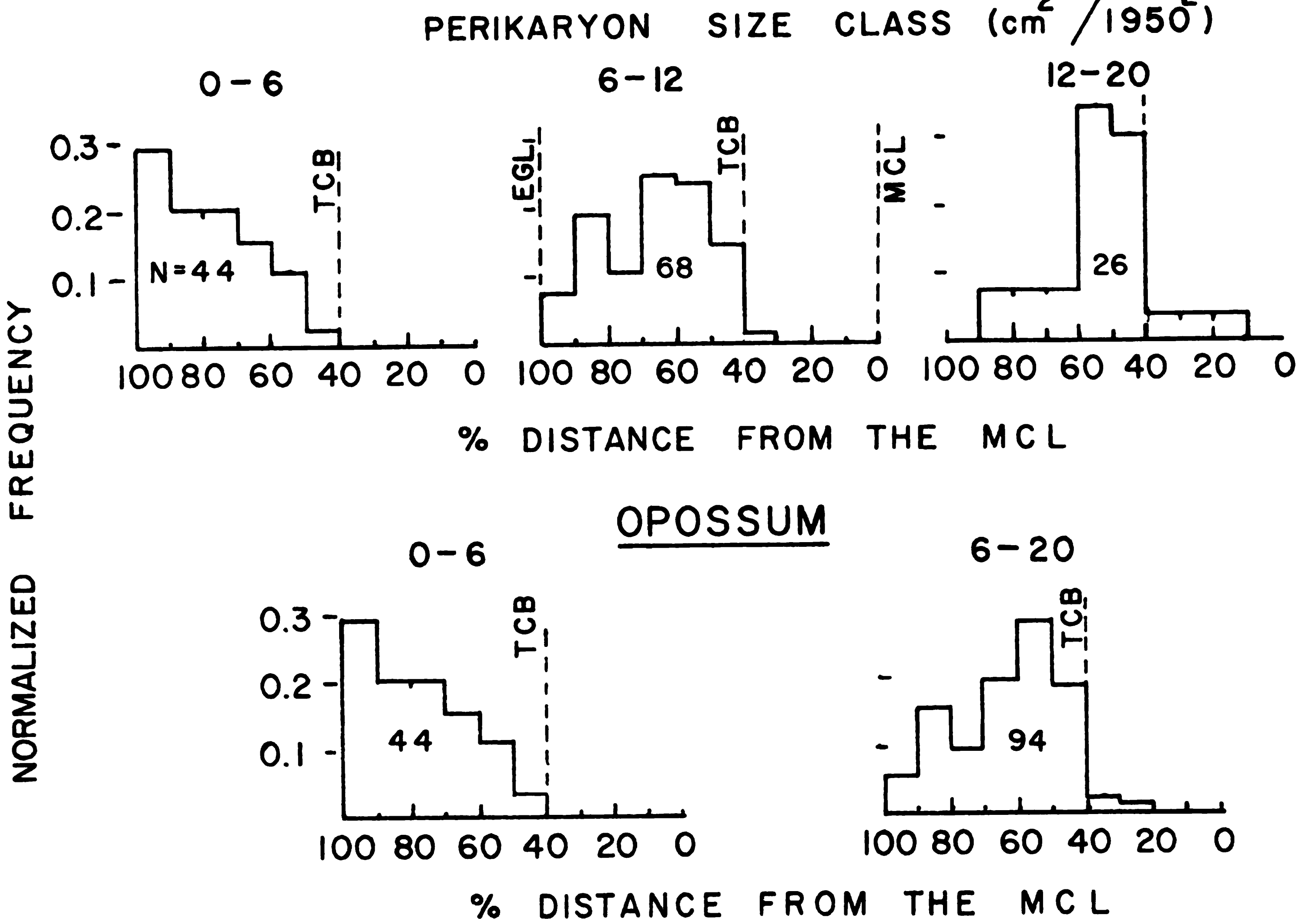


FIGURE 10.

FIGURE 11. Histograms of Different Size Classes of Perikarya in Rat and Rabbit. The disposition of size classes of perikarya of tufted cells found in rat and rabbit olfactory bulbs is shown here. Like opossum the cells with large perikarya are found most frequently at the inner boundary of the middle tufted cell zone in the external plexiform layer. But unlike opossum, the distribution profile of small perikarya coincides with that of the large in rabbit and rat.
TCB: tufted cell boundary.

HISTOGRAMS OF DENSITIES OF TUFTED CELL PERIKARYA

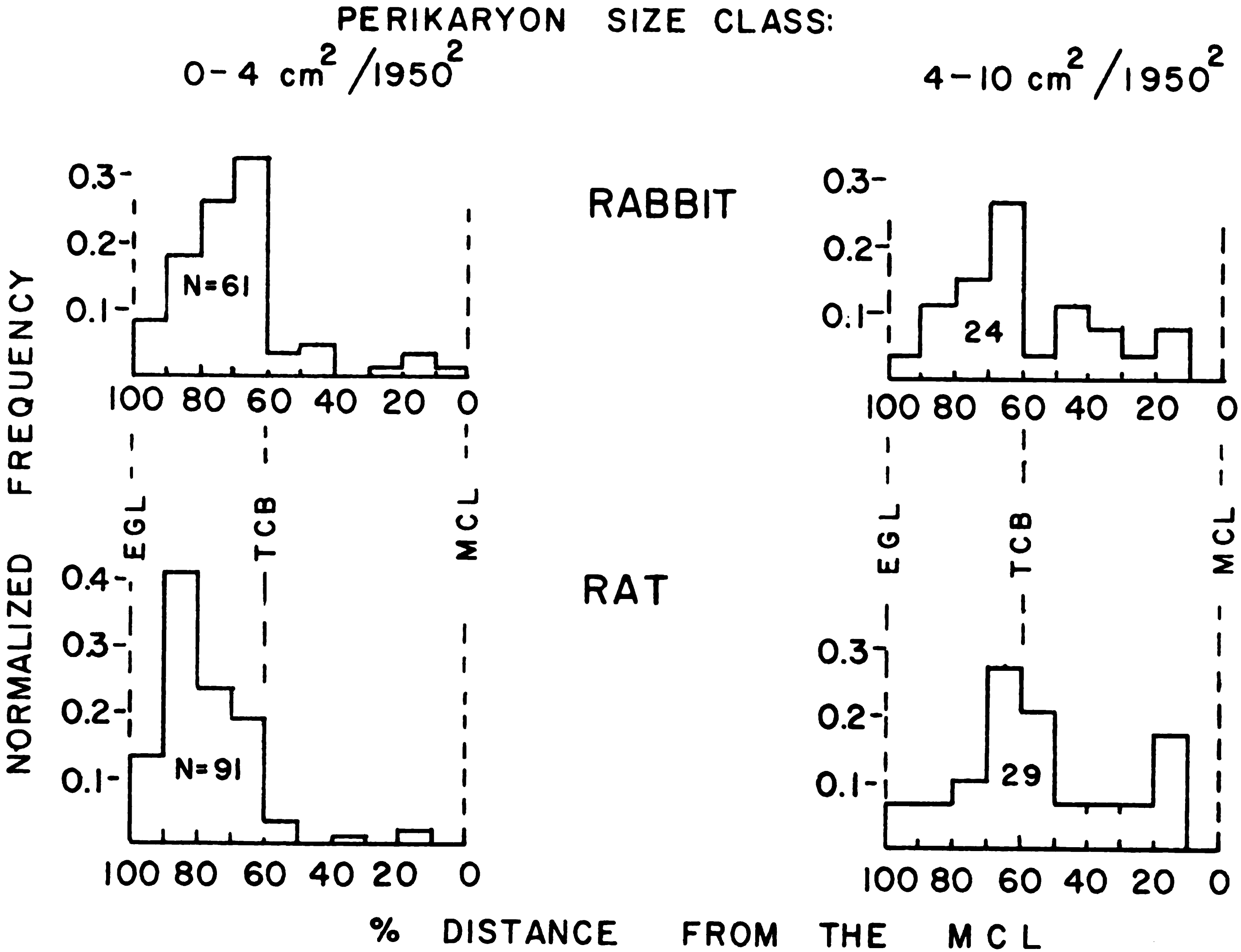


FIGURE 11.

DISCUSSION

Antidromically stimulated units in the EPL of the opossum olfactory bulb have a frequency-spatial distribution complying closely with the frequency-spatial distribution of the perikarya of tufted cells of the large type (Figure 9).

Another way of viewing the anatomical and electrophysiological data is that each is a sampling of the population of tufted cells (and mitral cells) in a different way. The anatomical sampling is complete; all perikarya existing within the areas observed were measured for size and position. In the electrophysiological sample a micropipette electrode probed the lamina of the olfactory bulb and sampled those perikarya which it encountered. Each perikaryon encountered was classified according to whether it was directly invaded by antidromic impulses or synaptically invaded. The question now becomes: is the sample obtained by the electrode the same or only part of the anatomical sample?

This can be tested by the Kolomogorov-Smirnov test (a non-parametric test; Siegel '56) to determine whether the two samples were drawn from the same population. There are two anatomical samples which are candidates to compare with the sample drawn by the recording electrode: all of the tufted cells within the EPL or those tufted cells with large perikarya($>6cm^2/\ 1950)^2$ or $154u^2$. At the $p=.05$ level neither sample is rejected although the sample of perikarya without the small size classes indicated a better fit.

From this evidence it can be concluded that at most a constant proportion of the tufted cells send their neuraxes through the LOT. Possibly the larger perikarya send neuraxes through the LOT in higher proportions of their total number than smaller perikarya but the evidence gathered in this study cannot resolve this.

The sampling bias of electrodes has often been raised (Rushton '49, Wiesel '60) but a study by Biedenbach and Stevens ('68) produces evidence that the micropipette electrode is most selective toward the density or packing of perikarya rather than the size of perikarya. If electrodes are selective of size they are selective of those perikarya whose diameters are smaller than the electrode tip diameter. Micropipette electrodes such as those used in this study should be equally selective of sizes of perikarya down to 1 um or less.

The next item which must be treated is why the data from the study of Nicoll ('70) reflect a quite different picture of units vs. anatomy. The histogram of units which Nicoll reports (Figure 9b) could not contain only units which followed stimulus frequencies to 110Hz (8 out of 37). For the same units compiling the histogram of depth Nicoll reports the latencies of these units, reproduced here in Figure 8b. To follow a stimulus of 110Hz a unit cannot have a latency greater than 9.1msec since the interval between stimuli is less than the latency.

The argument posed by Nicoll that the long latencies are due to smaller conduction velocities of the smaller neuraxes of the "small" tufted cells does not seem very compelling when viewed quantitatively. For myelinated neuraxes conduction velocity is directly proportional to the diameter of the neuraxis. The ratio of the two latencies, those of mitral cells and those of units found in the EPL predicts, approximately, the ratio of diameters of the neuraxes in question. For ease of comparison the latencies of 2msec for mitral cells and 15msec (ca. the mean found by Nicoll) for the units in the EPL will be compared. The ratio, 2/15 = 0.13, that is, the neuraxis of the elements recorded from in the EPL are 0.13X the diameter of the neuraxes of mitral cells.

While this may be the case, Nicoll draws further support from the generalization that neuraxes are closely related to the diameter of their perikarya. This appears to be generally so (Gouras '69) but Stone and Hollander ('71) and Stone and Freeman ('71) reveal that this is only qualitatively so; there is a wide range of conduction velocities, (ie. diameters) of neuraxes that perikarya of the same size may exhibit depending on what system is being investigated. Even though the precise diameter cannot be predicted from the conduction velocity the contention of Nicoll that long latencies imply small neuraxes and hence small perikarya can be checked for order of magnitude correctness.

Recalling the ratio of latencies found above (0.13) and referring to the size of perikarya shown for rabbit in Figure 4, 6.3cm^2 X (1950)^2 for mitral cell perikarya, the cross sectional area for cells in the EPL with a latency of 15msec is:

$$0.13 \text{ X } 6.7\text{cm}^2 = 0.87\text{cm}^2$$

No perikaryon (deemed a tufted cell) in the EPL of the rabbit material used in this study was of this areal dimension when traced. The smallest median size was 2.6cm^2/1950^2; no perikarya less than 1.8cm^2 was recorded.

Possibly the existence of these long latencies can be attributed to the effects of transection of the LOT by Nicoll weeks before he performed the recording experiment. Shepherd ('72) points out this possibility, that the long latencies may be due to the effects giving rise to chromatolysis of damaged neurons.

Given that the results found in this present study can be correlated with results found in rabbit by Nicoll, his results pose a very interesting possibility: that transecting a nerve which contains neuraxes of different origin and/or character, (not normally reflected by variability of latency under electrophysiological probing) leads to effects which cause the various neuraxes within the nerve to have different conduction velocities, hence variable latencies. This remains an open possibility since a study done by Pilar and Landmesser ('72) reports no significant change in conduction velocities of the neuraxes of the chick ciliary ganglion following transection (neuraxotomy) with survival times of 10 hours to 10 days.

CONCLUSION

The results of this study support the evidence found anatomically by Lohman and Mentink ('69) that tufted cells project neuraxes through the LOT. The results here are in contrast with those found by Nicoll ('70) but do support the basic electrophysiological evidence of neuraxes of tufted cells in the LOT. This study extends the information of tufted cell neuraxes in the LOT in that the perikarya of tufted cells projecting neuraxes through the LOT represent a constant proportion (possibly all) of the middle tufted cell population. There was some indication that the tufted cells with larger perikarya may be more strongly represented in the LOT. Contrary to previous experience of anatomical evidence for the projection of neuraxes of tufted cells being disproven by electrophysiological data, the data in this study strengthen and expand the contention of neuraxes of tufted cells within the LOT.

The LOT, then, has two efferent fiber systems from the main olfactory bulb formation projecting to the olfactory cortex. Does one infer from this a dual function? a system for redundancy? an olfactory input to be used not for purposes of perception of olfactory stimuli? or is this dual projection part of a two stage processing of olfactory input? These and probably more questions may be posed but at this juncture it may be best to examine in what ways one might gain some insight into the possible role or purpose of the tufted cells. Certain limitations must be kept in mind: the tufted cells are viewed in relation to the mitral cells whose explicit role is not known.

Spatial and Temporal Relationships

A thought-provoking review by Pinching ('72) treats spatial and temporal aspects of the cell organization of the olfactory bulb in rat. The overall picture is this:

--The arborization of the primary dendrites of tufted cells are confined to a restricted portion of a given glomerulus; the smaller is the perikaryon, the smaller the volume of the glomerulus occupied by the arborizations (illustrated in Figure 6A).

The internal tufted cells whose perikarya approach the size of those of mitral cells have arborizations which occupy more completely the volume of the glomerulus.

--The perikarya of external tufted cells situated periglomerularly lack accessory dendrites and apparently have neuraxes confined to the olfactory bulb.

Pinching suggests that these features may be interpreted in terms of thresholds of activation of any given neuron. Under this premise those neurons with small dendritic fields will be activated more easily than neurons with large dendritic fields. If so, then a picture of neuron activity emerges of the most external (hence small perikarya) neurons being activated first while the more deeply lying neurons (large internal tufted cells and mitral cells) are activated later. Such a view may also carry the implication that the neurons activated earliest have the least "refined" signal while those activated later have had their signals modulated and refined considerably (by internal granule cells, collaterals of tufted and mitral cell neuraxes).

This picture of neuron activity means that the neuraxes of the LOT are carrying signals of all stages of refinement. That is, the olfactory cortex is receiving the "raw" data and the "conclusion" of data analysis. Such a spectrum of refinement of signal is reasonable if one expects a refined signal for perception of olfactory stimuli and less processed signals which function as olfactory modulation of other (and unidentified) brain functions.

Projections

Using lesion-degeneration techniques Lohman and Mentink ('69) showed evidence of the projection of neuraxes of tufted cells through the lateral olfactory tract in rabbit. By superficial lesion to the olfactory bulb the perikarya in the mitral cell layer were spared and presumably only the perikarya in the external plexiform layer were affected. Degeneration of terminals of neuraxes from such lesions was sparse in the superficial half of the plexiform layer of the olfactory cortex. Degenerating fibers also due to superficial lesions were traced by Lohman and Mentink to the lateral part of the olfactory tubercle (see Figure 1).

Along with the projection of neuraxes of presumed mitral cells to the anterior olfactory nucleus there may also be neuraxes from tufted cells. It certainly is important to determine if neuraxes of tufted cells may have collaterals terminating in regions devoid of terminals due to mitral cell neuraxes.

Comparative View

As has been indicated in this study not all mammalian olfactory bulbs are alike with respect to tufted cell population. Several marsupials and some placentals have accentuated populations of tufted

cells. If such accentuation reflects a specialization of the tufted cells then examination of these animals may clearly reveal what are the projections of the tufted cells (at least in that animal).

The monotremes offer an intriguing "handle" on the importance of spatial relationships of perikarya in the olfactory bulb: the usual compact row of perikarya of mitral cells is absent in the spiny anteater or echidna (Tachyglossus aculeatus) and the duck-billed platypus (Ornithorhynchus anatinus). The only large perikarya which are present lie in presumably the EPL and by normal standards are tufted cells. Every other landmark feature is present. Are those large perikarya tufted cells or mitral cells, or both?

Such disposition of large perikarya in olfactory bulbs is usually observed in reptiles such as snakes. But other reptiles like turtle exhibit the large perikarya of the olfactory bulb in a broad row.

The large perikarya of the accessory olfactory bulb formation in mammals are disposed as tufted cells in the main olfactory bulb formation. In chinchilla, however, these perikarya are exhibited in a tight row. Position of perikarya and placement in a row appear to have some functional importance.

Phylogeny

Nieuwenhuys ('67) and Andres ('70) report that across the taxa of animals the tufted cells are absent until amphibians. It is also in amphibians that the presumed mitral cell perikarya gain secondary or accessory dendrites. Attainment of accessory dendrites allows extensive synaptic interaction with the internal granule cells. Such an additional or specialized function apparently reaches a culmination in some marsupials and placentals insofar that they exhibit many large perikarya of tufted cells which presumably have extensive accessory dendrites.

One very important aspect of the tufted cells seems to be the location of their perikarya. They are in common contact with signals from:

--the olfactory receptors via the olfactory nerve

--the externally directed processes of the internal granule cells

--bulbo-petal fibers contra- and ipsilateral from other areas of the brain.

The tufted cells may represent a signal processing system with quite different characteristics from that of the mitral cells.

SUMMARY

1. The median size of perikarya of middle and internal tufted cells of rat, rabbit and opossum were quantitatively shown to have a gradient of size (described by Cajal) with respect to radial position in the EPL--the larger cells nearer the MCL.

2. The relative density of perikarya of tufted cells in the EPL was shown to be highest in the outer half of the EPL forming a distinct tufted cell layer.

3. In opossum it was shown that the population of perikarya of middle tufted cells could be partitioned according to size classes. The large perikarya were exclusively located at the inner fringe of the tufted cell layer of 2 above. Such a partitioning was not evident in the sample of perikarya drawn from rat and rabbit.

4. Recordings with micropipette electrodes of units antidromically activated by stimulation of the LOT revealed that units were distributed in the outer zone of the EPL and at or near the MCL. These units were only those which followed frequency stimuli up to 100Hz.

5. The latency of activated units were predominantly about 2 msec.

6. Dye marks (Pontamine sky blue) iontophoretically placed where the second peak of the field potential became flat--ie. the inflection point of the sign inversion of the field potential--coincided with the line of large perikarya of the mitral cell layer.

7. The shrinkage factor which was determined to correct the altered dimensions of sections due to histological processing for celloidin was found to be 1.36.

8. The histogram of density of perikarya in the EPL was found to be the same as the histogram of units in 4 above under the Kolomogorov-Smirnov test at the p=0.05 level.

9. It was concluded that a constant proportion, if not all, of the middle and internal tufted cells project neuraxes through the LOT in opossum.

APPENDICES

APPENDIX I

Micropipette Electrodes

The preparation of micropipette electrodes followed these steps:

1) Boil glass tubes in doubly distilled water 15 minutes.

2) Rinse in isopropyl alcohol.

3) Reflux in isopropyl alcohol 15 minutes.

4) Dry in oven (50°C).

5) Insert ca. 24 glass fibers.

6) Boil in doubly distilled water 15 minutes.

7) Rinse in isopropyl alcohol (12 times).

8) Reflux in isopropyl alcohol 30 minutes.

9) Dry in oven (50°C).

The following covers these steps in detail.

For purposes of this experiment glass tubing was obtained from Corning Glass, special laboratory products #7740 (1mm OD; 0.5mmID) and cut to 7cm lengths.

Cleaning of the tubing is probably the most crucial operation in the preparation of glass micropipette electrodes. The cut glass tubes are then boiled in doubly distilled water (15-20 minutes).

A basket to hold the glass tubes for the cleaning procedure was made by taking a plastic syringe and cutting off the ends such that a cylinder remained. A portion of the reinforced top section of a nylon stocking was then stretched over one end of the syringe cylinder and held permanently in place by applying a tacking iron to the sides of the cylinder thus joining the nylon with the plastic of the cylinder. Thread was attached through holes at the top of the cylinder to provide a means of suspension of the cylinder during the cleaning process.

Following the boiling step the basket with tubes was rinsed briefly in isopropyl alcohol (Chen, V., Personal communication).

The next step was to place the basket in a distillation column which was arranged vertically. A round bottom flask half full of isopropyl alcohol and a heater below it completed the set up. At the top of the distillation column a stopper with a glass rod through it supplied a point of support for thread of the basket. Water was flowed in the cooling jacket of the condensor. The heater was regulated with a Variac Potentiometer and was adjusted so that during the initial stage of this step the region within the column where the isopropyl alcohol was condensing would be nearly at the top of the tubes. After three to five minutes of this the heat was reduced so that the point of condensation is at the bottom of the basket. In the set up used here the I.D. of the distillation column was only slightly larger (2-3mm) than the diameter of the basket. (NOTE: Use of glass boiling beads in the flask will prevent a "bump" or blow out of the contents when heat is first applied). The total time of this refluxing in isopropyl alcohol is somewhat arbitrary but was normally at least 20-25 minutes.

Following refluxing, the basket with glass tubes was removed and drained by repeated blotting of the bottom of the basket on several thicknesses of paper towel. The basket and tubes were then placed in a covered beaker and put into an oven (50°C) to dry.

When dry the glass tubes were ready to receive the glass fibers. (The purpose of the glass fibers within the tube is to facilitate the filling of the electrode tips. With the fibers the necessity for mild boiling under reduced pressure and filling with dye or electrolyte through several steps and days is eliminated). The fibers (10-12um in diameter) are resin coated so that static electricity does not render them unhandleable.About 24 fibers were easily placed into each tube by first dragging the bundle of 24 across the surface of a dish containing 2.8M KCL. The solution dried quickly rendering the bundle of fibers stiff and the fibers adherent to each other.

A length of fibers was inserted into the tubes prior to pulling such that the fibers extended 2-3mm from either end of the tube. Moving the fibers within each tube so that 4-5mm extended from one end this extended length of fibers became ca. 180^{o} as the tube was placed back into the basket. The result was that the tube with fibers had a "J" configuration with the curve of the "J" resting on the nylon mesh at the bottom of the basket. This manipulation prevented the glass fibers from falling through the nylon mesh during subsequent cleaning steps.

Although the tubes may pass through additional cleaning steps it is advisable to handle the tubes only by the ends when possible with forceps fitted with Tygon tubing of an appropriate diameter and length.

The steps of boiling and refluxing were repeated, the glass tubes with fibers were then ready for pulling into micropipette electrodes. The cleaned glass was kept in a tightly closed container. Proper filling of the electrodes was highly dependent on cleanliness.

Pulling the Micropipette Electrodes

A horizontal micropipette puller manufactured by Industrial Science Associates (63-15 Forest Avenue, Ridgewood, N.Y. 11227) was used to make the electrodes. In addition to adjusting the resistor governing the strength of the "weak pull" the puller was operated through a Variac. The Variac permitted finer control of the pull strength. A coil furnace was employed instead of the platinum foil primarily for the greater physical stability of the heating element. Nichrome wire of 0.6mm in diameter was wound around a 15 gauge hypodermic needle and fitted to the furnace holder.

Adjustments of the temperature of the heating element governed the amount of taper, length, and overall shape of the electrode. Regardless of the overall length of taper the final 5-10um tapered once more down to about 1-2um from a preceeding diameter of about 3-5um. The point at the end of the final 5-10um length was smaller than the light microscope can resolve and therefore was on the order of less than 0.5um. With the point of the electrode intact normal recording was carried out. If marking was done by ejecting dye out of the electrode tip iontophoretically the point part of the electrode was broken off. See Figure A1.

Breaking off the tip of the electrode but still leaving a 1-2um tip was done by taking the electrode in hand, holding it perpendicular to the corner of a protruding "Kimwipe" paper tissue and whisking the tip of the electrode across the surface of the "Kimwipe". Contact with the Kimwipe was only enough so that the resulting motion of the Kimwipe was barely perceptable. This delightfully simple procedure is very consistent and effective.

Filling Electrodes

After the micropipette electrodes have been "pulled" they were filled with the electrolyte desired. KCl solutions were made at a molarity of 2.8M rather than 3.0M since the latter is very close to saturation and slight changes in environmental conditions may cause minute precipitate which could foul the electrode tip, (ie. make its impedance very high), (F. Kutyna, personal communication). Whatever the electrolyte it was filtered through a millipore filter of no greater than 0.45um pore size.

Actual filling was done with a syringe (5cc) and a 28 gauge hypodermic. This is the gauge needle found suitable for tubing of 0.5mm I.D.; 28 gauge is actually 0.45mm O.D. Enough room is left between the needle and the inside of the micropipette tube so that fluid will flow but adequate suction is possible. Smaller hypodermic needles did not seem to perform as well.

The hypodermic needle is inserted in the barrel of the formed electrode (glass fibers have been pulled out--these break off near the first shoulder) all the way to the shoulder. It was found that first, injection of electrolyte followed by brief suction,followed again by injection expelled any bubbles near the shoulder. Bubbles of air further toward the electrode tip, not beyond the tip of the hypodermic, could be "worked" out by successive injection-suction-injection sequences. If a piece of plastic tubing was placed at the base of the needle and filled with electrolyte before placement of the needle into the electrode, a reservoir is available for the suction phases. Once convinced that the bubbles have been expelled, gentle injection pressure was applied while slowly slipping the electrode off the needle. The electrode was then placed in an appropriate holder and kept in a closed container saturated with water vapor.

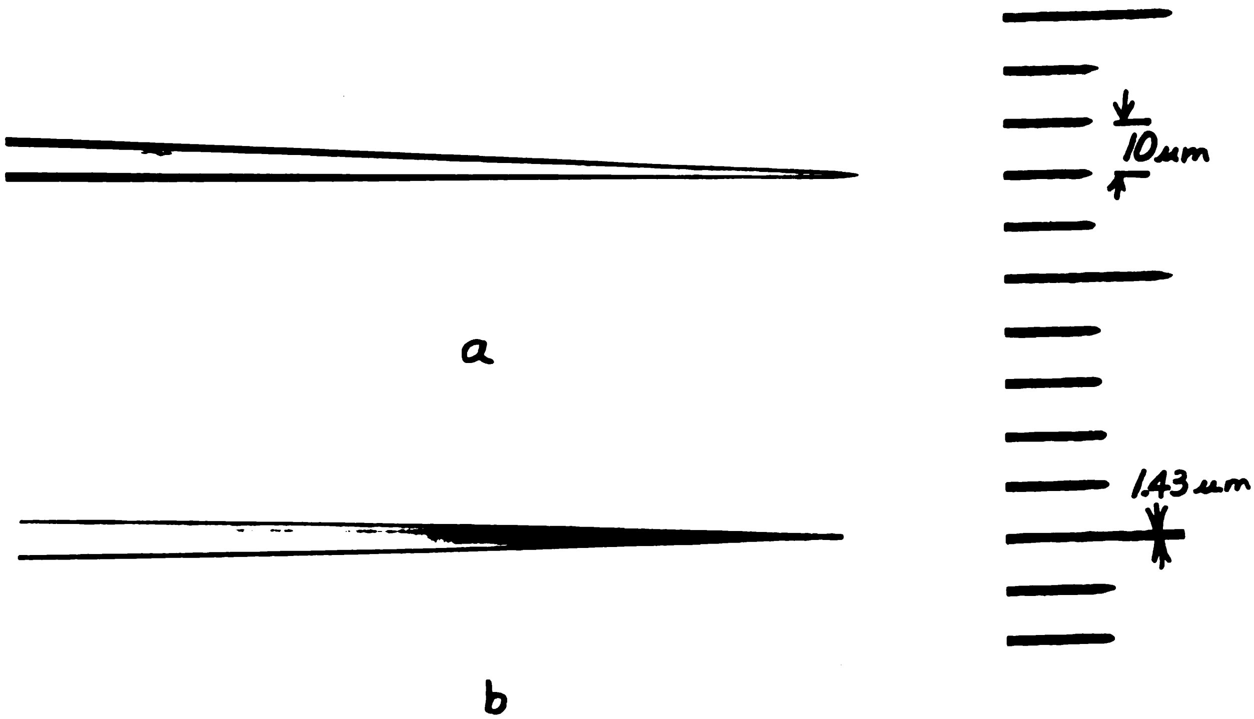


FIGURE A1. Electrode Tips. The tips of micropipette electrodes are shown in these photomicrographs. The electrode in (a) comes to a point of less than 1 um across. The electrode in (b) has had its tip broken off by whisking it tangentially across a corner of a Kimwipe paper tissue. The darker interior of (b) is due to the dye within the electrode; upon breaking the tip the density of dye coloration rapidly increases at the very tip; the boundary of dense color migrates gradually away from the tip. Eventually (ca. 30 minutes) the deep coloration advances the entire length of the taper. The scale to the right is in divisions of 10um. Each line is about 1.43um wide.

APPENDIX II

FIGURE A2. Layers of the Olfactory Bulb. The layers of perikarya are visualized in this photomicrograph of a section stained for Nissl substance with thionin. The olfactory bulb layers are:

GL: Layer of glomeruli: Incoming olfactory nerves synapse with primary dendrites of mitral and tufted cells.

EGL: External granule cell layer: A zone periglomerular in extent containing short axon cells and tufted cells lacking accessory dendrites.

TCL: Tufted cell layer: That outerzone of the classically defined external plexiform layer which contains the middle tufted cells.

EPL: External plexiform layer: A cell scarce zone except for internal tufted cells--the region in which the accessory dendrites form reciprocal synapses with the ascending processes of the internal granule cells.

MCL: Mitral cell layer: Defined by the narrow band of cells with large perikarya--the mitral cells. Often, granule cells form a basement layer for the mitral cells.

IPL: Internal plexiform layer: A cell scarce zone containing centripetal fibers of extrabulbar origin, collaterals of tufted cells coarsing horizontally and radially directed neuraxes of mitral and tufted cells.

DL: Discoid layer: Internal granule cells packed into groups with a discoid configuration.

PV: Periventricular layer: Consists of incoming and outgoing fibers and the ependymal cells of the olfactory bulb ventricle.

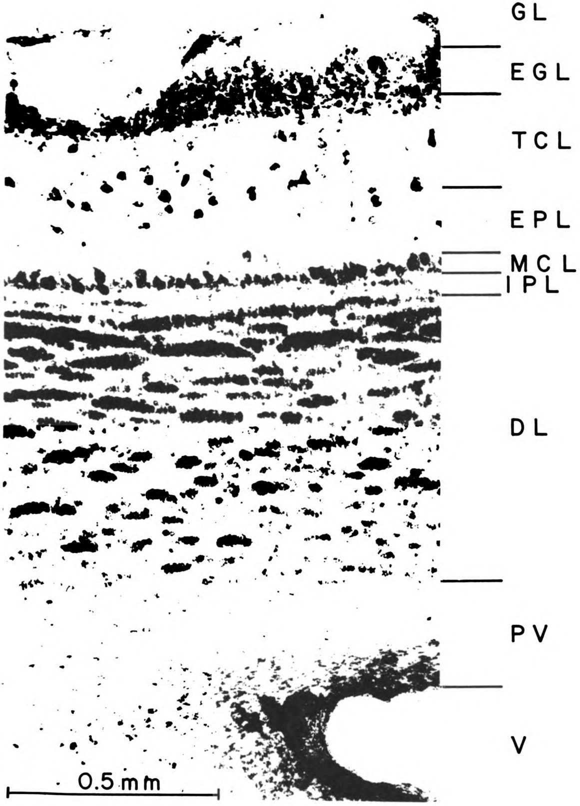
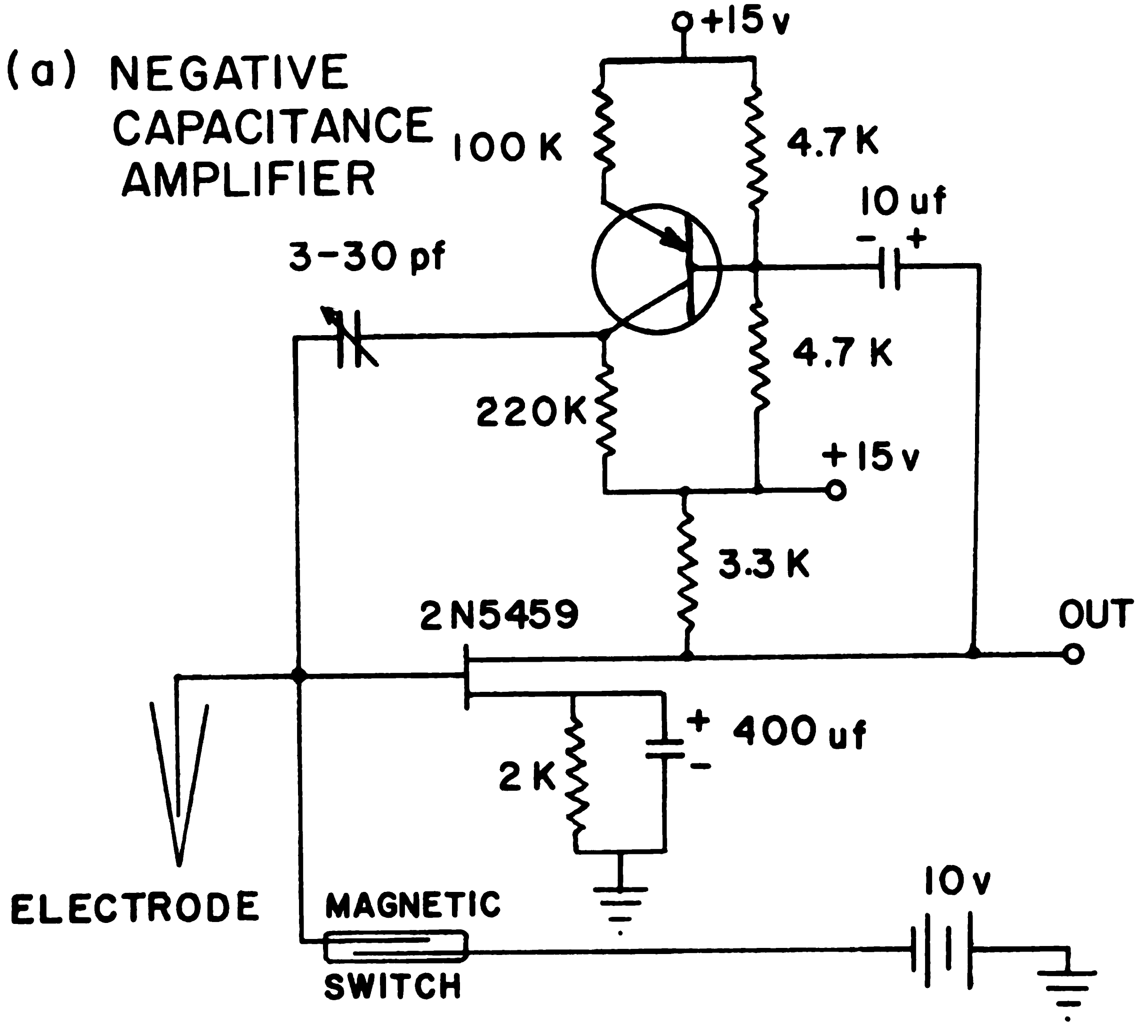


FIGURE A-2

APPENDIX III

FIGURE A3. Schematics of Electronic Circuits. Part (a) illustrates the circuit used in this study to construct a negative capacitance amplifier. A high impedance input was achieved by the use of a field effect transistor (FET) (2N5459). The output is fedback through an inverting circuit employing a transistor and through a variable capacitance to the input. Part (b) shows a circuit for measuring electrode resistance as suggested by the book by Bureš et al ('67). The square wave input was from the calibrator output of a Tektronix oscilloscope.


(a) NEGATIVE CAPACITANCE AMPLIFIER
+15 v
100 K
4.7 K
10 uf
3-30 pf
4.7 K
220K
+15 v
3.3 K
2N5459
OUT
400 uf
2 K
ELECTRODE
MAGNETIC SWITCH
10 v

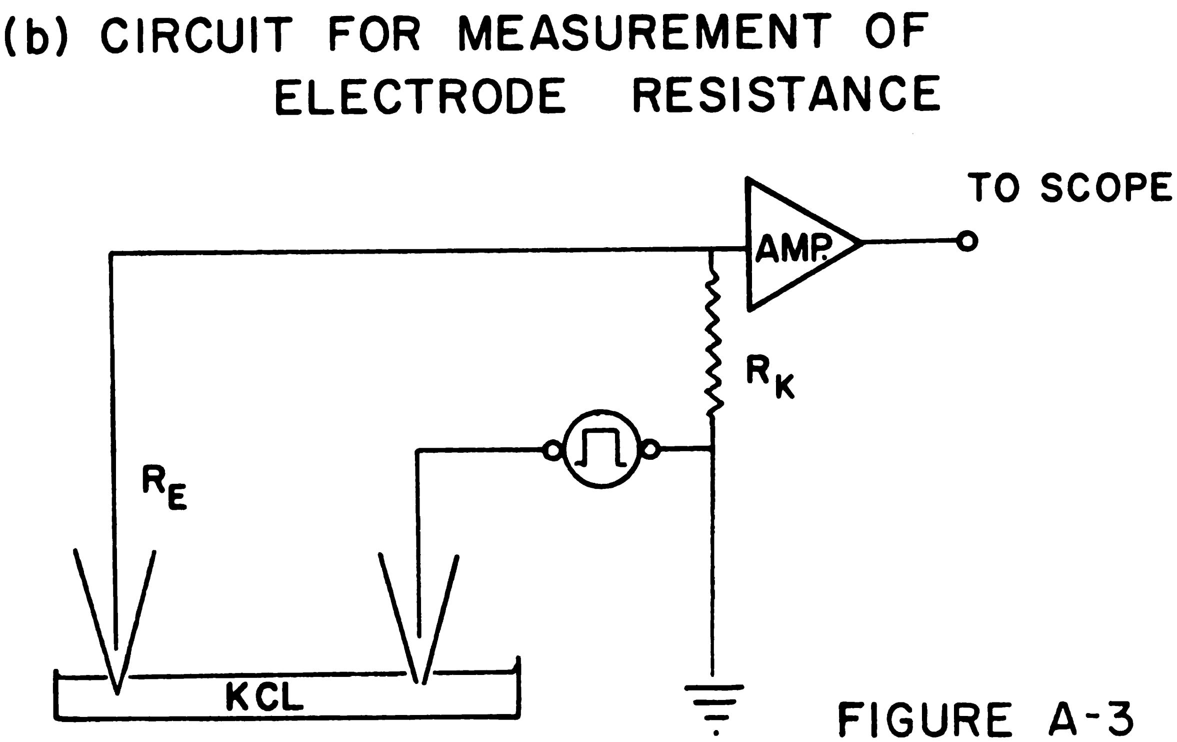

(b) CIRCUIT FOR MEASUREMENT OF ELECTRODE RESISTANCE
TO SCOPE
AMP.
R_K
R_E
KCL

FIGURE A-3

BIBLIOGRAPHY

BIBLIOGRAPHY

Andres, K. H. 1970 Anatomy and ultrastructure of the olfactory bulb in fish, amphibia, reptiles, birds and mammals. In Wolstenholme and Knight: Taste and Smell in Vertebrates. A Ciba Foundation Symposium. J.&A. Churchill, London. pp. 177-196

Biedenbach, M. A. and C. F. Stevens 1969 Electrical activity in cat olfactory cortex produced by synchronous orthodromic volleys. J. Neurophysiol. 32: 193-203.

Bureš, J., M. Petráň, and J. Zachar 1967 Electrophysiological Methods in Biological Research, translated by P. Hahn, Academic Press, N. Y.

Cajal, S. R. 1911 Histologie du Système. Nerveux de l'Homme et des Vertébrès. Vol. 2, Paris: Maloine, 647-672

Cajal, S. R. 1955 Studies on the cerebral cortex (Limbic structures) trans. by Kraft, L.M.; Lloyd-Luke, London.

Crosby, E. C. and T. Humphrey 1939 Studies of the vertebrate telencephalon. I. The nuclear configuration of the olfactory and accessory olfactory formations and of the nucleus olfactorius anterior of certain reptiles, birds and mammals. JCN 71: 121-213.

Geddes, L. A. 1972 Electrodes and the Measurement of Bioelectric Events. J. Wiley and Sons, N. Y.

Gouras, P. 1969 Antidromic responses of orthodromically identified ganglion cells in monkey retina. J. Physiol. 204: 407-419.

Hellon, R. F. 1971 The marking of electrode tip positions in nervous tissue. J. Physiol. 214: 12P

Humason, G. L. 1962 Animal Tissue Techniques, W. H. Freeman and Co., San Francisco, xv and 468 pp.

Lohman, A. H. M. and G. M. Mentink 1969 The lateral olfactory tract, the anterior commissure and the cells of the olfactory bulb. Brain Research 12: 396-413.

Nicoll, R. A. 1970 Identification of tufted cells in the olfactory bulb. Nature 227: 623-625.

Nieuwenhuys, R. 1967 Comparative Anatomy of Olfactory Centres and Tracts, In; Progress in Brain Research, Vol. 23, ed. Y. Zotterman, Elsevier Pub. Co., pp. 61-64.

Ochi, J. 1963 Olfactory bulb response to antidromic olfactory tract stimulation in the rabbit. Jap. J. Physiol. 13: 111-128.

Phillips, C. G., T. P. S. Powell and G. Shepherd 1963 Responses of mitral cells to stimulation of the lateral olfactory tract. J. Physiol. 168: 65-68.

Pilar, G. and L. Landmesser 1972 Axotomy mimicked by localized colchicine application. Science 177: 1116-1118.

Pinching, A. J. 1972 Spatial aspects of the neuronal connections in the rat olfactory bulb. In. Olfaction and Taste IV, ed. D. Scheider. Wissenschafliche Verlagsgesellshaft. MBH, Stuttgart.

Rall, W. and G. M. Shepherd 1968 Theoretical reconstruction of field potentials and dendrodendritic interactions in olfactory bulb. J. Neurophys. 31: 884-915.

Rushton, W. A. H. 1949 The structure responsible for action potentials in the cat's retina. Nature 164: 743-744.

Siegel, S. 1956 Nonparametric Statistics for the Behavioral Sciences, McGraw Hill Co., N. Y.

Stevens, C. F. 1969 Structure of cat olfactory cortex. J. Neurophysiol. 32: 184-192.

Stone, J. and R. B. Freeman 1971 Conduction velocity groups in the cat's optic nerve classified according to their retinal origin. Exp. Brain Res. 13: 489-497.

Stone, J. and H. Holländer 1971 Optic nerve axon diameters measured in the cat retina: Some functional considerations. Exp. Brain Res. 13: 498-503.

Switzer, R. C. 1972 Quantitative Relationships of Cell Populations in Olfactory Bulbs of Mammals. Masters Thesis, Mich. State Univ., E. Lansing, Michigan.

Valverde, F. 1965 Studies on the piriform lobe. Harvard Univ. Press, Cambridge.

Wiesel, T. N. 1960 Receptive fields of ganglion cells in the cat's retina. J. Physiol (London) 153: 583-594.