Quantitative Delineation of the Efferent Anatomy of the Medial Mammillary Nucleus of the Cat

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ABSTRACT A quantitative description based on fractional parts of the total neuron population discovered for the projecting neuron subpopulations of the medial mammillary nucleus in the cat is presented. Five neuron subpopulations send projections through the principal mammillary tract to the thalamic, subthalamic and tegmental structures and one intrinsic subpopulation has been identified. These subpopulations describe in detail the structural organization of the projecting neurons. Although the methodology and supportive research leading to these results is quite extensive the material presented in this paper is concerned with 15 cats in which appropriate lesions have been placed. These animals provide the data upon which the quantative description rests.

Quantitative knowledge of the relative sizes and extents of mutual overlapping of the neuron populations that constitute the efferently projecting cell groups, and also the relative size of the internal projecting neuron populations of the medial mammillary nucleus is a necessary stage in the determination of its neuronal organization and the relationship of the latter to structures to which the nucleus is directly connected. A previous report of neuron population data on the mammillary nuclei and related structures of the cat brain has been made by the author and collaborators (Fry et al., '64) and anatomic implications of some of this information have also been published (Fry, '66). However, the earlier data were not nearly sufficient to provide a basis for deriving the efferent organization of either the medial or lateral mammillary nucleus with any semblance of completeness. In the meantime, sufficient additional quantitative information has been acquired to present now a reasonably comprehensive picture of this organization for the medial nucleus. It is the purpose of this paper to summarize the pertinent data and draw their anatomic implications.

Quantitative neuron population work on the mammillary complex of the cat brain, previous to that of the author and collaborators, was restricted to the study of normal material (Guillery, '55; Powell et al., '57). Such information, while useful for comparing the total populations of interconnected nuclei, does not constitute the necessary data for identifying the neuron subgroups of a nucleus. The derivation of *efferent* anatomy, other than the qualitative aspect of identifying structures receiving projections, is obtained primarily from population data on brains in which retrograde degeneration follows the interruption of efferently projecting fiber tracts and/or nuclei receiving their terminations.

A considerable body of *qualitative* anatomic knowledge is available in the literature on both the efferent and afferent connections of the medial mammillary nucleus of the cat brain and information pertinent to this report is summarized in figure 1. (Abbreviations are listed in table 1.) A nucleus or region is designated in each case by a rectangle. A single circle containing the symbol 1 designates the entire population for the medial mammillary nucleus and the ventral tegmental nucleus. If the specific details of the neuronal projections are not known, the diagrammatic representation of the source of efferent projections and the termination of afferent connections is simply the rectangular border. However, for example, when information on the neuron population that gives rise to a specific projection exists the source of the fibers can be shown as indicated for the ventral tegmental nucleus. In this case, it was already suggested by qualitative ob-

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servations (Akert and Andy, '55) that the entire population projects to the mammillary complex. In the diagram, fiber groups efferent from the medial mammillary nucleus are shown by solid lines, those afferent to the nucleus by dotted lines. Except for the ventral tegmental nucleus, sources of the afferents are not indicated since this paper is concerned exclusively with the efferent organization. The presence of afferent connections on the diagram is useful for indicating some of the directions for the next phase of the work. The branching of fiber tracts, indicated in the figure, is a qualitative observation and, of course, individual branching fibers can be so identified in suitable preparations. However, in general, the relationship of branched fibers to specific neuron populations requires quantitative information for its elucidation (Fry, '66).

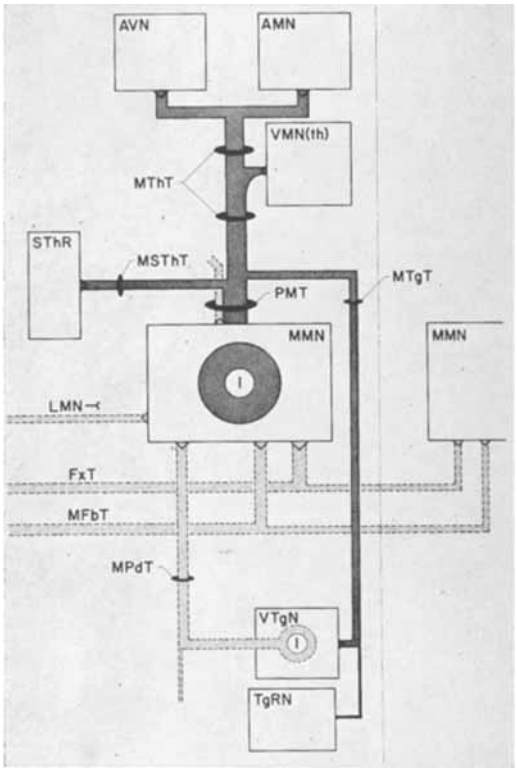

Fig. 1 Block diagram of the efferent and afferent connections of MMN of the cat brain based on qualitative anatomic knowledge.

TABLE 1

Abbreviations

	Nuclei
AMN	anterior medial (thal)
AVN	anterior ventral (thal)
LMN	lateral mammillary
MMN	medial mammillary
SThR	subthalamic region
TgRN	tegmental reticular
VMN	ventral medial (thal)
VTgN	ventral tegmental
	Fiber groups
FxT	fornix
MFbT	medial forebrain bundle
MPdT	mammillary peduncle
MSThT	proj. of MMN to subthal.
MTgT	mammillotegmental tract
MThT	mammillothalamic tract
PMT	principal mammillary tract

Efferent connections of the medial mammillary nucleus, MMN, in the cat brain are made via the principal mamillary tract, PMT, which forms in the dorsal part of the nucleus and bifurcates above it. Component branches are the compact mammillothalamic tract, MThT, which takes a dorsorostral course at the base of the thalamus, the less compact mammillotegmental tract, MTgT, whch courses caudally into the tegmentum, and a lateral coursing group of fibers which terminates in a subthalamic region (Nauta, '58). Along its anterior course MThT gives off terminations to the partially surrounding thalamic gray, specifically to the ventromedial nucleus of the thalamus, VMN (Fry et al., '63), but the more massive termination is in the anterior thalamic nuclear group with MMN projecting to the ipsilateral anterior ventral, AVN, and anterior medial, AMN, thalamic nuclei. The tegmentally directed MTgT terminates primarily in the tegmental nuclei of Gudden with MMN projections terminating in the ventral tegmental nucleus, VTgN, and also sparsely, according to Guillery (Guillery, '61), in the tegmental reticular nucleus TgRN. Evidence against the existence of efferents other than those projecting through PMT will be presented in this paper.

Afferent connections to MMN, to be treated quantitatively in a later paper, are made bilaterally via the fornix, FxT (Valenstein and Nauta, '59), and medial forebrain bundle, MFbT (Nauta, '58), to each of which a number of structures contribute fibers. Unilateral afferent projection is via

the mammillary peduncle, MTdT, and its source is primarily, if not exclusively VTgN. Work in progress also indicates that MMN receives projections from the adjacent LMN and evidence for fibers afferent to MMN via PMT has also been obtained. However, these latter do not appear to course in MThT as reported earlier by Le Gros Clark (Clark, '33), for the rat brain so this variation may constitute an interspecies difference.

MATERIALS AND METHODS

The experimental animals employed in this investigation were adult cats, the great majority females. Nine animals with unmodified brains and 15 with lesions provide the quantitative data. Several additional animals were used in the short term fiber degeneration work needed to complement the previously reported qualitative anatomic observations on this system (Fry et al., '63). The stepwise procedures for lesion production and subsequent histologic study of the brains have been previously described. The former involves internal brain landmarks as coordinate references for determining positions of structures (Fry and Fry, '63) and the use of focused ultrasound to produce the array of individual small lesions required to achieve the composite brain lesion with respect to shape and size, so as to match the particuar brain structure in which the lesion is to be placed (Fry et al., '64).

The histology and auxilliary preparations have also been described (Fry et al., '64), and only major features, subsequent changes and additional information will be mentioned here. The brains for the quantitative studies were paraffin embedded and sliced at an average section thickness of 10.0 μ. To achieve uniformity in the average thickness from section to section, it is essential to slice the block at a relatively constant rate. For the speed currently employed, 70 sections per minute, the average deviation in average section thickness is 2%. The brains for the silver impregnation preparations, Nauta and Fink-Heimer, were sectioned at 40 μ thickness.

In the quantitative work the Weil stain is routinely employed for the delineation of tracts, capsules, and diffuse distribution of fascicles. Sections so stained are superimposed on neighboring Nissl stained ones during the process of determining the boundaries of nuclear cross sections. For the earlier brains of the series reported here thionin was used as the cell body stain. However, since these sections always exhibited fading caused by the protracted exposure to light required for the two independent cell mappings and subsequent intercomparison, cresylecht violet has been used as the cell stain for the brains prepared later in the series. Since all brain sections are saved it is possible to stain a 10% uniformly spaced sample group of sections with thionin and an adjacent 10% uniformly spaced group of sections with cresylecht violet. For the MMN it has been shown that all 10% uniformly spaced sample groups when stained alike yield the same total neuron count for the nucleus. Comparison of neuron populations of the MMN on the same brain using the two stains shows no significant differences in the MMN total neuron population. No detectable fading of the cresylecht violet stained sections is apparent after the extensive population observations are completed.

The extent of the neuron pool included within MMN, for the brains reported upon herein, has been described previously (Fry et al., '64) but it will be outlined here. The boundary specification is stated in terms of the morphologic characteristics of normal material and it is in agreement with the distributions of retrograde cell loss and corresponding gliosis which occur within the nucleus in response to lesions in efferent pathways. Capsule fibers clearly define the nuclear boundary posteriorly and on its ventral and medial aspects over most of its antero-posterior extent. All divisions described by Bleier (Bleier, '61) are included. These include Bleier's central division which is in the dorsomedial aspect and is separated to some extent from the rest of the nucleus by fibers coalescing to form PMT. Also included is the small group of cells lying just medial to FxT and appearing in transverse sections as a relatively dense patch of gray matter centered approximately one-quarter mm from the anterior end of the nucleus. The few neurons seen in some sections, which lie as

a partial string of beads between the main masses of MMN and LMN, are included in MMN. Neurons present within the reticulum of fibers at the ventro-medial apex of LMN, and which resemble cells of neighboring MMN, and which are distinctly different from the larger dark staining cells of LMN, are also included in MMN. The cells lying interspersed among the fibers of PMT on the dorsolateral aspect of the nucleus, and which stain darker than neighboring cells of MMN are not included; they belong apparently to the supramammillary nucleus. Also excluded are the premammillary and anterior mammillary nuclei, see Bleier for terminology and morphological descriptions. The most difficult part of the boundary of MMN to identify in transverse sections is the anterior end. Four criteria are employed here: (1) the presence of diagonally oriented myelinated fibers lying across the face of the nucleus at the rostral end (this criterion is especially useful for nuclei with little or no cell loss). (2) a qualitatively observable reduction in neuron density on passing through the anterior boundary, (3) a change from an appearance of randomness in the cell configuration within anterior MMN to a preferred pattern of orientation just anterior to the nucleus, (4) qualitatively apparent differences in glial density on passing through the anterior end of the nucleus in cases where a major fraction of the neuron population has undergone retrograde degeneration.

Recording of nuclear boundary decisions is made on transparent plastic overlaying photomicrographs (magnification $\times$ 250) of the nuclei. The record of cell identification (nucleoli present) and classification (normal, questionable pathological, pathological) is also on these plastic sheets as previously described (Fry et al., '64).

The accuracy of the *neuron count* determined for the entire set of tissue sections through a nucleus from cell counts for a subset of sections is influenced by (1) overlooking of neurons, (2) relative number of questionable pathological cells, (3) variations in constructing the graph of cell count per section versus section number, (4) sample size, (5) inclusion of non-neural elements.

If essentially all the neurons with nucleoli in the tissue examined are identified then the cell count is an overestimate of the population because the nucleolar diameter is comparable to the section thickness. Therefore a correction factor, K_n, must be applied to the cell count in order to obtain the correct value. The calculation of this factor is subject to various sources of error which intoduce uncertainty into the determination of the neuron population in addition to the five already listed. However, since the quantity of primary interest in this paper is the percentage cell loss, rather than the absolute number of cells that have disappeared, it is only the accuracy of the ratio of the correction factors for the bilaterally corresponding medial mammillary nuclei that need be considered. Factors that influence the value and the accuracy of this ratio are (1) variations in average section thickness (affect the accuracy) (2) side to side differences in the distribution of nucleolar size (affect the value). The seven factors just listed are now considered primarily from the viewpoint of the possible magnitude of the uncertainty which each introduces into the accuracy of the determination of the percentage population difference between the medial mammillary nuclei in the same brain (normal or modified).

In order to minimize the number of non-neural elements that become included in the cell count the mapping procedure is performed completely independently by two different examiners. The results are compared by a third examiner who studies the non-common elements and decides on their classification. Of course, even if the difference between the cell counts of two mappers amounts to only a few per cent, which is the situation in many cases, this does not prove that the value of the cell count which results after comparison is then an accurate indication of the true cell count for the sections examined. This follows because additional examiners may identify nerve cells other than those mapped by the first two. Consequently, no value(s) for the overall correction factor, K, to convert cell counts to cell populations will be given, since the determination of the accuracy with which the mapped cell count represents the total number of nerve

cells present in the mapped sections has not been completed. The value of the correction factor, K_c, to convert from cells mapped to cells present in the sections is not needed for the purposes of this paper since: (1) the neurons of MMN in the cat are very similar in appearance throughout the extent of the nucleus, and (2) those cells present as residual populations, after retrograde degeneration has reached essential equilibrium, have nucleoli that are not greatly different in size from those in the unmodified nucleus in the same brain. That the mapped cells, if not accurately equal in number to those present in the sections, are a qantitatively reproducible fraction of the entire set is shown by the closeness of agreement between the cell counts of the bilaterally corresponding medial mammillary nuclei in unmodified brains (average difference 1.8%) and the reproducibility of the fractional depopulations in different brains with similar lesions (see the section on results). That part, K_n, of the overall correction factor, $K = K_n K_c$, due to the relative size of average nucleolar diameter, d_a, and the section thickness, T, is readily calculated from the simple Abercrombie (Abercrombie, '46) expression

$$K_n = \frac{T}{T + d_a}.$$

For the purpose of estimating nuclear population values from cell count data for MMN, neglecting temporarily the error of overlooking a fraction of the neurons present, an average value of K_n equal to 0.82 can be employed for all of the brains for which cell count values are presented.

On graphing the cell counts the number of normal and normal plus questionable cells for each section are plotted. The curve constructed to represent the count for all the tissue sections through the nucleus is drawn to average the two values plotted for each section for which the neurons were mapped since it is not possible to decide whether the questionable cells are projecting ones directly affected by a lesion or whether the changes are the result of secondary effects. In any case, if an animal is not sacrificed for a year after lesion production, the percentage of cells present that exhibit the questionable appearance, in a nucleus in which a major depopulation has occurred, amounts typically to approximately 2% of the population.

The variation in the values of the cell count obtained for a nucleus, introduced by differences in constructing a curve to fit the data points is less than 1% and typically a few tenths of a per cent when neuron counts are available for 10% of the sections spaced through the nucleus.

The importance of sample size was evaluated quantitatively by comparing the results obtained for the total cell count when samples of different sizes are employed. The variation discussed in the preceeding paragraph is also included in the following results. A typical case is summarized in table 2. Cell counts based on four mutually independent 5% samples, and two similarly independent 10% samples are compared with the count value obtained from a 20% sampling. The average deviation of the count based on mapping all neurons on 5% of the tissue sections, from the count based on the 20% sample, is 2.2% (the average of the 8 individual percentage deviations, 4 for each side). Similarly the average deviation for the 10% samplings is 1.0% . On the basis of this type of intercomparison a 10% sampling for MMN is

TABLE 2

Effect of sample size on neuron count of MMN — C 1144DA

Sample size	Code no.	Normal nucleus		Partially depop. nucleus	
		Cell count	Deviation	Cell count	Deviation
%			%		%
5	1	69800	0.4	30400	4.7
	2	70500	1.4	30700	3.8
	3	68100	2.0	31900	0.0
	4	67500	2.9	31200	2.2
10	1	70900	2.0	31800	0.3
	2	68500	1.4	31800	0.3
20	1	69500		31900	

employed now routinely but 5% samples were used for all the normal brains and those modified ones studied early in the series as indicated in the next section.

Uncertainty can be introduced into the calculation of the per cent cell loss for MMN by factors other than those affecting the determination of the neuron count. Imprecise knowledge of the average section thickness must be considered here because the ratio of correction factors for converting total counts to population values for the partially depopulated and the unmodified medial mammillary nuclei is involved and these factors depend on the section thickness. Only when the nucleolar size distribution is identical on both sides is the indicated ratio independent of the thickness, in this case its value is unity as is apparent from the following formula. Designate the cell counts for bilaterally corresponding medial mammillary nuclei by c_1 and c_2, the thickness of the sections by T and the average nucleolar diameters for the two nuclei by d_1 and d_2 respectively. Then if P_1 and P_2 represent the neuron populations calculated from these parameters the population loss, L, experienced by the modified nucleus (i.e., no. 2), expressed as a percentage of the population of the unmodified nucleus (i.e., no. 1), is

$$L = 100\left[\frac{P_1 - P_2}{P_1}\right] = 100\left[1 - \frac{c_2}{c_1}\left(\frac{T + d_1}{T + d_2}\right)\right]$$

The effect of an uncertainty in knowledge of the section thickness on L is determined by calculating the value of the latter for different values of T. For example consider an uncertainty in T of $\pm 5\%$, i.e., $\pm 0.5\ \mu$, in 10 μ which is considerably larger than that characteristic of the brain series reported here. Then for values of d_1 and d_2 of 2.5 μ and 2.0 μ respectively, and for $c_2/c_1 = \frac{1}{2}$ as a specific case, the difference in L is no greater than 0.2%. Similar calculations show that T can be chosen equal to 10.0 μ for calculating the cell loss in all cases without introducing an appreciable error.[2]

It follows from the expression for L that its value depends on the values of d_1 and d_2 except when these are equal. Since the determination of d_1 and d_2 constitutes an extensive undertaking, if it is done accurately for even one specimen, it is of interest to determine the change in L of the difference between d_1 and d_2 is neglected. This can be determined by specific examples as follows. If the difference between the average nucleolar diameters is as large as 10% and T = 10.0 μ, then the value of L calculated on this basis, for a cell count ratio of one-half differs from the value of L assuming the $d_2 = d_1$ by 0.8%. Since a comparison of averages of nucleolar diameter measurements made bilaterally on typical sections through normal and modified mammillary nuclei indicate no greater differences than 10%, the neuron loss values presented in this paper are calculated by neglecting differences in the average nucleolar diameter between the two sides. Under these latter circumstances the percentage loss in the neuron population is simply equal to the percentage reduction in the cell count, i.e.,

$$L = 100\left[\frac{P_1 - P_2}{P_1}\right] = 100\left[\frac{c_1 - c_2}{c_1}\right]$$

It should also be noted that population loss based on a summation of incremental population values calculated from the incremental cell counts corresponding to a number of size ranges of nucleoli (taken from the histogram for the nucleolar size distribution) differs by a negligible amount from the loss calculated by using an average nucleolar diameter for all the cells of the nucleus. Thus the presentation of methods of calculating cell loss given here has been confined to the latter since it is considerably simpler and no important loss of accuracy is entailed by its use.

RESULTS AND DISCUSSION

All the modified brains that provide the quantitative data for this report are listed in table 3. Those with interruption of efferent tracts from MMN, or with lesions in nuclei receiving efferent projections, are listed first and are grouped into categories based on lesion location. Data on two brains with lesions in afferent fiber groups, which delimit the distribution of the efferent projections of MMN are listed in the second major subdivision of the table.

[2] For normal brains the consideration under discussion is not relevant because there is no cell loss and consequently a knowledge of the section thickness is not necessary for the calculation of L.

TABLE 3

Data on modified brains. Neuron counts and losses for MMN

Cat		Histo. prep.	Lesion location		Survival Months	Sample size	Neuron count		Neuron loss
			Structure	Side					
no.	*sex*	*date*				%	*lt.*	*rt.*	%
			Efferents						
			MThT						
1168	F	6–67	anterior	R	12	10	9330	48500	48.0
1144DA	F	10–67	midcourse	R	16	20	69500	31900	54.1
826	F	7–62	posterior	L	12.5	5	25000	74200	66.3
			MTgT						
765	F	7–62	above MMN	L	14.5	5	72700	82000	11.4
837	F	7–62	above MMN	L	7.5	5	72300	77000	6.2
			MThT and MTgT						
770	F	5/62	post. MThT	L	13	5	26900	71700	62.4
			MSThT						
858	F	5–63	adjacent PMT	L	22	5	72200	86900	16.9
			Lat. to PMT						
1158	F	1–66	1 mm. Lat.	L	22	5	63000	63100	0.2
			PMT						
1166G	F	11–66	vert. ext.	R	11	10	74800	9690	88.5
1166C	F	6–67	vert. ext.	R	12	10	91300	11700	87.1
			Ant. Thal. N.						
984	F	10–67	AVN FxT (1/2 subcall.)	R	67	10	85100	54100	37.2
1003	M	4–63	AMN (incompl.)	R	12	10	80600	62500	22.4
			MMN						
1011	F	3–63	ant. MMN, LMN PMT	L	6	5	1990	88500	
			Afferents						
			MPdT						
1011A	F	6–65	almost compl.	L	15.5	5	72700	70900	2.4
			MFbT						
1060M	M	4–63	almost compl.	L	8.5	5	80500	83900	4.0

With respect to the efferents, the three brains of the first category have lesions in MThT. These lie in different positions along the course of the tract as illustrated in the sagittal diagram of figure 2. In this diagram the extent of the primary lesion along the tract is shown in each case. In C 1168 the lesion is confined to the portion of MThT anterior to VMN and the projection of MMN to this nucleus is not interrupted. The cell loss, 48.0%[3] is thus a measure of the magnitude of the projection of MMN to the anterior thalamus. In C 1144 DA the lesion extends from rostral to the anterior end of VMN posteriorly to interrupt fibers to the latter which leave MThT over three quarters of the basal extent of the nucleus. However, it does not interrupt the tract at the posterior basal part of VMN and, since a major fraction of the projection from MThT enters VMN in its posterior half, the cell loss, 54.1%, in MMN, although greater than that in C 1168, is considerably less than in C 826 in which the posterior end of the lesion lies just anterior to the bifurcation of PMT thus severing all MThT projecting fibers from the mammillary nuclei. In this latter case the cell loss in MMN, as measured in C 826, is 66.3%.

The second category of the table includes brains in which MTgT was inter-

[3] It is desirable to retain the digit in the tenths position in order to avoid round-off errors.

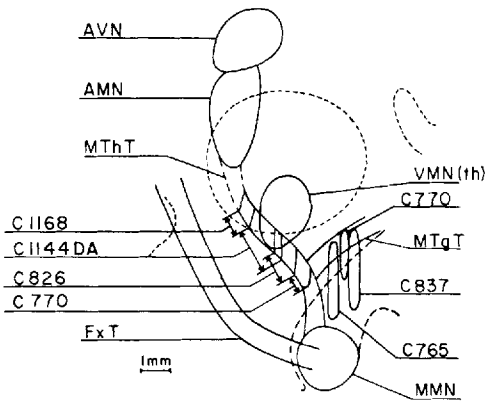

Fig. 2 Sagittal projection diagram of indicated brain structures showing positions of lesions in MThT and MTgT.

rupted, probably not completely as indicated on the sagittal projection diagram of figure 2. The uncertainty here is caused by the fact that the spatial extent of the distribution of MTgT in stained cross sections is not readily delimited and the two lesions involved here would have had to extend further dorsally in order to be certain of complete interruption. For the brains under consideration one of the animals was sacrificed early compared to the one year interval which is considered appropriate in this study for determining quantitatively retrograde cell loss. The shorter interval may account at least partially for the smaller, 6.2% cell loss determined for C 837 and, while awaiting the acquisition of additional data, it will be presumed that the 11.4% value obtained on C 765 is a more appropriate indication of the magnitude of the projection of MMN into MTgT than either the lower value or the average of the two.

The interruption of MThT in the posterior part of its course, and of MTgT in the same brain, was accomplished in C 770 and the resulting cell count data is listed in the third category in the table. In this case the cell loss, 62.4% , in MMN is not significantly different than the loss following the interruption of posterior MThT alone.

Since the fibers, MSThT, that project laterally from PMT into the subthalamus course diffusely, and since this fiber group has not been studied in detail, it is difficult to decide the extent of the interruptions unless very extensive lesions are employed. In C 858 the lesion is immediately adjacent to PMT as can be seen in figure 3 in the insert showing its transverse projection. The sagittal projection of the lesion is shown superimposed on the sagittal projection of MThT, MTgT and other structural features in the same figure and as can be seen the antero-posterior extent of the lesion is only 0.5 mm. Although considerable shrinkage could have occurred, during the 22 month survival period, it is unlikely that the entire lateral projection was severed and in this case the observed cell loss, 16.9% , would constitute a lower bound for the magnitude of the subthalamic projection from MMN. The result just indicated can be compared with that following a lesion separated 0.5 mm laterally from PMT and with the transverse and sagittal position and shape seen in figure 3. In this case, C 1158, no significant cell loss occurred in MMN and the result suggests a more anterior position for the lateral course of the diffuse subthalamic projection than at the mid-mammillary body level.

That the subthalamic projection is indeed somewhat larger than the loss observed in C 858 is shown conclusively by the results on C 1166G and C 1166C in which the entire ventrodorsal extent of PMT was interrupted in each case. The measured cell losses, 88.5% and 87.1% are in excellent mutual agreement. When the entire projection of MMN into MThT and MTgT, 64.4% , the average of the losses for C 826 and C 770 — is subtracted from the average, 87.8% ; of the losses in C 1166 G

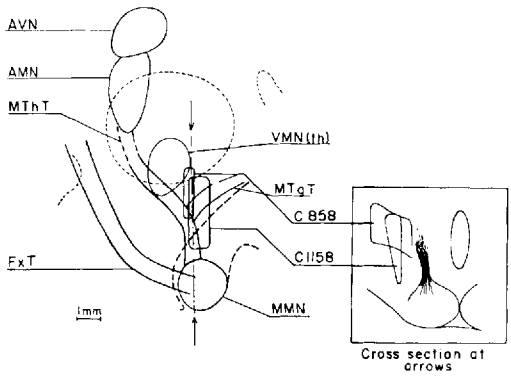

Fig. 3 Sagittal projection diagram of indicated brain structures and insert of a portion of a single transverse section, showing configurations of lesions lateral to PMT.

and C 1166 C the difference 23.4% is a lower bound for the size of the subthalamic projection. The loss in C 858 accounts for about three-fourths of the value just derived and thus the lesion in the latter case could well have interrupted all but one-fourth of the MMN projecting axons in MSThT.

That all of the externally projecting neurons of MMN project via PMT is shown by results subsequently presented here so the size of the internally projecting population of MMN is obtained as the difference between unity and 87.8% for a value of 12.2%.

The anterior thalamic projection of MMN is to AVN and AMN and the results obtained on C 984 and C 1003 bear on the structure of this relationship. In C 984 the lesion destroyed directly almost the entire neuron population of AVN, sparing only a very small fraction of the population in the lateral part of the nucleus. Thus essentially the entire projection from MMN was interrupted and this was accomplished without infringement on AMN. However, the interpretation of the loss, 37.2%, in MMN as caused entirely by the lesion in AVN is subject to some qualification since the lateral half of the subcallosal fornix was also interrupted on the side ipsilateral to the anterior thalamic lesion. Since some transneuronal cell loss probably occurs in MMN following interruption of the entire hypothalamic fornix in the adult cat (preliminary unpublished results of this laboratory indicate that this cell loss may amount to as much as 25% of the total population), and since the hypothalamic fornix in C 984 just anterior to MMN is obviously reduced in size (of the order of ⅓ reduction in cross section), part of the cell loss in MMN could be the result of such a transneuronal response. However, in the absence of a preparation with a lesion like that in C 984 but without the complication arising from a subcallosal fornix lesion, it is still possible, on the basis of observations on C 984, to support strongly the view that the MMN projection to AVN is three-eighths of the population. This follows from a bilateral comparison of the mammillothalamic tract cross sections in C 984. It is apparent, on examining the tracts in their course anterior to VMN, that the fiber population on the side ipsilateral to the lesion is certainly considerably less than half, and probably less than a third, that on the contralateral side. This implies that the projection to AVN is equal essentially to the entire cell loss in C 984 since the magnitude of the projection to AVN and AMN together is equal to one-half the population of MMN, as shown by the result obtained on C 1168, and the fiber population ratio would therefore be equal to approximately four in C 984 for the conditions indicated. The only other value for the ratio, which would appear acceptable as suggested by the overall view of the organization seen below, is approximately two which is ruled out by the sizes of the tract cross sections. (The population of fibers projecting from LMN via MThT is small compared to the population of fibers projecting from MMN).

In C 1003 the lesion destroyed directly over half the neuron population of AMN sparing the lateral part of the nucleus adjacent to the diagonally coursing fiber bundles which lie along its lateral aspect. There is no obvious reduction in the extent of the density of the myelin in this fiber group, which includes the projection to AVN via MThT. Therefore, unless some MThT fibers to AVN course diffusely through AMN to reach AVN, or unless projecting fibers to AVN have collaterals to AMN, there is no interruption of the MMN projection to AVN. With regard to the existence of collaterals it is apparent, since the cell loss in MMN in C 1003 is 22.4% and since the projections are: to AVN — three-eighths, and to AVN and AMN together — one-half, that a branched projection to AVN and AMN from somewhat more than 10% of the population of MMN is present. This conclusion is justified only if it is known that through-going fibers from this number of cells do not traverse AMN on the way to AVN without sending a branched connection to terminate on neurons of AMN. The latter situation will be assumed tentatively until brains with appropriately placed lesions and survival times following modification are available to settle the matter in a definitive fashion.

Population results following the placement of lesions in the afferent tracts, MPdT and MFbT are also included in table 3. These results bear on the question

of the possible existence of some efferent projections of MMN leaving via these fiber systems. However, in C 1011A no cell loss occurs in MMN in 15 months following complete severing of MPdT. Similarly C 1060 shows no significant cell loss in MMN after almost complete severing of MFbT in its course just anterior to the position of the mammillary bodies. In this latter case the survival time, eight and one-half months, is somewhat shorter than the one year or longer employed for most of the brains in this series, but the time is long enough so that some loss would be expected, if the results on other brains of comparable survival intervals following lesion production is any basis for judgment. In addition, the absence of efferently projecting fibers in MFbT is also supported by short term fiber degeneration studies. In brains prepared for the identification of degenerating fibers none are seen either in MFbT or in FxT two weeks after placement of lesions in MMN.

That the residual group of neurons remaining in MMN after PMT destruction is not a diffuse one projecting outside the medial and lateral mammillary complex is eliminated by the results seen in C 1011 in which the neural components of PMT and the entire anterior third of MMN were destroyed unilaterally. In this case the remaining two thirds of MMN contains an appreciable population of neurons and their anterior-posterior distribution curve (Fry et al., '64) is not inconsistent with the corresponding distribution curves seen in figure 4, which characterize C 1166C and C 1166G. Also bearing on the nature of the circuitry of the residual group is the lack of evidence in silver preparations of fibers terminating in LMN following lesions in ipsilateral MMN. All of the results just indicated appear to constitute conclusive proof that the neuron population remaining in MMN after complete destruction of PMT is an internal projecting one.

In order to proceed with the anatomic implications of the quantitative results listed in table 3 it is convenient to exhibit on a block diagram of the efferent pathways of MMN, figure 5, the neuron losses which occur in MMN following the various lesion configurations already described.

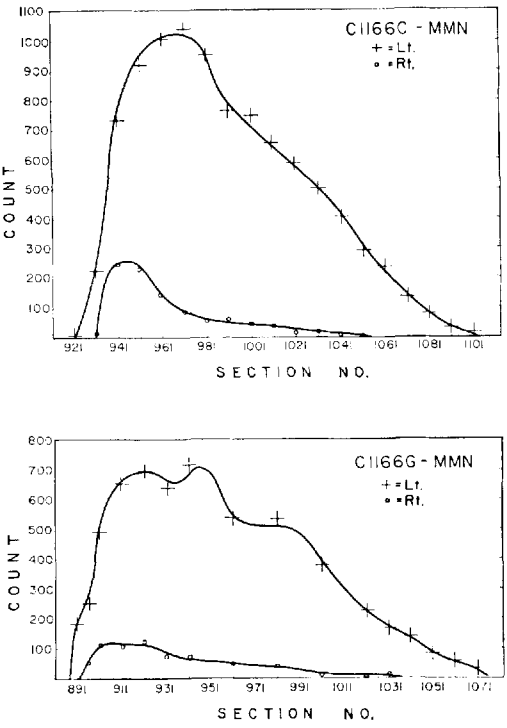

Fig. 4 Neuron count distribution graphs for C 1166C and C 1166G.

Consider first the bifurcation from PMT of the MMN projections that course in MThT and MTgT. The five different possible structural relations are illustrated in figure 6: (a) no fibers with branches in both MThT and MTgT exist, (b) all fibers entering MThT and MTgT have branches in each, (c) all fibers in MThT are branched with ones in MTgT but fibers exist in MTgT with no branches in MThT, (d) all fibers in MTgT are branched with ones in MThT but fibers exist in MThT with no branches in MTgT, (e) fibers with branches in MThT and MTgT exist in addition to those in MThT with no branches in MTgT and to those in MTgT with no branches in MThT. Case (a) must be included, although Ramón y Cajal (Ramón y Cajal, 1895) first reported branching in PMT fibers with one branch directed caudally and the other rostrally, since such branched fibers may arise entirely from LMN. A decision among the five cases can be made by recourse to pertinent quantitative data included in

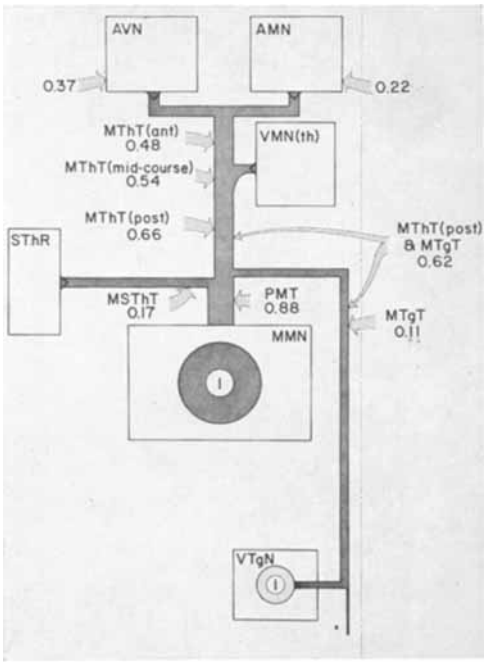

Fig. 5 Block diagram of efferent pathways of MMN and their terminals showing depopulation losses within the nucleus following lesions at the indicated sites.

figure 5. The specific data of interest here correspond to the brain modifications indicated by the diagrams in the upper part of figure 7, which also includes certain observed relations between these data. In the figure, interruption of a pathway is designated by an X, absence of knowledge of the structural relation in the pathway is shown by not completing the circuit connections, and the existence of efferent fibers other than those under consideration is designated by a short line segment. The three diagrams in the upper half of figure 7 designate the following brain modifications: (a) interruption of posterior MThT, (b) interruption of MTgT, (c) interruption of posterior MThT and of MTgT in the same brain. From the cell losses in MMN that occur in these three cases, designated by the symbol L_{MMN} in parenthesis subscripted with the letter corresponding to the particular lesion configuration, the two relations listed below the three diagrams are derived i.e., (1) the cell loss that occurs following the interruption of both MThT and MTgT, 62.4%, is not significantly different from the loss that occurs following the severing of MThT (posterior) alone, 66.3%, (2) the loss that occurs following the severing of MThT is much greater than that which occurs after interrupting MTgT. The anatomic implication of these results is illustrated in the diagram in the lower part of figure 7, i.e., case (d) of figure 6, since the configurations (a), (c), and (e) would result in greater cell loss following the interruption of both MThT and MTgT than that which would occur when MThT alone is severed and configuration (b) would result in the same cell loss when MThT and MTgT are individually severed. Thus the structure of the bifurcation is that all the MTgT projecting neurons from MMN (approximately 11% of the population) send a branch into MThT and that half the population of MMN projects into MThT with no branches in MTgT (a value of 54% is calculated as the difference between the total projection into MThT, average of the results obtained on C 826 and C 770, and the projection with a branch in MTgT).

The next circuitry feature considered is the projection to VMN from MMN. The quantitative data of C 1144 DA and C 1168, in addition to the cell losses just discussed,

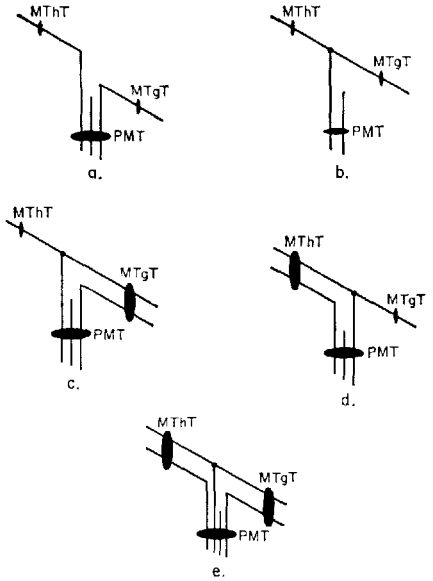

Fig. 6 Possible structural relations for the bifurcation into MThT and MTgT of MMN efferents in PMT.

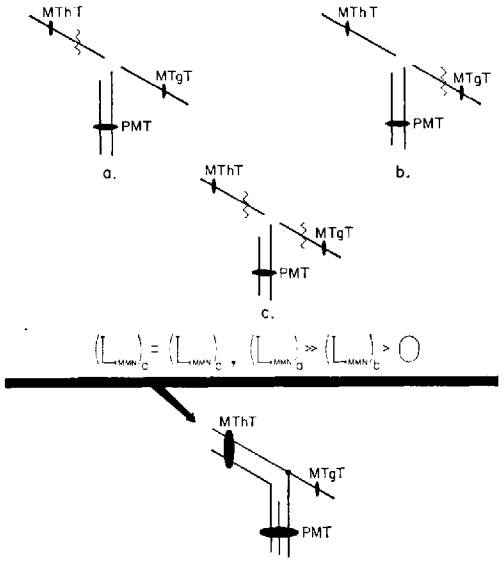

Fig. 7 Structure of the bifurcation of PMT efferents from MMN into MThT and MTgT.

i.e., that following interruption of posterior MThT and that which occurs when MTgT is severed, bear on this. As concluded in the previous paragraph, one half the neurons of MMN project into MThT with no branch in MTgT. As the diagram of figure 5 shows severing MThT in its course anterior to the projection to VMN also results in a loss of half the neurons from MMN, 48%. When MThT is interrupted in the region where the fibers projecting to VMN leave, a loss, 54%, is measured which is intermediate between that which occurs following interruption of the anterior, 48%, and posterior, 64%, courses. In addition to these differences in the cell losses a second important difference, bearing on the structure of the MMN projection to VMN, is in the transneuronal response of the neuron population of VTgN when the anterior and when the posterior courses of MThT are interrupted in different brains. Both types of information are employed in constructing figure 8 in which the conclusions regarding the structure of the bifurcating projection into MThT and MTgT are incorporated. In (a) the lesion designated is in MThT posterior to the projection into VMN and the neurons of VTgN respond as a single population, they are present but reduced in size (volume) by over 50%. There is no cell loss in VTgN. By contrast, as indicated in (b), when the lesion in MThT is anterior to VMN, the neurons of VTgN exhibit no transneuronal response. Of course, the transneuronal response in VTgN also occurs in case (c) that is, when MTgT is interrupted. The quantitative information of figure 5 shows that, within the accuracy of the data, the relation exhibited holds between the losses in MMN in each case. The transneuronal response pattern shows that the fibers which project to VTgN do not course in MThT beyond the projection to VMN and this result, in conjunction with the observed relation, indicates that the circuitry shown in the bottom of the figure is the appropriate configuration i.e., all those neurons that project to VTgN also project to VMN. The possibility of a branched projection between VMN and anterior MThT remains as indicated by the dotted lines in the figure. This latter possibility can be decided by quantitative data on a preparation in which the neuron population of VMN is destroyed, and MThT is not infringed, such a preparation will be available for study in about a year.

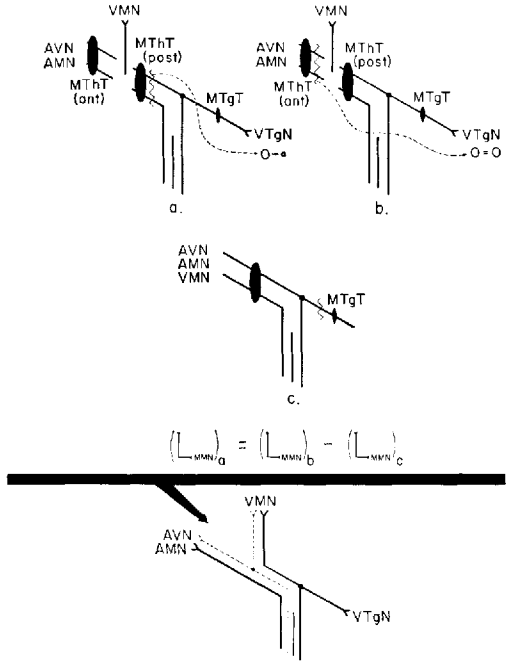

Fig. 8 Relation between efferents from MMN projecting to VMN and to VTgN.

The three types of preparations illustrated diagrammatically in figure 9 bear on the structure of the projection of MMN to the subthalamus, (a) interruption of the subthalamic component alone (b) interruption of PMT in entirety, (c) interruption of MThT in its posterior course. The magnitude of the subthalamic projection must be at least as large as the difference (24%) of the cell losses that occur following PMT interruption (88%) and the severing of MThT in its posterior course (64%). It is considered unlikely that it is larger than a quarter of the population for the following reasons. A lesion placed immediately adjacent to the lateral aspect of PMT must interrupt a major fraction of the subthalamic projection and a loss of 17% of the population occurred in the C 858, note the relation listed in the figure. The value, 17%, is a reasonable amount smaller than 24% and therefore does not suggest that the complete projection involves more than a quarter of the population. In addition, if the subthalamic projection is greater than a fourth the population of MMN, branching with tegmental or anterior thalamic projections must occur. That the former is not the case is shown by the absence of a transneuronal response in VTgN in C 858. The latter possibility cannot be decided, at least at present, on the basis of the presence or absence of a transneuronal response in the anterior thalamic nuclei since such a response is not qualitatively apparent even when MThT is completely severed. In the interim period, while awaiting additional direct evidence bearing on the possible existence of an anterior thalamic-subthalamic branched projection, since the indirect evidence does not favor its existence, it is most likely that the structure of the projection of MMN to the subthalamus is of the form indicated in the lower part of figure 9 i.e., no fibers, which are branched with others projecting into MThT or MTgT, accompany the unbranched component which arises from one quarter of the neuron population of MMN.

Other quantitative conclusions can be derived from the data of figure 5, first, the size of the internal projecting neuron population of MMN can be obtained in view of the absence of efferent fibers in the

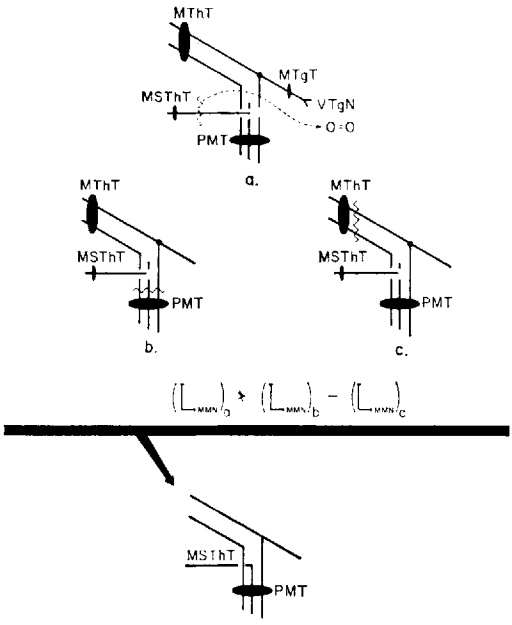

Fig. 9 Structure of MMN projection to subthalamus.

"afferent" tracts and of any diffuse outflow through the anterior boundary of the nucleus, by simply subtracting the loss, 88%, following PMT interruption from unity, the value obtained is 12%, i.e., one-eighth of the population. Second, the division between AVN and AMN of the anterior thalamic projection from MMN, which amounts to half the population of the nucleus, and which has already been considered in detail, is three-fourths to AVN with one-half unbranched and one-fourth branched with the projection to AMN. In addition to the branched group to AMN this nucleus also receives one-fourth of the projections to the anterior thalamus which is unbranched with fibers terminating in AVN. However it should be noted that revision of these conclusions regarding the branching pattern may follow the gathering of additional cell population data on brains with other modifications than are available at present for the investigation of this specific feature.

It is now possible to construct, on the basis of the analysis presented here, a circuit diagram, figure 10, for the efferent organization of MMN including the quan-

titative specification of the relative sizes of the neuron subpopulations that are involved. The numerical results indicate that within the accuracy of the data, all of the latter are equal to the fractional values designated in the figure.

It is apparent by comparison that the diagram of figure 10 constitutes a much more comprehensive understanding of the efferent neural circuitry of MMN than does that of figure 1. The only substantial indeterminacy remaining [4] is the possible existence of some branching of projections between VMN and the anterior thalamus. (This question can be settled in a straightforward fashion by determining the cell loss in MMN following destruction of VMN, without direct interruption of MThT, and, if necessary, by making similar determinations in brains with the lesion combinations AVN, and VMN, AMN and VMN). Of course brains with more complete direct interruption of the subthalamic projection would be useful in further substantiating the conclusion that this projection is no greater than one-fourth the neuron population of MMN and therefore that no branching with mammillothalamic projecting fibers exists.

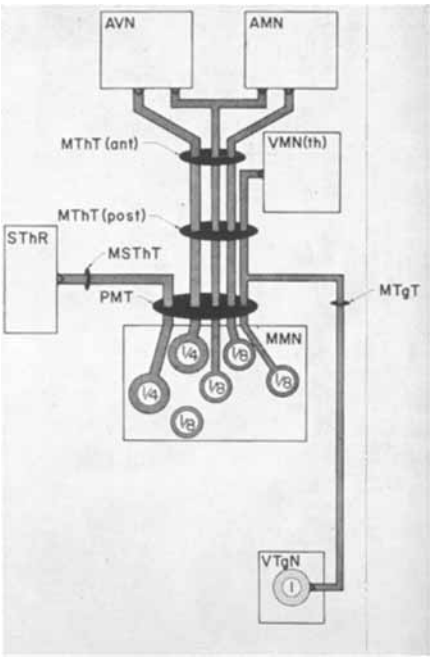

Fig. 10 Quantitative neuronal circuit diagram of the efferent anatomy of MMN.

Specific features of the efferent organization that has just been derived for MMN are of considerable interest. The absence of projecting fibers to the anterior thalamus with branches to the tegmentum contrasts with the tegmentally branched connections to VMN. It will be of considerable interest to determine how the connections within MMN differ for these efferently projecting populations, especially in view of the reciprocal relationship between MMN and VTgN. Another feature of considerable interest is the large size of the subthalamic projection and its non-overlapping with the other efferently projecting neuron populations. It would be desirable to characterize anatomically the extent of the subthalamic projection field. With the complexity already apparent at this stage of elucidating the organization of MMN, efferent connections of comparable magnitudes to structures in four diverse regions of the brain (anterior thalamus, ventromedial thalamus, sub-thalamus and tegmentum) and efferent connections (aside from those of the mammillary peduncle and from adjacent LMN) over two apparently quite complex fiber systems (fornix and medial forebrain bundle bilaterally) it might appear that the internal projecting population, amounting to only one-eighth the total population of the nucleus, is rather small in size to furnish the intraconnections which would be required for many types of internal organizations. Of course, the large size of the fornix projection indicates that most of the connections in this case are made directly on cells of the externally projecting neuron groups.

The elucidation of the organization of the efferent projections of MMN sets the stages for a systematic attack on the distribution of each afferent fiber system over the internal projecting group and over each of the efferent populations. This will include, for example, a determination of the distributions of the fibers of the fornix and medial forebrain bundle, at the level of the mammillary bodies, over the neurons of MMN, to be followed by a breakdown with respect to sources of origin. Achievement of

[4] Quantitative information on the possible projection to TgRN is lacking but this is reported as sparse.

this objective is logically followed by the determination of the connections of the internally projecting cell group. Other objectives of considerable interest include the application of the methods that have provided the data for this report to the elucidation of the efferent organizations of the anterior ventral and anterior medial thalamic nuclei and to the ventral tegmental nucleus. The structure of the lateral mammillary nucleus is obviously of parallel interest, not only from an inherent viewpoint, but also because it is now desirable to determine in what manner it is structurally related to the medial nucleus. Considerable progress has been made on the elucidation of the organization of the efferents of LMN but this work has not yet reached the definitive stage attained for MMN.

It is apparent that the medial mammillary nucleus in the cat brain is a center of considerable complexity where a wide variety of afferent projections may distribute over as many as six organizationally distinct neuron populations. One of these connects entirely internally and the others project to a wide spectrum of brain structures: the anterior thalamic nuclei that project in turn to the limbic cortex, the ventromedial nucleus of the thalamus with its widely dispersed projection fields (Smaha and Kaelber, '67), the ventral tegmental nucleus with its reciprocal connection to the medial mammillary nucleus and a presently imprecisely defined region of the subthalamus.

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