Cortical Afferents to the Entorhinal Cortex of the Rhesus Monkey

Abstract. *Although the entorhinal cortex is a major contributor of afferents to the hippocampus and dentate gyrus, knowledge of its own afferents has been vague. Regions of both the frontal and temporal lobes were found to contribute afferents to this region of the brain. These afferents form probable multisynaptic links in pathways connecting the classical sensory areas of the cortex and the limbic system.*

The limbic system has been studied extensively because of its central importance in memory, emotion, and the maintenance of the internal state of the organism (*1–3*). Several questions, however, regarding the anatomical organization of the limbic system have largely remained unanswered. These may be placed into two categories: (i) those dealing with specific neuroanatomical connections between structures that are considered components of the limbic system and (ii) those dealing with the broader topic of how the limbic system is interrelated with the rest of the brain.

This report is concerned with two such questions. The first is the specific anatomical question of the nature of afferents to the entorhinal cortex, which projects heavily to the hippocampus and dentate gyrus (*4*, *5*). The second, and broader, question focuses on the manner in which the limbic system has access to information from the classical sensory systems via the entorhinal cortex.

Our results implicate three cortical areas as direct sources of afferents to the entorhinal area. The first is located on the ventral portion of the temporal lobe and apparently corresponds to area TH of von Bonin and Bailey (*6*). The second corresponds to Brodmann's area 51, the prepiriform cortex (*7*). The third, located on the caudoventral portion of the frontal lobe, corresponds to Walker's areas 12 and 13 (*8*). Since these regions themselves are the recipients of afferent outflow from the major sensory systems, they are considered probable sources of sensory information for the limbic system.

Brain tissue studied came from 34 rhesus monkeys. In the brains of these animals, subpial ablations had been made on the ventral surfaces of the frontal lobes (orbitofrontal area) and temporal lobes (parahippocampal area). After 7- to 12-day survival periods, the Nauta (*9*) and Fink-Heimer (*10*) silver impregnation techniques were used for histological identification of axonal projections and terminal endings.

The entorhinal cortex of the monkey lies medial to the rhinal sulcus and occupies the caudal aspect of the uncal portion of the temporal lobe (*11*). The general structure of this cortex conforms to the six-layered arrangement of other cortices but, in comparison, is conspicuous because of the poor alignment of cell layers and the presence of cell-sparse laminae. The entorhinal cortex has generally been divided into two cytoarchitectonically distinguishable subareas, a medial area, 28a, and a lateral area, 28b (*7*). Of the cortical regions investigated in the monkey, the entorhinal region is unique in having the only direct and extensive connections with the hippocampus and dentate gyrus (*12*).

Cajal (*4*), in his classical description of the entorhinal cortex, was unable to identify the origin of afferent fibers entering its boundaries, and the source of these fibers has remained a mystery. Our results indicate that area TH, immediately caudal to the tip of the rhinal sulcus, is a significant source of ipsilateral afferents to the entorhinal area (Fig. 1a). After selective ablations of this region, terminal degeneration is observed in both areas 28a and 28b but is heaviest in the former. Ablations confined to temporal neocortical areas TE, TF, and TG lead to terminal degeneration in the entorhinal area as well, but only in the lateral portions of areas 28a and 28b, in the depths of the rhinal sulcus. Terminal degeneration is also observed in area TH after these ablations.

Evidence for a second source of afferents from the ipsilateral temporal lobe to the entorhinal area was observed after small ablations of area 51, the prepiriform cortex (Fig. 1a). Our observations indicate that area 51 supplies afferents mainly to area 28b, the more lateral subarea.

A third, and particularly significant, source of ipsilateral afferents to the entorhinal area was found after ablations

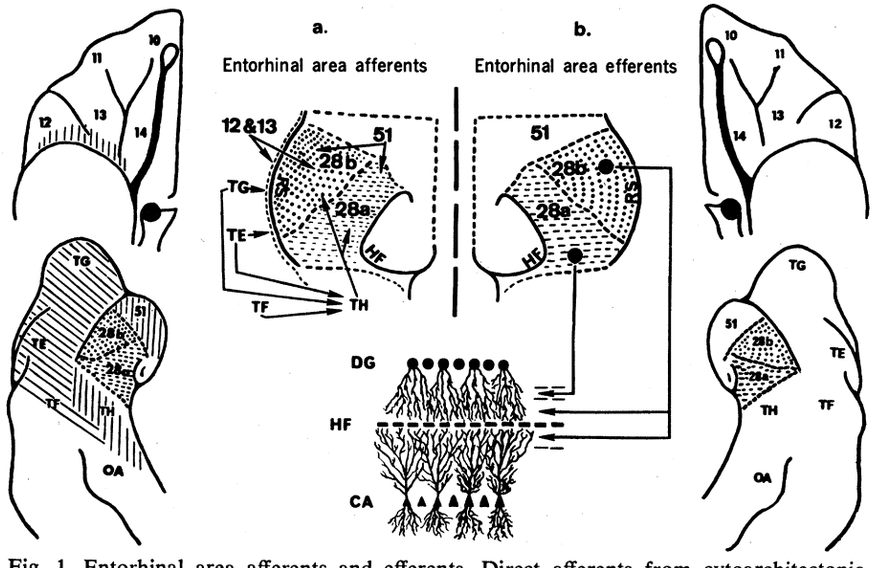

Fig. 1. Entorhinal area afferents and efferents. Direct afferents from cytoarchitectonic regions of the ventral surfaces of the frontal and temporal lobes to the entorhinal cortex (areas *28a* and *28b*) are depicted by hatching (a). Diagonal hatching indicates afferent sources that terminate in the depths of the rhinal sulcus (*RS*). Vertical hatching indicates afferent sources that terminate in more widespread entorhinal cortex subareas. Stippling of the entorhinal cortex on the ventral surfaces of the temporal lobe indicates that it is the only cortical area of the ventral frontal and temporal lobes which projects directly (b) to the hippocampus (*CA*) and dentate gyrus (*DG*). The medial subarea, *28a*, projects preferentially to the dentate gyrus. The lateral subarea, *28b*, projects more diffusely to dendritic regions on both sides of the hippocampal fissure (*HF*). In each case evidence of termination is restricted to a specific dendritic zone of the dentate gyrus granule cell and the hippocampal pyramidal cell. Other labels are *OA*, occipital neocortical area; *TE*, *TF*, *TG*, and *TH*, temporal neocortical areas; *10* to *14*, orbital frontal neocortical areas; and *51*, prepiriform cortex.

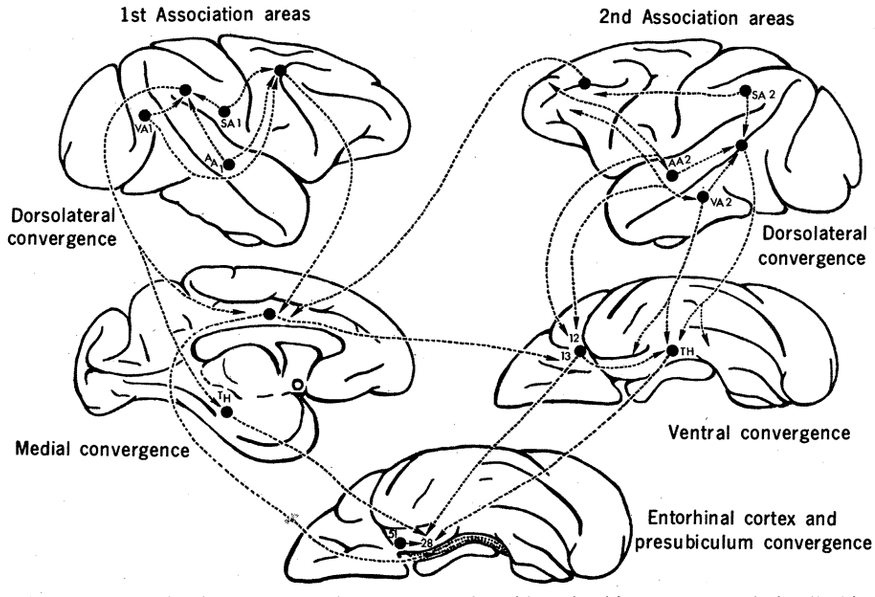

Fig. 2. The probable multisynaptic pathways via which the hippocampus of the limbic system has access to sensory information. These pathways are depicted on the dorsolateral, medial, and ventral views of the rhesus monkey brain. Sites of origin (circles) and termination (arrows) refer only to general cytoarchitectonic regions and not to specific points. Primary association areas are labeled *VA1*, the area for vision; *SA1*, that for somesthesis; and *AA1*, that for audition. Secondary association areas are labeled similarly, except that the digit is changed to 2. For other labels see legend to Fig. 1.

of the caudal portions of orbitofrontal areas 12 and 13 (Fig. 1a). Like the prepiriform area, these project to area 28b, and terminal degeneration appears to stop sharply at the junction with area 28a.

These observations in the monkey of direct cortical afferents to the entorhinal cortex are the first such evidence obtained by modern neuroanatomical research procedures. To reiterate, the ventral portions of the frontal and temporal lobes both contribute afferents to the entorhinal area. Area 28a receives afferents from area TH, sparse afferents from the prepiriform area, and no afferents from the orbitofrontal areas. In contrast, area 28b receives a heavy supply of afferents from the prepiriform and orbitofrontal areas. The lateral border of areas 28a and 28b also receives afferents from areas TE, TF, and TG, or virtually the entire rostroventral portion of the temporal lobe. These three temporal neocortical areas have an additional important connection with area TH, which as noted, is a major source of afferents to area 28a (*13–15*).

The differential distribution of afferents to the medial and lateral subareas of the entorhinal cortex led us to see whether these areas project differentially to the hippocampus and dentate gyrus. Our results indicate that in the monkey, area 28a projects preferentially to a restricted dendritic zone of the dentate gyrus. In contrast, area 28b projects more diffusely to the apical dendritic zone of both hippocampal pyramidal cells and dentate gyrus granule cells (Fig. 1b). These observations seem in good agreement with the results of Hjorth-Simonsen and Jeune (*16*) for the rat.

With the possible exception of the presubiculum, whose connections remain to be analyzed, the entorhinal area is a particularly significant source of cortical afferents to the hippocampus. Therefore, afferents reaching this region of the brain may be important in influencing the hippocampus and other subcortical structures to which it projects. When these specific neuroanatomical findings are considered along with those of intercortical connections from primary sensory and association areas on the convexity of the hemisphere (*14, 17*), a model of probable connections between sensory and limbic systems can be derived. The importance of such connections has been emphasized (*2, 3, 18*). These connections, along with other subcortical connections, are vital for the integration of the internal and external environments of the organism (*19*).

The primary sensory areas of the neocortex project to adjacent association areas. These can be grouped into two categories according to their afferent and efferent connections. First association areas from the primary sensory modalities of vision (area VA1), audition (area AA1), and somesthesis (area SA1) constitute one category, while second assocation areas for these senses—areas VA2, AA2, and SA2—constitute the other. Both types of association areas have projections that appear to be significant to the question of connections between the sensory and limbic systems. These relations are summarized in Fig. 2.

The first association areas project to multimodal convergence areas in the frontal and parietal lobes (*14, 17*). Both multimodal regions project in turn to the cingulate gyrus on the medial surface of the hemisphere, which contributes a heavy supply of afferents to the presubiculum (*20*). Also, the parietal convergence area projects to regions on the ventral surface of the temporal lobe. Thus, for projections from first association areas, the probable connections between sensory and limbic systems are relayed first in multimodal convergence areas and then in the cingulate gyrus and ventral temporal lobe before reaching the presubiculum and entorhinal cortex (Fig. 2).

In comparison, the projections from second association areas tend to terminate in the dorsolateral and orbitofrontal regions of the frontal lobe and the ventral regions of the temporal lobe (*14, 17*). These regions, likewise, are in close proximity to those having direct connections with the entorhinal cortex (Fig. 2).

These multisynaptic connections to the entorhinal area, along with those to this region from the olfactory system via the prepiriform area, indicate that the entorhinal cortex is a final cortical link between the sensory systems of the neocortex and the hippocampus and dentate gyrus of the limbic system. These pathways support the suggestion that multimodal information and not modality-specific information may be one of the relevant sources of input reaching the entorhinal cortex.

The hippocampus is a primordial brain area, which is found in all vertebrates from newer reptiles to man. In the course of evolution, the hippocampus shows no tendency to become vestigial; indeed, it reaches its greatest absolute size and elaboration in higher primates and man (*11*). Our results are consistent with these observatons, since some of the afferents we have described

are from cortical areas considered phylogenetically the most recent.

The ventromedial portions of the temporal lobe are thought to contain structures vital for the higher-order functions of memory and the acquisition of new learning in man (*1*, *21*). The striking memory deficits observed after damage to these regions of the brain are, perhaps, more understandable because the sensory information converging into these regions appears to be highly refined.

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Transfer Factor from Guinea Pigs Sensitive to Dinitrochlorobenzene: Absence of Superantigen Properties

Abstract. *Transfer factor from guinea pigs sensitive to dinitrochlorobenzene was not bound to an immunoadsorbent column that is specific for the dinitrophenyl determinant. The absence of the dinitrophenyl determinant on transfer factor suggested that the factor does not function as superantigen. The duration of the adoptive sensitivity, the small molecular weight, and the polypeptide or polynucleotide (or a combination) composition of the transfer factor are consistent with a derepressor function of the molecule.*

Early attempts to transfer delayed hypersensitivity with disrupted leukocytes suggested that a subcellular factor was involved (*1*). Since then, the passive transfer of delayed reactivity in man and in nonhuman primates with subcellular material (transfer factor) has been well established (*2*). Although transfers of delayed sensitivity with cell-free material in rodents have been inconsistent, methods have been described (*3*, *4*) for the collection, isolation, and testing of transfer factor from guinea pigs and rabbits sensitive to 2,4-dinitrochlorobenzene (DNCB). Essential steps for the collection of transfer factor from guinea pigs include (i) high donor-to-recipient ratios (at least 6 : 1); (ii) rapid collection of sensitive cells without their being washed; and (iii) incubation of cells at high concentrations [10^9 cells per 10 ml of Hanks balanced salt solution (HBSS)] at 37°C, without addition of serum or antigen. When these procedures are followed, incubation fluids, collected after 4 hours, were effective in transferring to other animals the contact sensitivity to DNCB (*4*).

Transfer factor has been described as a heat-sensitive, dialyzable, polypeptide or polynucleotide (or a combination) that is insensitive to deoxyribonuclease, ribonuclease, and trypsin (*5*, *6*). Accordingly, it has been suggested (*2*) that the specificity of transfer factor is imparted by either a superantigen or derepressor action. If transfer factor served as an immunogen, then transfer factor from animals sensitive to DNCB would carry the dinitrophenyl (DNP) determinant. Our studies (i) demonstrate that transfer factor from guinea pigs sensitized to DNCB does not bind to an immunoadsorbent that possesses covalently linked antibody to DNP, and (ii) describe additional physiochemical properties of transfer factor from guinea pigs. The results are interpreted as being consistent with a derepressor, rather than a superantigen, function for transfer factor.

Hartley guinea pigs were sensitized by six daily applications of 2 percent DNCB applied topically to a clipped area of the neck (*1*). Fourteen days after their initial treatment, the animals were skin tested with 1 percent DNCB, and were given 15 ml of mineral oil intra-abdominally. Lymph node and peritoneal exudative cells were collected (*3*, *4*) 48 hours later. Both the pooled lymph node cells and the pooled peritoneal exudative cells (each from 12 to 16 donors) were incubated separately for 4 hours in HBSS at 37°C, without addition of antigen (*4*). The incubation fluids were dialyzed for 18 hours, against 20 volumes of 0.9 percent saline, at 4°C. The dialyzate was concentrated by lyophilization and was applied to a Sephadex G-75 column (2.5 × 100 cm) equilibrated with 0.1*M* tris(hydroxymethyl)aminomethane (tris), *p*H 8; the effluent was monitored at an absorbance of 260 nm. Transfer factor was associated with a fraction eluted about 350 ml beyond the void volume of the column. Transfer factor activity was evaluated by contact skin tests of 1 percent DNCB in olive oil, which were applied