Hippocampal Efferents Reach Widespread Areas of Cerebral Cortex and Amygdala in the Rhesus Monkey

Abstract. *The subiculum of the primate hippocampal formation stands at the end of a polarized sequence of intrinsic hippocampal efferents and is the source of efferents to the medial frontal cortex, the caudal cingulate gyrus, and the parahippocampal area and amygdala in the temporal lobe. In addition, the subiculum sends subcortical efferents to the septum and diencephalon.*

In man, extensive damage to the hippocampal area produces severe memory deficits (*1*), while more localized damage such as Ammon's horn sclerosis, may be a causal factor in temporal lobe epilepsy (*2*). While details of these assertions remain open to debate, there is little doubt that the hippocampus and related structures of the parahippocampal area are involved in some aspects of both temporal lobe epilepsy and memory disorders. This conclusion leads to the often unspoken assumption that the hippocampus must be closely linked with the remainder of the primate cerebral cortex. Although anatomical pathways for cortical afferents to the primate hippocampus have recently been described (*3*), there has been no clear anatomical evidence for direct efferents from the hippocampus to the cerebral cortex; the major hippocampal outflow has been thought to travel in the fornix to subcortical areas. As a consequence, it has long been assumed that efferent influences of the hippocampus upon the cerebral cortex must be mediated by the fornix through the subcortical relays of the "Papez circuit" to the cingulate gyrus and from there by multisynaptic pathways to the rest of the cortex.

Nevertheless, this subcortical pathway fails to account for many of the data which suggest that the hippocampus has relatively direct efferent influences upon the cortex. For example, in man, localized hippocampal seizure activity can spread to temporal lobe neocortex directly rather than via subcortical relays in the Papez circuit (*4*). Similarly, electrical stimulation of the hippocampus in monkeys evokes movements of the face and mouth similar to certain automatisms of temporal lobe epilepsy. These evoked movements are not blocked by transection of the fornix, but are abolished if the hippocampus is surgically separated from the adjacent temporal lobe cortex (*5*). Finally, it is generally agreed that, in man, destruction of the subcortical hippocampal efferents by transection of the fornix often fails to reproduce the memory loss that would be expected if this were the principal route by which efferent outflow from the hippocampus reached the cortex (*6*).

Our studies in ten rhesus monkeys (*Macaca mulatta*) have shown that hippocampal efferents originate in the subiculum and travel directly, without subcortical relays, to cortical areas in the frontal and temporal lobes and cingulate gyrus, as well as to the amygdala. Injections of tritiated amino acids (*7*) were made into specific subfields of the hippocampal formation and autoradiographic procedures were used to detect the anterograde axoplasmic transport of the ^3H (*8*). To identify the cells of origin of some of these hippocampal efferents, we injected the cortex in three additional monkeys with 0.8 to 2.0 μl of a 20 percent solution of horseradish peroxidase (HRP). A histochemical procedure which produces a highly visible blue reaction product was used to detect the retrograde axoplasmic transport of the HRP (*9*).

The isotope injections involved all subfields of the hippocampal formation (*10*) at least once. Consistent with findings in rodents (*11*), injections in the ammonic subfields (CA1, CA2, CA3, and CA4) and the subicular subfields (prosubiculum, subiculum, and presubiculum) resulted in ^3H occurring over the septal nuclei, but only the subicular injections produced ^3H over the nucleus accumbens, the lateral dorsal and anterior thalamic nuclei, and mamillary bodies. In addition, after injections into the subicular subfields (Fig. 1A), ^3H was observed over a widespread area of the cerebral cortex (Fig. 1B).

In the frontal lobe, ^3H was observed throughout area FL of Bonin and Bailey (*12*), on the medial surface adjacent to the genu and rostrum of the corpus callosum, but not including the induseum griseum. The ^3H was also observed within the gyrus rectus and as far laterally as

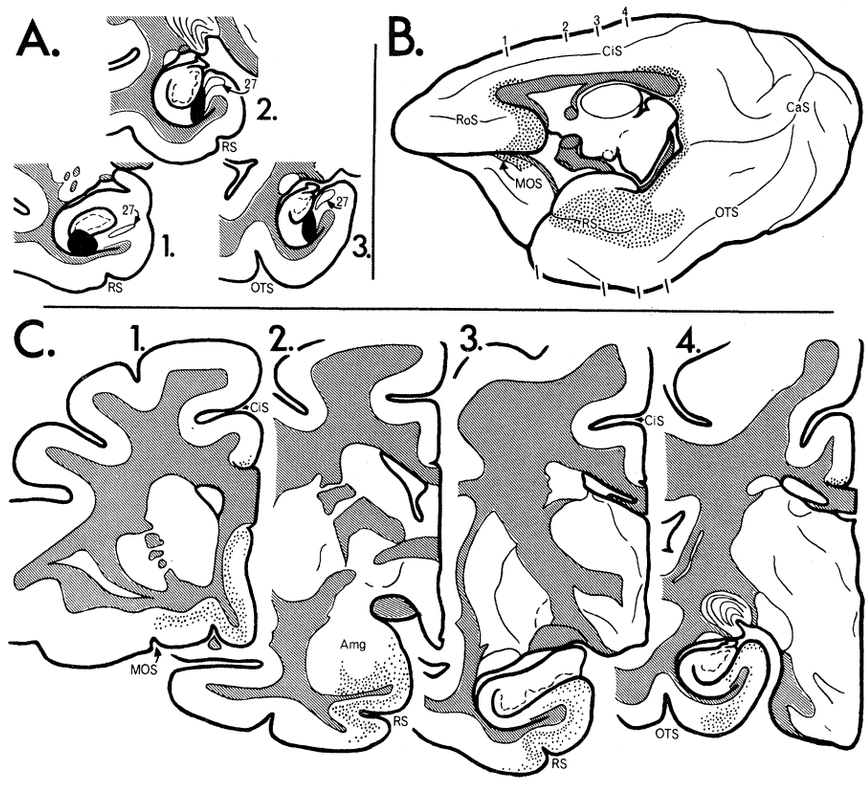

Fig. 1. (A) Three representative cross sections showing sites of isotope injections into the subicular fields at rostral, middle, and caudal levels of the hippocampal formation. (B) A ventromedial view of the monkey hemisphere showing the cortical areas in which ^3H was observed after ^3H-labeled amino acids had been injected into the subiculum. (C) The distribution of ^3H shown in cross sections at the four levels indicated in (B). Abbreviations: *Amg*, amygdala; *CaS*, calcarine sulcus; *CiS*, cingulate sulcus; *MOS*, medial orbital sulcus; *OTS*, occipital temporal sulcus; *RS*, rhinal sulcus; *RoS*, rostral sulcus; *27*, the presubiculum.

the depth of the medial orbital sulcus on the orbital surface of the frontal lobe (Fig. 1C, section 1). In two monkeys, injections of HRP were made into area FL and part of area 14. In both cases retrogradely labeled neurons were found only in the subiculum and prosubiculum within the hippocampal formation.

In the caudal cingulate gyrus, after injections of ^{3}H into the subiculum and presubiculum, the label was observed in the granular layer of retrosplenial cortex (Fig. 1C, section 4) but not in the induseum griseum. In one additional monkey, an HRP injection was made in the caudal cingulate gyrus including the rostral retrosplenial cortex, and retrogradely labeled neurons were found only in the subiculum within the hippocampal formation.

In the temporal lobe, the origin and termination of hippocampal efferents were more diverse. An isotope injection in the prosubiculum and adjacent part of CA1 resulted in ^{3}H throughout the deep pyramidal cell layer (V) of entorhinal cortex (area 28) and adjacent prorhinal cortex on the medial bank of the rhinal sulcus. Injections in the subiculum produced similar results but, in addition, ^{3}H was observed in perirhinal cortex (area 35) on the lateral bank of the rhinal sulcus as well as an adjacent part of the inferotemporal neocortex (Fig. 1C, sections 2 and 3). When an injection also involved the presubiculum, ^{3}H was also found in the superficial pyramidal cell layer (III) of entorhinal cortex. In the caudal parahippocampal area, behind the rhinal sulcus and entorhinal cortex, subicular injections produced ^{3}H over a distinct neocortical area (Fig. 1C, section 4) which may constitute either a caudal extension of perirhinal cortex (area 35) or a subdivision of area TF of Bonin and Bailey (*12*). Finally, following all of these subicular injections, transported ^{3}H was observed within the mediobasal amygdaloid nucleus (Fig. 1C, section 2).

That cortical efferents from the hippocampal formation originate in the subicular subfields gains additional importance from the pattern of intrinsic hippocampal efferents we observed. These seem to constitute a directionally polarized, sequential pathway whereby input to the hippocampus passes successively through the dentate gyrus and ammonic pyramidal subfields to the subiculum where the majority of all hippocampal efferents, both cortical and subcortical, now appear to originate. As previously demonstrated in the monkey, cortical afferents to the dentate gyrus, the ammonic pyramidal cells, and the prosubiculum originate in the adjacent entorhinal and prorhinal areas and terminate throughout the molecular layer of these fields (Fig. 2A) (*13*). After a lesion was made in the dentate granule cells, degeneration was observed within CA4 and among the proximal dendrite of CA3 (Fig. 2B).

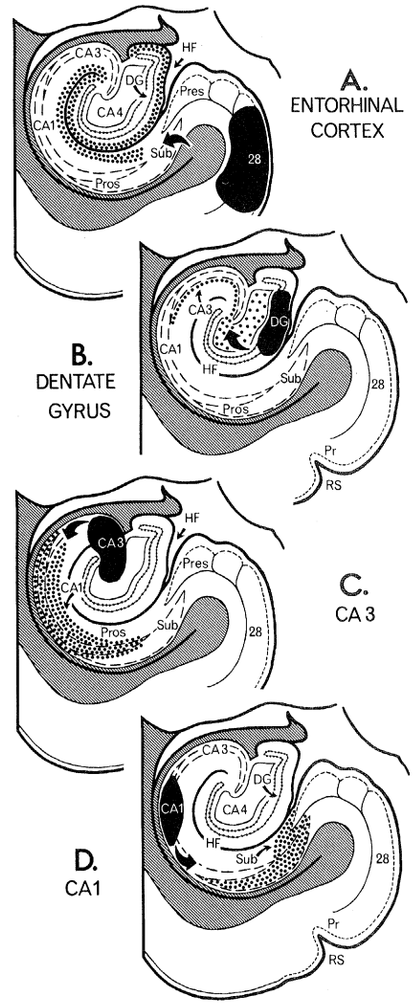

Fig. 2. The pattern of intrinsic hippocampal efferents illustrated at an intermediate level of the monkey hippocampal formation. (A) The pattern of afferent input from the entorhinal cortex (area *28*) to the molecular layer of the dentate gyrus (*DG*) and the CA3, CA2, and CA1 subfields (CA2 corresponds to the region where the large ammonic pyramids of CA3 overlap the smaller CA1 pyramids) as demonstrated by anterograde silver degeneration methods (*13*). The afferent input to the prosubiculum (*Pros*) originates from the prorhinal (*Pr*) cortex [see (B)] in the medial bank of the rhinal sulcus (*13*). (B) The pattern of fiber and terminal degeneration observed in CA4, CA3, and CA2 after a small lesion was made in the dentate gyrus. (C) The pattern of transported ^{3}H observed over CA1 and the prosubiculum after injection of ^{3}H-labeled amino acids into CA3 and CA4. (D) The ^{3}H observed in the subiculum (*Sub*) after an injection of ^{3}H-labeled amino acids into CA1. Abbreviations: *HF*, hippocampal fissure; *Pres*, presubiculum; *RS*, rhinal sulcus.

When the latter region was injected with ^{3}H, the label was observed throughout the proximal dendritic zone and pyramidal cell layer of CA1 and the adjacent prosubiculum (Fig. 2C). Finally, after an injection in CA1, ^{3}H was densely distributed throughout the deeper layers of the prosubiculum and in the entire pyramidal cell layer and molecular layer of the subiculum (Fig. 2D). In the last two cases, except for occurring within these intrinsic pathways, the ^{3}H was confined to the septal nuclei, and this extrinsic subcortical projection was less dense and less extensive than the intrinsic projections. Since the intrinsic pathways are excitatory (*14*), the subiculum may be the final recipient of the extensively processed output of the dentate gyrus and hippocampus. Hence, it is of considerable interest that in the monkey the subiculum is the sole source of direct efferents from the hippocampal formation to the cerebral cortex as well as the source of subcortical fornix efferents which reach beyond the septal area to the diencephalon. While the often prescient observations of Cajal (*15*) suggested that subicular efferents entered the fornix and contributed to subcortical hippocampal projections, he was unable to discern the cortical projections that we have described.

While the functional implications of these direct connections from the subiculum to the cortex are manifold, a number of points deserve mention. First, these connections provide a plausible anatomical explanation for some of the paradoxical clinical and experimental observations cited above. For example, seizures originating within the hippocampus could spread to temporal lobe cortex either directly from the subiculum or by way of subicular efferents to the amygdala. Furthermore, the lack of human memory disorders following transection of the fornix may be indicative of the relative importance in memory disorders of these direct cortical connections. In this regard, comparative neuroanatomical studies have reported that the hippocampal formation in primates, despite its "early" phylogenetic origins, is a "progressive" structure which increases in size in higher primates and reaches its greatest size in man (*16*). Moreover, within the hippocampal formation, the subicular and adjacent CA1 subfields appear to be responsible for this expansion, and the other ammonic subfields and the dentate gyrus are relatively decreased (*16*). This is of particular interest, since it suggests that in man the expansion of the subicu-

lum parallels the expansion of the cerebral cortex, and that even more widespread hippocampal efferent connections with the cerebral cortex may exist.

In the rhesus monkey the cortical areas that receive direct hippocampal efferents in turn send efferents to nearby association areas which appear to be either "polysensory" or many synapses removed from primary sensory cortex. Thus the medial and orbital frontal cortices send efferents to dorsolateral prefrontal association cortex. Retrosplenial cortex sends efferents to the inferior parietal lobule, recently shown to be generalized association cortex (*17*). Both the prefrontal and parietal association areas in the monkey may correspond to similar regions in the human where damage produces severe deficits in attention (*17*). Similarly, the perirhinal cortex, the caudal parahippocampal area, and the amygdala send efferents to large parts of the frontal and temporal lobe association cortex, where experimental lesions in monkeys produce deficits in complex discrimination learning and social behavior. If the subiculum is, as we suggest, a final common pathway for hippocampal efferents to the cerebral cortex of the monkey, then it is quite possible that in both the rhesus monkey and man, the hippocampus is only one synapse removed from specific areas of the cerebral cortex and only two synapses removed from important areas of association cortex.

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7. Typical injections contained equal part of tritiated leucine, proline, lysine, and occasionally a commercial amino acid mixture (composition similar to algal protein hydrolyzate). The injections ranged in size from 10 to 50 μc of ^{3}H in 0.2 to 1.5 μl of saline.
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10. As used in this report, the term hippocampal formation includes the following structures: the dentate gyrus (fascia dentata); the hippocampus (cornu Ammonis) including the four ammonic subfields, CA1, CA2, CA3, and CA4, and the subicular subfields, the prosubiculum, subiculum, and presubiculum.
11. L. W. Swanson and W. M. Cowan, *J. Comp. Neurol.* **172**, 49 (1977). In addition to subcortical efferents, these investigators report efferents from the subiculum to the anterior limbic cortex and the perirhinal cortex in the rat. These last two projections undoubtedly correspond, at least in part, to some of the hippocampal efferents we have observed in the monkey. However, Swanson and Cowan also report that, in the rat, efferents from the CA3 subfield also reach the cortex, but we have found no evidence of this in the monkey after our CA3 injections.
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18. We thank K. Barry and E. Kotopoulis for technical assistance and N. Geschwind, M.-M. Mesulam, D. N. Pandya, and B. A. Vogt for reviewing the manuscript. Supported by NIH grant NS-09211 and the Benevolent Foundation of Scottish Rite Freemasonry, Northern Jurisdiction.

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Premotor Cortical Ablations in Monkeys: Contralateral Changes in Visually Guided Reaching Behavior

Abstract. *In rhesus monkeys (*Macaca mulatta*), ablation of the premotor and supplementary motor areas and the adjoining rostral half of the precentral gyrus impairs the capacity of the contralateral arm to reach around a transparent obstacle to a visible food reward, and results in a tendency of this arm to reach straight to where the food is visible. This may reflect a disinhibition of brainstem pathways which steer the arm and hand straight to a visual target.*

In a previous study in rhesus monkeys (*1*), a unilateral posterior parietal leucotomy combined with a commissurotomy leaving the optic chiasm intact produced a defect in visually guided relatively independent movements of the contralateral hand and fingers (*1*). In addition, when food was held outside of the cage in front of these animals, they quickly learned to reach with the intact arm—that is, contralateral to the intact hemisphere, through a hole low in the cage front, while with the affected arm contralateral to the leucotomy they generally tried to reach straight for the food by squeezing the hand and arm through the spaces between the cage bars (*1*). Since the intrahemispheric occipital and posterior parietal fibers to the frontal lobe transected by the leucotomy terminate mainly in the arcuate gyrus, the premotor and supplementary motor areas, and the adjoining rostral part of the precentral gyrus (*2*), it was expected that the same defects as caused by the leucotomy would be produced by an ablation of these frontal areas. The present report demonstrates that in keeping with this expectation, ablation of these frontal areas impairs the capacity of the animals to reach around an obstacle with the contralateral arm in order to obtain a visible food reward, and results in a tendency of this arm to reach straight to where the food is visible.

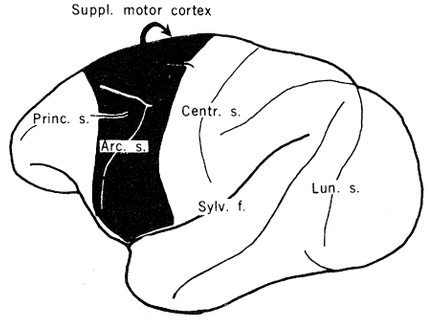

Fig. 1. Extent of the "premotor" cortical ablation, based on sketches made during surgery. Abbrevations: *Suppl. motor cortex,* supplementary motor cortex; *Princ. s.*, principal sulcus; *Arc. s.*, arcuate sulcus; *Centr. s.*, central sulcus; *Sylv. f.*, sylvian fissure; and *Lun. s.*, lunate sulcus.

The visually guided reaching behavior with the arm, as well as the visually guided relatively independent movements of the hand and fingers (*3*), were studied in three rhesus monkeys (*Macaca mulatta*). In these monkeys a unilateral "premotor" ablation was made involving the arcuate gyrus, the premotor and supplementary motor areas, and the adjoining rostral part of the precentral gyrus (Fig. 1); more than 6 months later a commissurotomy was performed without sectioning the optic chiasm.

Two to three weeks after the "premotor" ablation, the reaching behavior was tested as follows. The cage front was replaced by a transparent plastic plate with a hole in the middle. From trial to trial slices of apple were stuck on