19. Expression of the founder cell marker gene, *vestigial*, can be detected extending dorsally into the ectopic muscles forming in the region of the heart (M. K. Baylies and M. Bate, unpublished results).
20. The temperature sensitivity of the *twist* allelic combination $twist^{v50}/twist^{ry50}$ has been described (*29*) [M. Leptin, J. Casal, B. Grunewald, R. Reuter, *Development 1992 Supplement* 23 (1992)]. At 18°C, embryos carrying this allelic combination survived until adulthood, whereas at 29°C, these embryos died during embryogenesis. We have sequenced each allele (K. Lewis and M. K. Baylies, unpublished results), and the changes are as follows: for $twist^{v50}$, $Pro^{22}$ (Ccc) to $Ala^{22}$ (Gcc); for $twist^{ry50}$, $Gln^{251}$ (cAg) to $Leu^{251}$ (cTg).
21. Reduction of Twist activity does not necessarily lead to the ectopic induction of visceral or cardiac mesodermal markers. Thus, whereas larger amounts of Twist inhibit visceral and cardiac mesoderm formation, reduced amounts of Twist may not in themselves be sufficient to initiate the formation of visceral or cardiac muscle. We suggest that there are additional factors that must be present if visceral or cardiac mesoderm is to form (*27, 33*).
22. The *daughterless-GAL4* line [A. Wodarz, U. Hinz, M. Engelbert, E. Knust, *Cell* **82**, 67 (1995)] expresses Gal4 ubiquitously, starting at cellularization and continuing throughout the remainder of embryogenesis. Ectopic Twist expression under the control of *daughterless GAL4* prevents ectodermal differentiation and eliminates the ectodermally derived cuticle. In situ hybridizations were performed with a *Distal-less (Dll)* cDNA fragment (*32*). *Dll* is expressed solely in ectoderm and in ectodermally derived tissues [S. M. Cohen, *Nature* **343**, 173 (1990)]. To assay for the epidermis and nervous system, we made cuticle preps of, and stained with horseradish peroxidase antibody, respectively, the experimental embryos (*32*). In addition, no cell death, as assayed by acridine orange incorporation [K. White *et al.*, *Science* **264**, 677 (1994)], was found in early embryos, which excludes the possibility that ectopic Twist causes ectodermal cell death.
23. M. K. Baylies and M. Bate, unpublished results.
24. Intrinsic and extrinsic influences upon the mesoderm (*33*) include the following: *even-skipped/fushi tarazu* (M. Bate, E. Rushton, M. K. Baylies, in preparation), *wingless* (*32*), and *decapentaplegic* (*27*) [M. Frasch, *Nature* **374**, 464 (1995)].
25. Homologs of *twist* include the following: Xtwist [N. D. Hopwood, A. Pluck, J. B. Gurdon, *Cell* **59**, 893 (1989)]; Mtwist [C. Wolf *et al.*, *Dev. Biol.* **143**, 363 (1991)]; Meso-1 [M. A. Blanar *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 5870 (1995)]; and bHLH-EC40 [E. E. Quertermous, H. Hidai, M. A. Blanar, T. Quertermous, *ibid.* **91**, 7066 (1994)].
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34. We thank M. Ruiz-Gomez, A. Martinez-Arias, E. Rushton, H. Skaer, and M. Taylor for critical reading of the manuscript. The anti-myosin was provided by D. Kiehart; the anti-fasciclin III, by N. Brown; the anti-Zfh1, by G. Rubin; the anti-Twist, by M. Leptin; and the full-length *twist* cDNA, by N. Brown. The *daughterless-GAL4* line was kindly provided by E. Knust. Sequencing was courtesy of the Wellcome Trust Facility, grant number 17424. Special thanks to A. Prokop and N. Brown for advice and help with the figures. This work was supported by a NATO Postdoctoral Fellowship to M.K.B. and a Wellcome Trust grant to M.B.

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# Structural Evidence for Functional Domains in the Rat Hippocampus

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**The hippocampus has two major outputs: multisynaptic pathways to the cerebral cortex and a massive descending projection directly to the lateral septal part of the basal ganglia. Here it is shown that the descending output is organized in such a way that different hippocampal regions map in an orderly way onto hypothalamic systems mediating the expression of different classes of goal-oriented behavior. This mapping is characterized by a unidirectional hippocampo-lateral septal projection and then by bidirectional lateral septo-hypothalamic projections, all topographically organized. The connectional evidence predicts that information processing in different regions of the hippocampus selectively influences the expression of different classes of behavior.**

Multimodal information processing in the hippocampus (Ammon's horn) is important for memory formation (*1*), and the results of this processing are sent to two major forebrain locations (*2*). One projection reaches broad parts of the cerebral cortex via the parahippocampus and apparently influences the long-term storage of memories. The other projection is topographically organized and descends through the precommissural fornix to the lateral septal nucleus (LS). It was shown several years ago (*3*) that the better known postcommissural fornix projection to the mammillary body arises not in the hippocampus proper but rather in the adjacent subicular complex of the parahippocampus. The present work was designed to clarify the possible functional significance of the descending output of the hippocampus, which remains enigmatic, by reexamining the structural organization of input and output pathways of the LS.

First, we carefully examined the spatial distribution of several neurotransmitter-related mRNAs. LS neurons are known to express the neuropeptides enkephalin (*4*) and somatostatin (*5*), as well as glutamic acid decarboxylase (GAD) (*6*), the enzyme responsible for synthesizing the neurotransmitter γ-aminobutyric acid (GABA). Serial section in situ hybridization analysis through the rostrocaudal extent of the LS, with adjacent sections labeled for the expression of enkephalin and somatostatin mRNA, reveals that somatostatin hybridization is concentrated dorsocaudally, whereas enkephalin hybridization is concentrated rostroventrally (Fig. 1A), although the two cell types are not segregated completely. In contrast, GAD is expressed abundantly throughout the LS, including a distinct ventromedial part with very little enkephalin or somatostatin mRNA expression. This evidence suggested that the LS may be divided into rostral (LSr), caudal (LSc), and ventral (LSv) parts, respectively.

To test this hypothesis, we examined neural inputs and outputs of the three parts with anterograde and retrograde tracing methods. First, we made 22 simultaneous injections of the anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHAL) and the retrograde tracer fluorogold (FG) into various parts of the LS and confirmed the results with 13 single PHAL and 4 single FG injections (in 39 animals in all). Second, we reexamined a collection of more than 100 anterograde autoradiographic experiments that were done with $^3$H–amino acid injections throughout the hippocampal formation and used in previous work (*3, 7, 8*).

The double injections were used to plot LS connections with the hypothalamus. PHAL injections in the LSc labeled a dense pathway to and through the far lateral hypothalamus that terminates densely in the lateral supramammillary nucleus (Fig. 1, B and D), and accompanying FG injections retrogradely labeled neurons in the same two hypothalamic regions. Thus, the LSc establishes bidirectional connections with the far lateral hypothalamus and lateral supramammillary nucleus. In contrast, PHAL injections in the LSr labeled major inputs to the medial preoptic nucleus (lateral part), anterior hypothalamic nucleus and adjacent perifornical region of the lateral hypothalamic area, ventrolateral ventromedial nucleus and adjacent tuberal nucleus, and posterior hypothalamic nucleus (Fig.

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1D); again, FG-labeled neurons were seen in all of these areas, indicating bidirectional connections. Finally, one PHAL injection was confined almost entirely to the tiny LSv, and in this experiment, labeled terminals were concentrated in the medial part of the medial preoptic nucleus, periventricular zone, ventral premammillary nucleus, and caudal perifornical region of the lateral hypothalamic area. Previous work showed that the medial preoptic and ventral premammillary nuclei project massively to the LSv (9).

Hippocampal retrograde labeling after FG injections in the LSc was concentrated in field CA3, whereas after LSr injections it was concentrated in field CA1 and the subiculum, and after LSv injections it was concentrated in the ventral tip of the CA1-subiculum field (Fig. 1D). The autoradiographic experiments confirmed this organization of hippocampal projections and revealed further details (*10*). A dorsoventral gradient in projections from the hippocampus to the LS (Fig. 1C, dashed yellow lines) is known (*7*), and the present reanalysis showed that the dorsal half of the hippocampus projects to a very small dorsal part of the LS, whereas progressively more ventral parts of the hippocampus innervate progressively larger parts of the LS (Fig. 1C; yellow lines a, b, and c).

These results suggest that the hippocampus may be divided into three broad domains by virtue of topographically organized descending projections to three parts of the LS, and evidence discussed below indicates that the CA1-subiculum domain may be further subdivided. The CA3 field domain projects selectively and bilaterally to the somatostatin-GABA–rich LSc, which in turn projects massively to the lateral supramammillary nucleus. The latter projects directly back to the dentate gyrus and field CA3 (*11*), which is important because the hippocampus does not participate in the classic Papez circuit

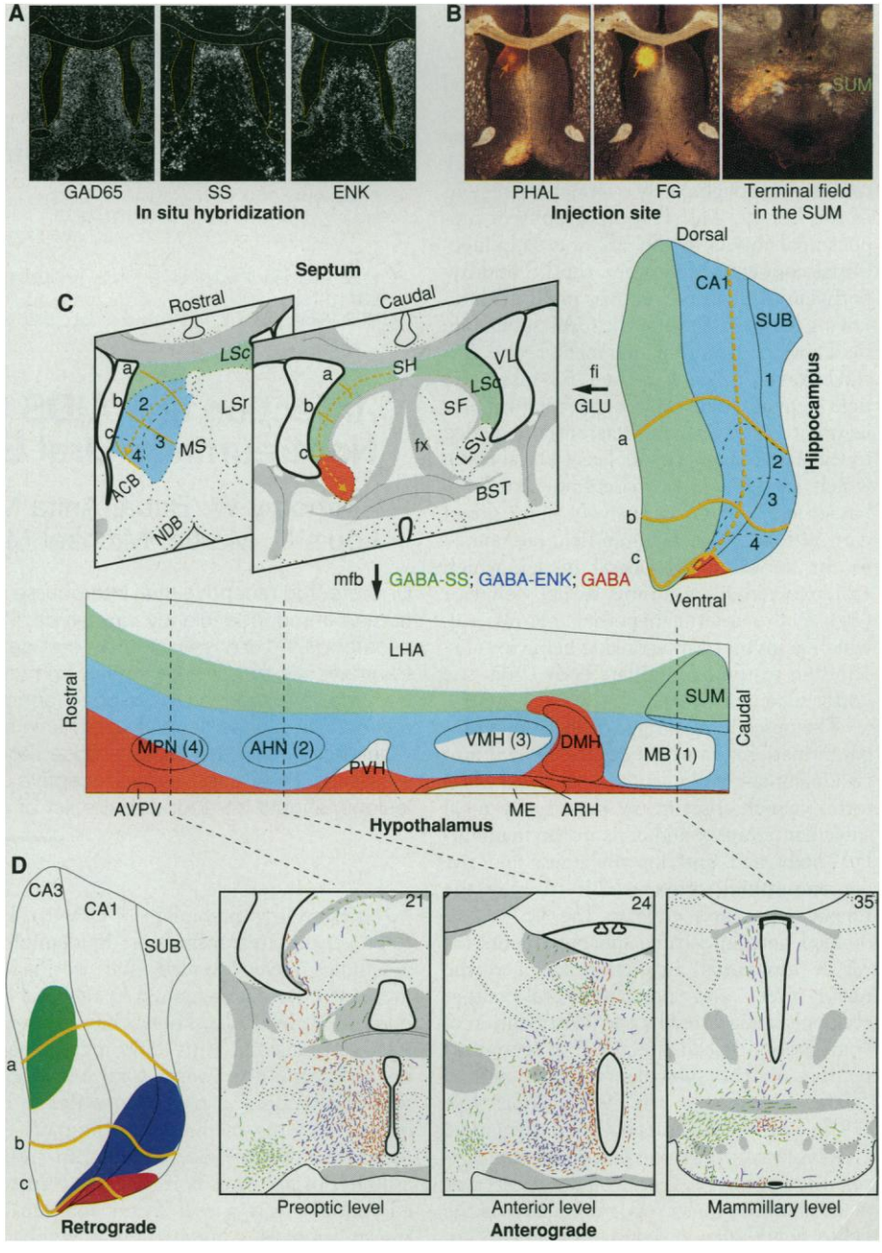


**Fig 1.** Chemoarchitecture and connections of the LS with the hippocampus and hypothalamus. (**A**) Pattern of in situ hybridization with probes to GAD, somatostatin (SS), and enkephalin (ENK) mRNA in adjacent frontal sections through mid-rostrocaudal levels of the LS. Major anatomical features are outlined in red. (**B**) Appearance of a double PHAL (left panel) and FG (middle panel) injection site (arrows) in adjacent frontal sections through the left LS, and the distribution of a terminal field (bright gold fibers) in the supramammillary nucleus (SUM, right panel). (**C**) Schematic organization of left hippocampal projections through the fimbria (fi, at arrow) to the LS, and of left LS projections through the medial forebrain bundle (mfb, at arrow) to the left hypothalamus. Dashed yellow arrows indicate the longitudinal (dorsoventral) axis of the hippocampus and LS; whereas solid yellow lines a, b, and c indicate three transverse levels through both structures. The graticule formed by these yellow lines indicates the topological organization of the hippocampo-septal projection, and numbers 1 through 4 indicate preferential connections with particular hypothalamic medial zone nuclei. Domain 1 (dorsal subiculum, SUB) projects directly through the postcommissural fornix to the cellular core of the mammillary body (*7*). (**D**) Results of double PHAL-FG injections restricted to the LSc (green) and LSr (blue) and of separate PHAL or FG injections centered in the LSv (red). The distribution of pyramidal cells retrogradely labeled with FG in the left hippocampus is shown on the left, and the distribution of axons anterogradely labeled with PHAL at three levels of the hypothalamus is shown in the three panels on the right. In situ hybridization was carried out exactly as described previously (*22*) (the GAD65 probe was 400 base pairs (bp) long, the SS probe was 520 bp, and the ENK probe was 970 bp; all were used at $10^7$ cpm/ml). The double PHAL-FG injection method was also carried out exactly as described (*23*). The unfolded hippocampal map used in (C) and (D) was constructed as described (*8*). The hypothalamic frontal sections in (D) are from levels 21, 24, and 35 of Swanson (*24*). Abbreviations: ACB, nucleus accumbens; AHN, anterior hypothalamic nucleus–anterior perifornical region; ARH, arcuate nucleus; AVPV, anteroventral periventricular nucleus; BST, bed nuclei stria terminalis; DMH, dorsomedial nucleus; fx, fornix; Glu, glutamate; LHA, lateral hypothalamic area; MB, mammillary body; ME, median eminence; MPN, medial preoptic nucleus; MS, medial septal nucleus; NDB, nucleus diagonal band; PVH, paraventricular nucleus; SF, septofimbrial nucleus; SH, septohippocampal nucleus; VL, lateral ventricle; and VMH, ventromedial-tuberal nuclei.

interrelating the subicular complex, mammillary body, and anterior thalamus (*2*, *7*). A second hippocampal domain includes most of the CA1-subiculum field, which projects selectively and unilaterally to the enkephalin-GABA–rich LSr, which in turn projects to hypothalamic medial zone nuclei known to be involved in the expression of social behavior (agonistic and reproductive). And a third domain is restricted to the ventral tip of the CA1-subiculum field, which projects selectively and unilaterally to the GABA-estrogen receptor–rich (*12*) LSv, which in turn projects to the medial part of the medial preoptic nucleus and hypothalamic periventricular zone as a whole, which is known to preferentially influence ingestive behavior specifically as well as endocrine-autonomic responses that are associated with all types of behavior (*13*).

Evidence for subdivision of the second domain comes primarily from PHAL studies demonstrating that hypothalamic medial zone nuclei receiving LSr inputs project back to distinct though partly overlapping regions of the LSr (*9*, *14*). The topography of hippocampal inputs to the LS, and of bidirectional connections between the LS and hypothalamus described above, imply the following relation between the CA1-subiculum field and the hypothalamic medial zone. Via the LSr, ventral parts of the CA1-subiculum field (leaving aside the most ventral tip projecting to the LSv) are preferentially related to the medial preoptic nucleus (lateral part), which is involved in masculine sexual behavior (*15*); and progressively more dorsal parts of the CA1-subiculum field are related to the ventromedial-tuberal nuclei, which are involved in feminine sexual behavior (*16*); to the anterior hypothalamic nucleus, which is involved in agonistic behavior (*17*, *18*); and to the mammillary body (from the dorsal subiculum) (*7*).

The descending output of the hippocampal formation as a whole can be divided into two major components: the postcommissural fornix, which arises in the parahippocampal subicular complex and ends in the mammillary body and anterior thalamus; and the precommissural fornix, which arises in the hippocampus and ends in the LS and is divided here into three subcomponents (*3*, *7*). A functional distinction between the major post- and precommissural output channels is suggested by the identification of "navigation," "head direction," or "compass" neurons in the subicular complex and of "place" neurons in the hippocampus [see (*19*)]. Because all hippocampal pyramidal cells project through the precommissural fornix to the LS (*20*), it would appear that place neurons project topographically via the LS to hypothalamic systems thought to coordinate somatic (behavioral), endocrine, and autonomic responses associated with specific classes of motivated behavior. Cajal (*21*) pointed out that the LS is a medial part of the striatum (in the basal ganglia) innervated by hippocampal cortex, and it will now be important to determine the extent to which motor functions of the circuitry outlined here are similar in principle to those being clarified for the adjacent nucleus accumbens (ventral striatum) and caudoputamen (dorsal striatum).

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# Infection and AIDS in Adult Macaques After Nontraumatic Oral Exposure to Cell-Free SIV


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**Unprotected receptive anal intercourse is a well-recognized risk factor for infection with human immunodeficiency virus–type 1 (HIV-1). Isolated human case reports have implicated HIV-1 transmission by oral-genital exposure. Adult macaques exposed nontraumatically to cell-free simian immunodeficiency virus (SIV) through the oral route became infected and developed acquired immunodeficiency syndrome (AIDS). The minimal virus dose needed to achieve systemic infection after oral exposure was 6000 times lower than the minimal dose required to achieve systemic infection after rectal exposure. Thus, unprotected receptive oral intercourse, even in the absence of mucosal lesions, should be added to the list of risk behaviors for HIV-1 transmission.**


Understanding the biology of HIV-1 is key to preventing its transmission. Epidemiologic studies have revealed that worldwide, most infections are acquired by mucosal exposure (*1*). In adults, known risk behaviors involving mucosal virus entry include vaginal and rectal intercourse. Oral infection is well documented in neonates, who can acquire the virus by breast feeding (*2*, *3*). Moreover, the presence of blood in gastric aspirates of neonates born to HIV-1–infected mothers was a risk factor for vertical transmission (*4*). These findings implicate the neonatal alimentary tract as a portal of virus entry during birth. Although some case reports have described seroconversion in adults after oral-genital sex only (*5*), a quantitative assessment of the relative risk of oral infection in adults has not been possible, as the route of virus entry is difficult to assess because of recall bias and problems inherent in face-to-face interviews.

Infection of rhesus monkeys (*Macaca mulatta*) with the SIV has proven to be the best system to model human HIV-1 transmission, viremia, and disease (*6*). HIV-1 and SIV are closely related: They share genome structure (*7*), modes of transmis-