Published in final edited form as:

J Comp Neurol. 2006 July 1; 497(1): 101–114. doi:10.1002/cne.20985.

An Analysis of Direct Hippocampal Cortical Field CA1 Axonal Projections to Diencephalon in the Rat

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Abstract

The hippocampal formation is generally considered essential for processing episodic memory. However, the structural organization of hippocampal afferent and efferent axonal connections is still not completely understood, although such information is critical to support functional hypotheses. The full extent of axonal projections from field CA1 to the interbrain (diencephalon) is analyzed here with the *Phaseolus vulgaris*-leucoagglutinin (PHAL) method. The ventral pole of field CA1 establishes direct pathways to, and terminal fields within, the anterior hypothalamic nucleus, ventromedial hypothalamic nucleus, lateral hypothalamic and lateral preoptic areas, medial preoptic area, and certain other hypothalamic regions, as well as particular midline thalamic nuclei. These results suggest that hippocampal field CA1 modulates motivated or goal-directed behaviors, and physiological responses, associated with the targeted hypothalamic neuron populations.

Indexing terms

hypothalamus; lateral hypothalamic area; subiculum, thalamus

Introduction

Field CA1 of Ammon's horn is an integral component of the hippocampal formation (as defined by Blackstad, 1956; see Swanson et al, 1987), and it displays unique structural, physiological, and molecular features among the various hippocampal cortical areas (Zhao et al., 2001). Although the fundamental organization of intrahippocampal circuitry has been relatively well known for some time (Blackstad, 1956; Anderson et al., 1971), the scope and depth of extrahippocampal projections are not yet fully appreciated. With the autoradiographic method Swanson and Cowan (1975, 1977a,b) established that the century old notion of Ammon's horn (fields CA1-CA3) as the source mammillary body inputs via the descending column of the fornix is incorrect—they arise in the subicular complex—and confirmed earlier reports of field CA1 axonal projections directly to the subiculum, entorhinal area, and lateral septal nucleus (Cajal, 1909-1911; Lorente de Nó, 1934; Blackstad, 1956; Nauta, 1956, 1958; Raisman et al., 1966; Hjorth-Simonsen, 1973; Meibach and Siegel, 1977). They also demonstrated that the general projection pattern of field CA1 is not homogenous throughout. Instead, there is a differential, topographic origin of specific

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Associate Editor: Paul Sawchenko

projections along field CA1's longitudinal axis, commonly referred to as the dorsoventral or septotemporal axis. Further evidence for this connectional heterogeneity was provided by the discovery of a ventral field CA1 axonal projection to rostroventral regions of the medial prefrontal cortex using anterograde and retrograde tracers (Swanson, 1981).

Thus, through the 1980s known outputs of field CA1 could be divided into two major classes, one descending through the fimbria and precommissural fornix directly to the lateral septal nucleus, and the other consisting of association projections to other cortical areas—directly to the subiculum and entorhinal area of the hippocampal formation, and to the medial prefrontal cortex; and indirectly via multisynaptic pathways to broad regions of the temporal lobe, starting with “relays” in parahippocampal regions of the temporal lobe adjacent to the hippocampal formation (see Swanson et al., 1987). This view was expanded in the first neuroanatomical study devoted exclusively to field CA1 projections (van Groen and Wyss, 1990a). Using PHAL, they described field CA1 projections directly to the subiculum, postsubiculum, parasubiculum, and entorhinal area of the hippocampal formation; the retrosplenial area, perirhinal area, and medial prefrontal region of the cerebral cortex; the olfactory bulb and anterior olfactory nucleus; parts of the amygdala and hypothalamus; and even some cortical commissural projections.

The analysis presented here represents the first systematic reexamination of field CA1 projections since then, and focuses on interbrain targets. Inputs to the cerebral nuclei and cerebral cortex are presented in following papers. The pioneering study of van Groen and Wyss (1990a) has been improved in three ways: considerably smaller injection sites, many of which are clearly restricted entirely to field CA1 as they and we define it, were made; many more experiments, which together involve virtually all regions of field CA1, were prepared; and more comprehensive series of histological sections throughout the forebrain were collected for a higher resolution analysis. The results indicate that field CA1 extrinsic projections are even more extensive, and topographically organized, than recognized previously—and that some projections reported by van Groen and Wyss (1990a) arise in the two cortical areas on either side of field CA1—field CA3 and the subiculum.

Materials and Methods

The materials and methods were similar to those described previously (Gerfen and Sawchenko, 1984; Groenewegen and Wouterlood, 1990; Canteras and Swanson, 1992; Smith, 1992). Experiments were performed according to the NIH *Guidelines for the Care and Use of Laboratory Animals*, and all protocols were approved by the University of Southern California Institutional Animal Care and Use Committee. The experiments described here were chosen from a collection of over 60 PHAL injections throughout field CA1. Adult male Harlan Sprague-Dawley rats (250–325 g) received a single, stereotaxically placed iontophoretic injection of a 2.5% solution of PHAL (Vector Laboratories, Burlingame, CA), prepared in 0.01 M sodium phosphate-buffered saline (NaPBS), pH 7.4, into various regions of field CA1 through a glass micropipette (10–15 μ m tip diameter) by applying a positive current (5 μ A, 7 second on/off intervals) for 7–10 minutes. Animals were anesthetized for stereotaxic surgery with an equal mixture of ketamine and xylazine solutions (100 mg/ml ketamine and xylazine) given 1 ml/kg body weight, intramuscularly as needed.

After surviving 14–17 days, the rats were deeply anesthetized with pentobarbital (35–50 mg/kg body weight, intraperitoneally) and perfused transcardially with 150 ml of 0.9% NaCl followed by 400 ml of ice-cold 4% paraformaldehyde in 0.1 M borate buffer (pH 9.5). Brains were removed, postfixed overnight at 4°C in the same fixative containing 10% sucrose, and frozen. Then, serial 30 μ m-thick sections (1-in-4) were cut in the transverse

plane on a sliding microtome. One complete series of sections was processed to detect PHAL using the immunohistochemical procedure described elsewhere (Gerfen and Sawchenko, 1984). An adjacent series of sections was stained with thionin to determine quickly cytological boundaries. Brightfield and darkfield photomicrographs were taken with a CCD camera (Diagnostics Instruments, Sterling Heights, MI). Darkfield photomicrographs also were taken with a Wild Leitz 35 mm camera mounted on a Wild M3Z stereozoom microscope; film was scanned with a Nikon 35 mm Film Scanner (LS-1000) into Adobe PhotoShop 5, where standard methods were used to compose and adjust brightness and contrast on a Mac PowerPC G4. Parceling of the rat brain, terminology for describing morphologic features of PHAL-labeled axons, and mapping strategies and procedures follow Swanson (2004), unless indicated otherwise. More information on the new lateral hypothalamic area (LHA) parceling scheme was recently published (Swanson et al., 2005).

The overall spatial distribution of each injection site is plotted onto an unfolded map or “flatmap” of the hippocampal formation (Fig. 1). The unfolded map represents the surface of a solid model of the hippocampal formation that was reconstructed from a series of transverse sections (Swanson et al., 1978; Petrovich et al., 2001). A plot display of injection sites on the unfolded map allows rapid, qualitative analysis and comparison of injection site distribution.

Results

A major finding is the presence of PHAL-labeled axons in broad regions of the hypothalamus. These projections are labeled solely by injections that label neurons in the ventral pole of what has traditionally been regarded as field CA1 in the rat. Inputs to the hypothalamus are represented by an iontophoretic injection of PHAL (Experiment HIPPO103) confined to the ventral pole of field CA1, and centered next to the medial border with field CA2 (Figs. 1-3), away from the lateral border with the subiculum. Labeled hypothalamic projections are predominantly ipsilateral, with contralateral exceptions as noted.

From the injection site, PHAL-immunoreactive axons enter the alveus and extend rostrally through the fimbria, eventually to innervate mainly the hypothalamic lateral zone and medial nuclei. The most prominent group of labeled axons travels through the postcommissural column of the fornix in a fairly tight bundle, with fibers exiting at various levels to innervate particular hypothalamic cell groups (Fig. 3D-I). These fibers may represent collaterals of parent axons continuing farther caudally through the column of the fornix, although this cannot be stated unequivocally with the present method. Near the rostral pole of the thalamus, a small group of axons diverges from the postcommissural fornix to enter the medial corticohypothalamic tract and innervate regions near the third ventricle. The second most prominent group of labeled axons extends ventrally from the lateral septal nucleus—a major striatal target of field CA1 by way of the precommissural fornix—to enter the lateral preoptic area (Fig. 3A). These fibers appear to generate a terminal field in the lateral preoptic area, and to be part of a larger group that also innervates laterally adjacent regions of the substantia innominata.

Inputs through the column of the fornix

The lateral zone—The LHA receives the strongest field CA1 projection of any hypothalamic component. LHA medial regions receive a moderate projection of varicose fibers and terminal boutons from the rostral end of the LHA to about mid-rostrocaudal levels of the ventromedial nucleus, where the projection becomes considerably denser (Fig. 3D-G). Rostroventrally, a tight group of PHAL-labeled fibers is observed in the ventromedial corner of the LHA anterior region's ventral zone, and a few labeled fibers are also seen in the

intermediate zone (Fig. 3D-F). Rostr dorsally, a moderate plexus of varicose fibers with boutons occupies the LHA juxtaparaventricular region and an adjacent medial expanse of the anterior region's dorsal zone, dorsal to the anterior hypothalamic nucleus (Fig. 3E,F). At the level of the ventromedial nucleus, the terminal field becomes significantly denser (Fig. 3G). Here, the LHA's juxtadorsomedial region, and dorsal and ventral zones of the juxtaventricular region, all contain a moderately dense terminal field.

Innervation of the LHA's perifornical tier includes a moderate terminal field in the posterior zone, a light terminal field in the premammillary zone, and a few labeled axons in the anterior zone of the subfornical region (Fig. 3F-H).

Overall, field CA1 inputs tend to avoid LHA regions lateral to the fornix. However, it is worth noting that in the anterior group a few labeled axons extend ventrolaterally from the fornix to end with a small number of boutons in lateral expanses of the anterior region's ventral zone, ventral to the substantia innominata and medial to the medial amygdalar nucleus (Fig. 3E,F). Medially, there is also a light projection to the retrochiasmatic area, between the anterior nucleus and the optic tract and supraoptic commissures (Fig. 3E); in fact, some of these fibers are in the supraoptic commissures and cross the midline to innervate the contralateral retrochiasmatic area. At this same level, a small plexus of varicose fibers is also observed in the subparaventricular zone of the periventricular region. Caudal to the retrochiasmatic area, rostral regions of the tuberal nucleus's subventromedial part are moderately innervated, whereas the terminal field diminishes to just a few scattered fibers toward the caudal pole (Fig. 3F-G).

The medial nuclei—At anterior hypothalamic levels, labeled axons are dispersed in the anterior hypothalamic area ventral to the bed nuclei of the stria terminalis. The central part of the anterior hypothalamic nucleus is moderately innervated with varicose fibers and a small number of terminal boutons (Fig. 3E,F). A few axons enter the anterior and posterior parts of the nucleus, but none are observed in the dorsal part.

The anterior part of the ventromedial nucleus is moderately innervated, whereas the central part is lightly innervated, and only a few isolated fibers are observed in the ventrolateral part (Fig. 3G,H). In experiments with larger injection sites, significantly denser terminal fields in the anterior and central parts of the ventromedial nucleus are observed (Fig. 4). Interestingly, labeled fibers are never observed in the dorsomedial part of the ventromedial nucleus (or in the prominent, overlying dorsomedial nucleus of the periventricular region).

The field CA1 projection to hypothalamus ends with the column of the fornix in the mammillary body. The medial mammillary nucleus receives a light input that extends as a few axons across the midline to medial regions of the contralateral medial nucleus. A few labeled axons also extend into adjacent cell groups, including the ventral tuberomammillary, dorsal premammillary, and lateral supramammillary nuclei. Finally, ventral regions of the posterior hypothalamic nucleus receive a light input, and a few scattered axons are also observed in the contralateral posterior nucleus (Fig. 3H-F).

Inputs through the medial corticohypothalamic tract

In addition to its projection through the column of the fornix, field CA1 sends a tiny bundle of axons into the medial corticohypothalamic tract, a divergence that occurs just after the fornix descends caudal to the anterior commissure (Figs. 3D and 5). These axons appear to innervate lightly the hypothalamic periventricular region, and the rostral thalamus (see below). Because neurons in ventral regions of the adjacent subiculum are the only reported source of the medial corticohypothalamic tract (see Canteras and Swanson, 1992), this is indeed a new observation. On leaving the medial corticohypothalamic tract, a few labeled

axons traverse the anterior parvocellular and anterior magnocellular parts of the paraventricular hypothalamic nucleus (Fig. 3D). Axons in the former are virtually free of boutons and varicosities, whereas those in the latter appear to generate small numbers of varicosities, terminal boutons, and boutons of passage. Fibers continue rostroventrally from the paraventricular nucleus to innervate lightly lateral regions of the medial preoptic area (Fig. 3B,C). This pathway generates a light projection with terminal boutons to the anteroventral preoptic nucleus, and sends a few solitary fibers to the anterodorsal preoptic nucleus.

In some experiments, sparse labeling is observed in the lateral part of the medial preoptic nucleus, and in the anteroventral paraventricular nucleus. In addition, a few scattered, varicose fibers are observed in the lateral zone of the paraventricular nucleus's posterior magnocellular part. The latter fibers actually appear to arrive via the descending column of the fornix at the level of the anterior hypothalamic nucleus, rather than via the medial corticohypothalamic tract (Fig. 3E).

Inputs through the precommissural fornix

There is a relatively moderate projection in the lateral preoptic area, adjacent to fibers in the medial preoptic area (Fig. 3B,C). While many fibers in the lateral preoptic area generate terminal boutons and boutons of passage, roughly half of them continue laterally into the adjacent substantia innominata. It appears likely that this projection to and through the lateral preoptic area—or some fraction of it—represents a separate projection pathway arising from axons that extend ventrally from the lateral septal nucleus; other methods will be needed to determine the precise structural organization of this complex part of the descending projection system from ventral field CA1. In any event, whereas labeled field CA1 axons in caudal levels of the preoptic region typically run parallel to one another, fibers projecting specifically through the lateral preoptic area appear to descend caudoventrally from the septal region instead of rostroventrally through the medial preoptic area (from the column of the fornix; see above). Many fibers in the lateral preoptic region end abruptly in its caudoventromedial tip. Cajal (1909-1911) referred to axons descending from the region of the lateral septal nucleus to the preoptic region as lateral arcuate fibers.

Inputs to the thalamus

Field CA1 inputs to the thalamus arrive through two routes and are very light—often appearing only as scattered fibers—but are distinctive enough to warrant attention. The paraventricular thalamic, paratenial, and interanteromedial nuclei receive very light projections that extend caudally from the medial corticohypothalamic tract, and some of these fibers extend across the midline into the contralateral paraventricular and interanteromedial nuclei. In addition, scattered fibers are seen in the nucleus reuniens and anteromedial nucleus (Fig. 3E,F). A second projection extends dorsally from the column of the fornix, at the level of the anterior hypothalamic nucleus, lightly to innervate rostromedial regions of the zona incerta (Fig. 3E-G). This projection selectively avoids the zona incerta region with a concentration of dopaminergic cell bodies (the A13 cell group). Caudal and lateral regions of the zona incerta are devoid of labeled axons. At this level, scattered fibers are also observed bilaterally in the nucleus reuniens and anteromedial nucleus of the thalamus.

Additional observations

Eight experiments involving ventral regions of field CA1 label projections to the hypothalamus (Fig. 1), and all of those confined to field CA1 label the same overall projection pattern. For example, Experiment HIPPO161 has a large injection site centered in the most ventral tip of field CA1 (Fig. 1). The projection pattern observed in this experiment

is virtually identical to that observed in Experiment HIPPO103 (described above), although the density of terminal fields in the former is clearly greater than in the latter.

The anatomical boundaries of field CA1 used in the present study are derived from the original work of Blackstad (1956) and discussed further in Swanson and colleagues (1987). The ventral end of field CA1 appears to be triangular, and comes to a point with no transverse dimension. Therefore, only differences along the longitudinal axis are possible in the ventral end of field CA1 with the method used here. No reliable topographic organization of projections was detected from the ventral region of field CA1 indicated in Figure 1, which occupies about 10% of field CA1's surface area as measured from the flatmap.

Discussion

The present results document for the first time axonal projections from the ventral end of hippocampal field CA1 to widespread hypothalamic cell groups, as well as the existence of inputs directly to the thalamus. Novel observations include moderate inputs to the lateral preoptic area, medial regions of the LHA, and the anterior part of the ventromedial nucleus. Light terminal fields are also generated in other regions of the LHA (including its retrochiasmatic area and tuberal nucleus, and the ventral region's lateral zone), and the medial preoptic and anteroventral periventricular nuclei, central part of the ventromedial nucleus and subparaventricular zone, and the medial mammillary and posterior hypothalamic nuclei. Very sparse projections are observed consistently in the anterodorsal preoptic, anteroventral periventricular, paraventricular hypothalamic, ventrolateral part of the ventromedial, dorsal premammillary, and lateral mammillary nuclei. Another novel observation is the presence of PHAL-labeled fibers from ventral field CA1 in the medial corticohypothalamic tract.

The only previously described input from ventral field CA1 to the interbrain is a dense cluster of labeled fibers caudally in the anterior hypothalamic region (van Groen and Wyss, 1990a), which is confirmed here as a moderate projection to the central and posterior parts of the anterior hypothalamic nucleus. However, we did not confirm their report of an input to the anterior part of the dorsomedial hypothalamic nucleus. Instead, a terminal field in the laterally adjacent juxtadorsomedial region of the LHA is labeled, and it selectively avoids the dorsomedial nucleus. This minor difference is probably explained by their much larger injection sites (30 minutes of continuous current was used to deposit tracer), which may well have spread to include adjacent regions of the ventral subiculum (Fig. 1)—an area known to innervate strongly the dorsomedial nucleus (Canteras and Swanson, 1992; Kishi et al., 2000). Kishi and colleagues (2000) also illustrated, but did not comment on, retrogradely labeled neurons in ventral field CA1 after cholera toxin b injections in the anterior hypothalamic nucleus, which we confirmed with PHAL. Many parts of the hypothalamus identified here as receiving inputs from ventral field CA1 may have been identified because the analysis was at much higher resolution (1-in-4 vs. 1-in-10 series of sections) than previous work (van Groen and Wyss, 1990a).

This is also the first explicit description of a field CA1 projection to thalamus. Risold and colleagues (1997) illustrated but did not comment on retrogradely labeled neurons in ventral field CA1 after fluorogold injections into the region of the thalamus containing the paratenial, paraventricular, and reuniens nuclei. In the present material, light inputs were also noted to the interanteromedial and anteromedial nuclei, and to rostromedial regions of the zona incerta (apparently avoiding the dopaminergic A13 group).

Whereas the very small PHAL injections employed here are useful for labeling neuron populations restricted to very small areas like ventral field CA1, and for analyzing the topographic organization of projections, a disadvantage is that the overall densities of pathways and terminal fields are under-estimated. All projections described here arise from only a very small (currently unknown) percentage of the total neuron population in ventral field CA1. Thus, the total output of ventral field CA1 to hypothalamic cell groups is relatively substantial compared to the rather sparse distributions labeled by the current experiments. This is confirmed by the considerably denser projection to anterior hypothalamus reported by van Groen and Wyss (1990a) and by the relatively denser terminal fields labeled by our larger injections (see Fig. 4).

Previously, neurons in ventral subiculum were the only known source of fibers projecting through the medial corticohypothalamic tract (see Swanson and Cowan, 1975; Canteras and Swanson, 1992). The origin of some medial corticohypothalamic tract fibers in ventral field CA1—along with the other interbrain projections described here—is supported by the precise localization of PHAL injection sites, and by at least four critical, obvious differences between projections from the ventral poles of field CA1 and the subiculum (see Table 1). First, the dorsomedial hypothalamic nucleus is clearly innervated the latter but not former (Canteras and Swanson, 1992; Kishi et al., 2000). Second, the ventromedial hypothalamic nucleus and ventromedial LHA are more densely innervated by field CA1 than by the subiculum (Kishi et al., 2000). Indeed, the LHA is the predominant target of ventral field CA1, whereas the hypothalamic medial nuclei are the chief targets of the subiculum (Canteras and Swanson, 1992; Kishi et al., 2000). Third, the latter (Canteras and Swanson, 1992) but not the former (Fig. 2B,C) very densely innervates the ventral part of the lateral septal nucleus (Fig. 2B,C; Canteras and Swanson, 1992). And fourth, axons from ventral field CA1 travel mostly through the postcommissural fornix to innervate hypothalamic targets with fewer axons traveling through the medial corticohypothalamic tract, whereas in contrast, axons from the ventral subiculum travel mostly through the medial corticohypothalamic tract with fewer axons traveling through the postcommissural fornix (Canteras and Swanson, 1992; Kishi et al., 2000). Because there are no published molecular markers to distinguish unambiguously ventral field CA1 from ventral subiculum (Zhao et al., 2001; Lein et al., 2004), previous connection reports combined with injection placement of the injections here (Figs. 1, 2, and 3J) provide the best assurance that only cells contained within field CA1 were labeled.

Moreover, the present study compares the projection patterns of 8 experiments in the ventral one-third of field CA1. All of the experiments with an injection restricted to field CA1 exhibited similar projection patterns. In experiments where the deposit of tracer spread to the adjacent subiculum, the projection pattern exhibited clear differences (not illustrated) that have been described in previous reports. In addition, we obtained by chance a PHAL injection site strictly confined to the ventral pole of field CA3, and no hypothalamic projections were labeled (not illustrated). Nevertheless, new molecular markers may eventually help determine—together with additional functional and structural evidence—whether the ventral tenth of what is now regarded as field CA1 is in fact part of field CA1, whether it is part of the ventral subiculum, or whether it is a combination of the two, perhaps with partial interdigitation of layers or cell types. Currently, the most unambiguous distinction between ventral regions of field CA1 and the subiculum is the presence of a stratum oriens in the former and not the latter (Blackstad, 1956; Swanson et al., 1987).

To begin understanding the functional significance of field CA1-hypothalamus projections, it is helpful to review briefly what is known about the possible function of each innervated cell group. Overall, hypothalamic cell groups, like many in the lower brainstem, initiate behavior in response to sensory or to cerebral cortex inputs, which may arrive directly or via

the cerebral nuclei (basal ganglia). Characteristically, hypothalamic cell groups coordinate somatomotor and visceral (autonomic and neuroendocrine) motor responses typical of various classes of motivated or goal-oriented behavior (see Risold et al., 1997). The cerebral hemispheres (including Ammon's horn) modulate and regulate hypothalamic output in conjunction with initiating and directing the foraging or exploratory behavior associated with obtaining specific goal objects (Risold et al., 1997; Swanson, 2000).

Historically, views of the LHA's role in behavior have been complex and somewhat vague. In golden hamsters, dorsomedial regions—roughly equivalent to dorsal and intermediate zones of the LHA anterior region that receive a clear ventral field CA1 input—are heavily connected with the anterior hypothalamic and lateral septal nuclei, positioning them with defensive behavior control circuitry (Ferris et al., 1990; Canteras, 2002). In contrast, ventromedial LHA regions—including the juxtadorsomedial, juxtaventromedial, and posterior zone of the subfornical region—appear to be involved in feeding behavior (Khan et al., 1999; Watts et al., 1999; Mullett et al., 2000; Holland et al., 2002; Petrovich et al., 2002; Goto et al., 2005). And finally, LHA regions near the supraoptic and paraventricular nucleus that receive direct inputs from ventral field CA1 may influence magnocellular neuroendocrine system responses (Simerly and Swanson, 1988; Risold et al., 1997; Thompson and Swanson, 1998).

The hypothalamic medial nuclei recently were included in a behavior control column playing a critical role in the expression of ingestive (eating and drinking), reproductive (sexual and parental), and defensive (fight or flight) behaviors (Swanson, 2000). The anterior hypothalamic nucleus clearly is essential for aspects of defensive behavior (see Ferris et al., 1990; Ferris et al., 1994; Bamshad and Albers, 1996; Bamshad et al., 1997; Ferris et al., 1997; Kollack-Walker et al., 1997; Risold et al., 1997; Canteras et al., 2001; Canteras, 2002) and previous anatomical work demonstrated a massive field CA1 to lateral septal nucleus to anterior hypothalamic nucleus projection (Risold et al., 1994, 1997) as well as a considerably more massive field CA1 to subiculum to anterior hypothalamic nucleus projection (Hjorth-Simonsen, 1973; van Groen and Wyss, 1990a; Canteras and Swanson, 1992; Kishi et al., 2000). The present results indicate that the ventral end of field CA1 also projects directly to the anterior hypothalamic nucleus. Because the septo-hypothalamic projection is GABAergic, and field CA1 projections are glutamatergic, the anatomical results suggest that field CA1 sends a direct excitatory projection to the anterior hypothalamic nucleus, and two indirect, much more substantial, projections to the nucleus—one excitatory, by way of the subiculum; and one inhibitory, by way of a “relay” in the lateral septal nucleus.

Ventral field CA1 also projects directly to the anterior part of the ventromedial nucleus, although there appears to be no clear evidence about its functional role. However, the dorsomedial part is involved in defensive behavior and the ventrolateral part in reproductive behavior (see Swanson, 2000; Canteras, 2002). Because the ventrolateral and central parts are lightly innervated, and the dorsomedial part is selectively avoided, by field CA1 inputs, it might be suggested that the anterior part is involved in reproductive behavior control networks (Canteras et al., 1994; Simerly, 1996; Petrovich et al., 2001; Simerly, 2002), although this hypothesis must be tested experimentally. Another possible route for field CA1 influences on reproductive behavior is by way of direct inputs to medial preoptic regions, some of which are sexually dimorphic (Simerly and Swanson, 1986; Albers and Rawls, 1989; Ibáñez et al., 2001; Sheehan and Numan, 2002; Simerly, 2002; Stack et al., 2002). Again, this possibility requires experimental analysis.

Caudally, the mammillary nuclei (medial, lateral, and supramammillary) maintain very dense bi-directional connections with the septo-hippocampal system, and many studies have

demonstrated mammillary effects on septo-hippocampal physiology and vice versa (see Wyss et al., 1979; Swanson et al., 1987; Allen and Hopkins, 1989; Pan and McNaughton, 1997; Vertes and Kocsis, 1997; Naber and Witter, 1998; Stackman and Taube, 1998; Kiss et al., 2000; Vertes and McKenna, 2000). Up until the 1970s it was universally believed that field CA1 projects massively to the mammillary body through the fornix—in fact, that Ammon's horn is the sole origin of the postcommissural fornix (see Nauta, 1958; Raisman et al., 1966). Use of anterograde and retrograde tracing methods radically altered this view: instead it was claimed that the postcommissural fornix arises from the ventral subiculum not Ammon's horn (see Swanson and Cowan, 1975; Swanson et al., 1987). The present results indicate that the ventral pole of field CA1, representing about 10% of its total surface area, does indeed contribute to the postcommissural fornix input to the mammillary body—and to the medial corticohypothalamic tract as well, also like the ventral subiculum—although these projections of ventral field CA1 are relatively light. As can be appreciated from Figure 1, the region of field CA1's ventral pole that generates hypothalamic inputs is exceptionally small and difficult to approach stereotaxically (in three dimensions its surface is a curving triangle, with two sides about three mm long, and the other side about one mm long), and it was not examined specifically in any of the earlier structural analyses. For further documentation of the hippocampal flatmap in terms of transverse section analysis, see Figure 6 in Petrovich and colleagues (2001).

Several other hypothalamic regions in receipt of direct field CA1 inputs also project to the cerebral cortex, directly and/or indirectly via the thalamus. It is premature even to speculate about the functional significance of this circuitry, but such regions include the ventromedial nucleus (Canteras et al., 1994; Risold et al., 1997; McDonald, 1998; Pikkarainen et al., 1999; Pitkänen et al., 2000; Petrovich et al., 2001); lateral hypothalamic area (Haglund et al., 1984; Saper, 1985), tuberomammillary nucleus (Köhler et al., 1985; Panula et al., 1989), and posterior hypothalamic nucleus (Vertes et al., 1995; Rempel-Clower and Barbas, 1998; Gerashchenko et al., 2001). In fact, virtually the entire hypothalamus projects topographically upon anterior, midline, and medial regions of the dorsal thalamus, and those parts of the latter receiving any sign of direct inputs from ventral field CA1 (the anteromedial, interanteromedial, paratenial, paraventricular, and reuniens nuclei) all project to the hippocampal formation of the cerebral cortex (reviewed in Risold et al., 1997).

Our results thus suggest that ventral field CA1 influences hypothalamic neural activity and associated behaviors by way of direct axonal projections. However, these influences are probably minor compared to a multitude of stronger disynaptic pathways from field CA1 to hypothalamus. First, by far the densest projection of field CA1 is to the adjacent subiculum (Hjorth-Simonsen, 1973), including ventral field CA1 to ventral subiculum (in preparation). As already mentioned, the ventral subiculum projects densely to widespread parts of the hypothalamus through the column of the fornix and medial corticohypothalamic tract (see Swanson and Cowan, 1975; Canteras and Swanson, 1992). Indeed, the possible functional significance of hippocampal influences on hypothalamic function is exemplified by the observation that non-cortical projections of the ventral subiculum are essentially the same as those of the suprachiasmatic nucleus (Canteras and Swanson, 1992). Second, ventral field CA1 projects to the parasubiculum (Swanson and Cowan, 1977; van Groen and Wyss, 1990a; in preparation), which in turn projects massively to the mammillary body (Swanson and Cowan, 1975; van Groen and Wyss, 1990b). Third, ventral field CA1 projects heavily to ventral regions of the medial prefrontal cortex (Swanson, 1981; Gabbott et al., 2002), in particular the infralimbic area (Brodman's area 25), which in turn has by far the densest hypothalamic projections of any prefrontal or insular cortical area (Brittain, 1988; Hurley et al., 1991). Fourth, field CA1 also innervates parts of the insular and amygdalar regions that in turn innervate the hypothalamus (van Groen and Wyss, 1990a; Wyss and van Groen, 1992; in preparation). And fifth, the major descending projection from field CA1 as a whole

is to the lateral septal nucleus—and the latter establishes a massive set of bi-directional connections with the hypothalamus, as discussed above.

Field CA1 is thus positioned to modulate hypothalamic neural activity through direct projections (Fig. 6) and through cortical regions that in turn project directly to the hypothalamus. Furthermore, an extensive literature indicates that cortical areas receiving direct field CA1 inputs are strongly interconnected—and that the cerebral nuclei (basal ganglia) parts they innervate in turn massively innervate the hypothalamus (see McDonald et al., 1999; Swanson, 2000). In short, a major part of the cerebral hemisphere is devoted to regulating and modulating hypothalamic activity and associated behaviors. This is not surprising in view of the fact that the hypothalamus is essential for life and for maintaining the species—with hippocampal field CA1 playing a critical role in elaborating cerebral cortical influences.

There is some rather indirect, recent evidence supporting the hypothesis that motivated behavior is regulated by the hippocampal formation (Andrade et al., 2000; Tracy et al., 2001), and more direct evidence for hippocampal influences on hypothalamic functions like pituitary-adrenal control (Jacobson and Sapolsky, 1991; de Kloet et al., 1993; Joëls and de Kloet, 1994; Bhatnagar et al., 1997; Herman and Cullinan, 1997; Lee and Davis, 1997), pituitary-gonadal control (Jennes et al., 1995; Lathe, 2001), penile erection (Chen and Chang, 2001), and ovulation (Lathe, 2001). The structural data presented here concerning the topographic organization of direct field CA1 axonal pathways to specific hypothalamic neuron populations can be used to formulate more incisive experimental analyses of hippocampo-hypothalamic functions. How current models of hippocampal involvement in episodic memory and navigation—with presumed underlying mechanisms involving LTP and LTD—interface with the “function” of field CA1 (that is, its descending output to influence physiology and behavior) remains a tantalizing mystery.

Acknowledgments

Grant sponsor: National Institutes of Health (2R01NS16686 and MH12765).

Abbreviations

aco	anterior commissure, olfactory limb
act	anterior commissure, temporal limb
AHA	anterior hypothalamic area
AHN	anterior hypothalamic nucleus
AHNa	anterior hypothalamic nucleus, anterior part
AHNc	anterior hypothalamic nucleus, central part
AHNd	anterior hypothalamic nucleus, dorsal part
AHNp	anterior hypothalamic nucleus, posterior part
AL	pituitary gland, anterior lobe
AMd	anteromedial nucleus thalamus, dorsal part
AMv	anteromedial nucleus thalamus, ventral part
AQ	cerebral aqueduct
ARH	arcuate nucleus hypothalamus

AV	anteroventral nucleus thalamus
AVP	anteroventral preoptic nucleus
AVPV	anteroventral periventricular nucleus
BA	bed nucleus accessory olfactory tract
BAC	bed nucleus anterior commissure
BMA	basomedial nucleus amygdala
BSM	bed nucleus of the stria medullaris
BST	bed nuclei of the stria terminalis
BSTif	bed nuclei of the stria terminalis, posterior division, interfascicular nucleus
BSTpr	bed nuclei of the stria terminalis, posterior division, principal nucleus
BSTsz	bed nuclei of the stria terminalis, posterior division, cell-sparse zone
CA1	field CA1, Ammon's horn
CA1slm	field CA1, stratum lacunosum-moleculare
CA1so	field CA1, stratum oriens
CA1spd	field CA1, pyramidal layer, deep
CA1sps	field CA1, pyramidal layer, superficial
CA1sr	field CA1, stratum radiatum
CA2	field CA2, Ammon's horn
CA3	field CA3, Ammon's horn
CA3slu	field CA3, stratum lucidum
chp	choroid plexus
CM	medial nucleus thalamus
COApm	cortical nucleus amygdala, posterior part, medial zone
CP	caudoputamen
cpd	cerebral peduncle
DGlb	dentate gyrus, lateral blade
DGlb-mo	dentate gyrus, lateral blade-molecular layer
DGlb-po	dentate gyrus, lateral blade-polymorph layer
DGlb-sg	dentate gyrus, lateral blade-granule cell layer
DMHa	dorsomedial nucleus hypothalamus, anterior part
ECT	ectorhinal area
em	external medullary lamina thalamus
ENT	entorhinal area
ENTl1-6	entorhinal area, lateral part, layers 1-6
EPv	endopiriform nucleus, ventral part
fi	fimbria

FF	fields of Forel
fr	fasciculus retroflexus
FS	fundus of the striatum
fx	columns of the fornix
fxpo	postcommissural fornix
fxpr	precommissural fornix
GPe	globus pallidus, external segment
GPi	globus pallidus, internal segment
IAD	interanterodorsal nucleus thalamus
IAM	interanteromedial nucleus thalamus
IL	pituitary gland, intermediate lobe
int	internal capsule
IVF	interventricular foramen
laf	lateral arcuate fibers
LGvl	lateral geniculate complex, ventral part, lateral zone
LGvm	lateral geniculate complex, ventral part, medial zone
LHA	lateral hypothalamic area
LHAa	lateral hypothalamic area, anterior region
LHAad	lateral hypothalamic area, anterior region, dorsal zone
LHAai	lateral hypothalamic area, anterior region, intermediate zone
LHAav	lateral hypothalamic area, anterior region, ventral zone
LHAd	lateral hypothalamic area, dorsal region
LHAjd	lateral hypothalamic area, juxtadorsomedial region
LHAjp	lateral hypothalamic area, juxtaparaventricular region
LHAjvd	lateral hypothalamic area, juxtaventromedial region, dorsal zone
LHAjvv	lateral hypothalamic area, juxtaventromedial region, ventral zone
LHAM	lateral hypothalamic area, magnocellular nucleus
LHAp	lateral hypothalamic area, posterior region
LHApc	lateral hypothalamic area, parvicellular region
LHAs	lateral hypothalamic area, supraforfical region
LHAsf	lateral hypothalamic area, subforfical region
LHAsfa	lateral hypothalamic area, subforfical region, rostral zone
LHAsfp	lateral hypothalamic area, subforfical region, posterior zone
LHAsfpm	lateral hypothalamic area, subforfical region, premammillary zone
LHAvl	lateral hypothalamic area, ventral region, lateral zone
LM	lateral mammillary nucleus

LPO	lateral preoptic area
LSc	lateral septal nucleus, caudal part
LSr	lateral septal nucleus, rostral part
LSv	lateral septal nucleus, ventral part
MA	magnocellular preoptic nucleus
mct	medial corticohypothalamic tract
ME	median eminence
MEex	median eminence, external lamina
MEin	median eminence, internal lamina
MEA	medial nucleus amygdala
MEPO	median preoptic nucleus
ml	medial lemniscus
MM	medial mammillary nucleus
MMme	medial mammillary nucleus, median part
mp	mammillary peduncle
MPN	medial preoptic nucleus
MPNc	medial preoptic nucleus, central part
MPNI	medial preoptic nucleus, lateral part
MPNm	medial preoptic nucleus, medial part
MPO	medial preoptic area
MRNm	midbrain reticular nucleus, magnocellular part
MS	medial septal nucleus
mtg	mammillotegmental tract
mtt	mammillothalamic tract
NC	nucleus circularis
NDB	nucleus of the diagonal band
NL	pituitary gland, neural lobe
och	optic chiasm
opt	optic tract
OV	vascular organ of the lamina terminalis
PA	posterior nucleus amygdala
PAG	periaqueductal gray
PERI	perirhinal area
PH	posterior hypothalamic nucleus
pm	principal mammillary tract
PMd	dorsal premammillary nucleus

PMv	ventral premammillary nucleus
PR	perireuniens nucleus
PRE	presubiculum
PS	parastrial nucleus
PSCH	suprachiasmatic preoptic nucleus
PSTN	parasubthalamic nucleus
PT	paratenial nucleus
PVa	anterior periventricular nucleus hypothalamus
PVH	paraventricular nucleus hypothalamus
PVHam	paraventricular nucleus hypothalamus, anterior magnocellular part
PVHap	paraventricular nucleus hypothalamus, anterior parvicellular part
PVHdp	paraventricular nucleus hypothalamus, dorsal parvicellular part
PVHmpd	paraventricular nucleus hypothalamus, medial parvicellular part, dorsal zone
PVHmpv	paraventricular nucleus hypothalamus, medial parvicellular part, ventral zone
PVHpml	paraventricular nucleus hypothalamus, posterior magnocellular part, lateral zone
PVHpvt	paraventricular nucleus hypothalamus, periventricular part
PVi	intermediate periventricular nucleus hypothalamus
PVp	posterior periventricular nucleus hypothalamus
PVpo	preoptic periventricular nucleus
PVT	paraventricular nucleus thalamus
RCH	retrochiasmatic area
RE	nucleus reuniens
REcd	nucleus reuniens, caudal division, dorsal part
REcm	nucleus reuniens, caudal division, median part
REcp	nucleus reuniens, caudal division, posterior part
REd	nucleus reuniens, rostral division, dorsal part
REl	nucleus reuniens, rostral division, lateral part
REm	nucleus reuniens, rostral division, medial part
REv	nucleus reuniens, rostral division, ventral part
RH	rhomboid nucleus
RT	reticular nucleus thalamus
SBPV	subparaventricular zone hypothalamus
SCH	suprachiasmatic nucleus
SF	septohippocampal nucleus

SI	substantia innominata
sm	stria medullaris
smd	supramammillary decussation
SMT	submedial nucleus thalamus
SNc	substantia nigra, compact part
SNr	substantia nigra, reticular part
SO	supraoptic nucleus
SOr	supraoptic nucleus, retrochiasmatic part
SPFm	subparafascicular nucleus thalamus, magnocellular part
SPFpm	subparafascicular nucleus thalamus, parvicellular part, medial division
st	stria terminalis
STN	subthalamic nucleus
SUB	subiculum
SUBv	subiculum, ventral part
SUMl	supramammillary nucleus, lateral part
SUMm	supramammillary nucleus, medial part
sup	supraoptic commissures
TEa	temporal association areas
TMv	tuberomammillary nucleus, ventral part
TR	postpiriform transition area
TUi	tuberal nucleus, intermediate part
TUl	tuberal nucleus, lateral part
TUsv	tuberal nucleus, subventricular part
V3	third ventricle
V3m	third ventricle, mammillary recess
V3p	third ventricle, preoptic recess
VAL	ventral anterior-lateral complex thalamus
VISC	visceral area
VL	lateral ventricle
vlt	ventrolateral hypothalamic tract
VM	ventral medial nucleus thalamus
VMH	ventromedial nucleus hypothalamus
VMHa	ventromedial nucleus hypothalamus, anterior part
VMHc	ventromedial nucleus hypothalamus, central part
VMHdm	ventromedial nucleus hypothalamus, dorsomedial part
VMHvl	ventromedial nucleus hypothalamus, ventrolateral part

VPL	ventral posterolateral nucleus thalamus
VPLpc	ventral posterolateral nucleus thalamus, parvicellular part
VPM	ventral posteromedial nucleus thalamus
VPMpc	ventral posteromedial nucleus thalamus, parvicellular part
ZI	zona incerta
ZIda	zona incerta, dopaminergic group
zl	zona limitans

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Fig. 1. Distribution of injection sites centered in hippocampal field CA1. Relative locations of PHAL injections from 62 experiments, plotted onto an unfolded map of the rat hippocampal formation (adapted from Petrovich et al., 2001). Injection sites filled with black illustrate experiments with injection sites limited to, or contained within, the ventral pole of field CA1. This triangular region occupies about 10% of field CA1's total surface area.

Fig. 2.

The ventral field CA1 PHAL injection site (black oval regions) in Experiment HIPPO103 (A-C). Panel A is a photomicrograph through the rostral end of the injection site, panel B through the middle, and panel C through the caudal end of the injection site. Panels D-F are photomicrographs of nearby thionin (Nissl)-stained sections to show cytoarchitectonic features of the transverse histological sections (paired with panels A-C, respectively). As currently understood, the major architectonic feature that distinguishes field CA1 from the subiculum is the presence of a clear stratum oriens in the former. See Figures 1 and 3-I for relative location of this injection site. Scale = 200 μ m.

Fig. 3.

A-I: Distribution of PHAL-labeled axons (red lines) plotted onto a series of rat brain reference drawings (adapted from Swanson, 2004), cut in the transverse plane and arranged from rostral (A) to caudal (I)—after an injection centered in the ventral pole of hippocampal field CA1 (red area in I).

Fig. 4.

A-B: Darkfield photomicrographs showing the distribution of PHAL-labeled axons in the hypothalamus at the level of the anterior hypothalamic nucleus (A), and the ventromedial hypothalamic nucleus (B). C-D: Brightfield photomicrographs demonstrating the location of the injection site (C) and an adjacent thionin-stained section (D) in Experiment HIPPO161. Scale bars: 200 μ m.

Fig. 5. Darkfield photomicrograph showing the divergence of PHAL-labeled axons from the fimbria to the medial corticohypothalamic tract, in a transverse section from an experiment with an injection confined to the ventral pole of hippocampal field CA1. Figure 3D is at approximately the level of this photomicrograph. Scale bar: 200 μ m.

Field CA1 projection to interbrain **Fig. 6.**

Summary diagram to indicate the general organization of projections from the ventral pole of hippocampal field CA1 to the interbrain (diencephalon). Projections to other parts of the forebrain are not shown. The flatmap of the rat central nervous system is based on Swanson (2004); line thickness is roughly proportional to relative projection strength.

Table 1

Comparison of projections from ventral field CA1 in the present study and projections from the ventral subiculum as reported previously, in the rat¹

	SUBv [Canteras & Swanson (1992)]	SUBv [Kishi et al. (2000)]	CA1v [Cenquizca & Swanson (2005)]
HYPO			
<i>AHNa</i>			*
<i>AHNc</i>			*
<i>AHNd</i>		*	
<i>AHNp</i>	*	*	*
<i>ARH</i>	*	*	
<i>ADP</i>			*
<i>AVP</i>	*		*
<i>AVPV</i>			*
<i>DMH</i>	*	*	
<i>LHA (all parts)</i>			*
<i>LPO</i>			*
<i>MePO</i>	*		
<i>MM</i>	*	*	*
<i>MPO</i>	*	*	*
<i>MPNI</i>	*		*
<i>PH</i>	*	*	*
<i>PMd</i>			*
<i>PMv</i>	*	*	
<i>PVp</i>	*		
<i>PVH (all parts)</i>			*
<i>RCH</i>	*		*
<i>SCH</i>		*	
<i>SPVZ</i>	*	*	*
<i>SUMI</i>			*
<i>SUMm</i>	*	*	
<i>TMd</i>	*	*	
<i>TMv</i>	*		*
<i>TUsv</i>		*	*
<i>VMHa</i>			*
<i>VMHc</i>	*	*	*
<i>VMHdm</i>		*	
<i>VMHvl</i>	*	*	*
THAL			
<i>AM</i>			*
<i>IAM</i>			*

	SUBv [Canteras & Swanson (1992)]	SUBv [Kishi et al. (2000)]	CA1v [Cenquizca & Swanson (2005)]
<i>LH</i>	*		
PVT	*		*
PT	*		*
RE	*		*
<i>ZI</i>			*

¹ Clear differences between projections of field CA1 and subiculum are emphasized by *italicized* abbreviations.