Theta Rhythm of the Hippocampus: Subcortical Control and Functional Significance

Robert P. Vertes
Walter B. Hoover
Gonzalo Viana Di Prisco
Florida Atlantic University

The theta rhythm is the largest extracellular synchronous signal that can be recorded from the mammalian brain and has been strongly implicated in mnemonic processes of the hippocampus. We describe (a) ascending brain stem–forebrain systems involved in controlling theta and nontheta (desynchronization) states of the hippocampal electroencephalogram; (b) theta rhythmically discharging cells in several structures of Papez’s circuit and their possible functional significance, specifically with respect to head direction cells in this same circuit; and (c) the role of nucleus reuniens of the thalamus as a major interface between the medial prefrontal cortex and hippocampus and as a prominent source of afferent limbic information to the hippocampus. We suggest that the hippocampus receives two main types of input: theta rhythm from ascending brain stem–diencephaloseptal systems and information bearing mainly from thalamocortical/cortical systems. The temporal convergence of activity of these two systems results in the encoding of information in the hippocampus, primarily reaching it from the entorhinal cortex and nucleus reuniens.

Key Words: supramammillary nucleus, median raphe nucleus, medial prefrontal cortex, Papez’s circuit, nucleus reuniens, LTP, memory

The theta rhythm of the hippocampus is a large-amplitude (1-2 mV) nearly sinusoidal oscillation of 5 to 12 Hz in the behaving rat (Bland, 1986; Vertes & Kocsis, 1997). It is the largest extracellular synchronous signal that can be recorded in the mammalian brain. Theta is selectively present during two behavioral states: waking behaviors that are thought to be critical for the survival of the species and throughout rapid eye movement (REM) sleep (Vanderwolf, 1969; Winson, 1972). It is well documented that rhythmically discharging cells of the medial septum/vertical limb of the diagonal band nucleus (MS/DBv) that fire synchronously with theta are responsible for its generation in hippocampal formation (Brazhnik & Fox, 1997; Brazhnik & Vinogradova, 1986; Gogolak, Stumpf, Petsche, & Sterc, 1968; Lamour, Dutar, & Jobert, 1984; Leung & Shen, 2004; Petsche, Gogolak, & Van Zwieten, 1965; Petsche, Stumpf, & Gogolak, 1962; Stewart & Fox, 1989; Vertes & Kocsis, 1997; Vinogradova, 1995). Low-frequency MS/DBv stimulation drives theta (James, McNaughton,
Rawlins, Feldon, & Gray, 1977; Kramis & Vanderwolf, 1980; McNaughton, Azmitia, Williams, Buchan, & Gray, 1980; McNaughton et al., 1977), and reversible or irreversible MS/DBv lesions, or the disruption of the rhythmic discharge of MS/DBv cells, eliminates the theta rhythm in the hippocampus as well as in parahippocampal structures such as the entorhinal and cingulate cortices (Donovick, 1968; Gray, 1971; Leung & Borst, 1987; Mitchell, Rawlins, Steward, & Olton, 1982; Sainsbury & Bland, 1981). The MS/DBv has been designated the “pacemaker” for the hippocampal theta rhythm (Leung & Shen, 2004; Numan, 2000; Vertes & Kocsis, 1997; Vinogradova, 1995).

It is also well established that the brain stem reticular formation (RF), through actions on the MS/DBv, serves a direct role in the generation of theta. In their original report that identified the theta rhythm in the curarized rabbit, Green and Arduini (1954) showed that theta could be elicited by natural sensory stimuli or by electrical stimulation of the brain stem RF. Shortly thereafter, Petsche and colleagues (1962, 1965) demonstrated that septal pacemaking cells could be driven by input arising from the brain stem RF, leading them (Petsche et al., 1965) to conclude that the septum transforms “the steady flow of pulses from the RF into a discontinuous pattern of discharges” that are then transferred “to the pyramidal cells of the hippocampus thus inducing the theta rhythm.”

Although these early studies indicated a role for the brain stem RF in elicitation of theta, they did not identify specific RF locations responsible for this effect. Stimulation was generally delivered to the midbrain RF (for review, see Vertes, 1982). In a series of reports, we showed that the nucleus pontis oralis (RPO) of the rostral pontine RF was critical for the generation of theta. We found that cells of RPO in the behaving rat discharged at high tonic rates (50-75 Hz) selectively during waking motor behavior and REM sleep (theta-associated states in the rat; Vertes, 1977, 1979) and that electrical stimulation of pontis oralis, but not that of surrounding regions of the brain stem, generates theta (Vertes, 1980, 1981). Other studies have similarly shown that RF stimulation, centered in RPO, both activates septal pacemaking cells and elicits theta (Bland, Oddie, Colom, & Vertes, 1994; Brazhnik, Vinogradova, & Karanov, 1985; Macadar, Chalupa, & Lindsley, 1974; McNaughton, Richardson, & Gore, 1986; Oddie, Bland, Colom, & Vertes, 1994).

In line with the proposal of Petsche et al. (1965), the foregoing suggested, then, that RPO is the primary source of tonic drive to the septal pacemaking cells in the elicitation of theta. To further assess this, we examined anatomical connections of RPO with the medial septum. Specifically, we made injections of the retrograde tracer, WGA-HRP, into the MS/DBv and analyzed patterns of labeled neurons in the brain stem (Vertes, 1988). Although labeled cells were identified in several regions of the brain stem, prominently including monoaminergic nuclei, exceedingly few were found in RPO or other reticular nuclei of the brain stem (Vertes, 1988). This suggested that the influence of RPO on the MS/DBv in the generation of theta was mediated by an intervening nucleus (or nuclei) or di- or polysynaptic pathways between RPO and the MS/DBv.

Although retrograde tracer injections in MS/DBv failed to produce labeling in RPO, interestingly, they gave rise to pronounced cell labeling in the supramammillary nucleus (SUM), dorsal to the mammillary bodies (Vertes, 1988). Approximately 150 to 200 labeled neurons per 50-μm section were seen through the heart of SUM. This suggested that the SUM may be a link between the brain stem and septum involved in the generation of theta. As discussed below, this has subsequently been confirmed by several lines of evidence: (a) SUM receives projections from RPO and, in turn, projects significantly to the septum and hippocampus; (b) SUM cells fire rhythmically with theta; (c) electrical stimulation-induced or pharmacological activation of SUM drives theta; and (d) the suppression of SUM disrupts the rhythmic discharge of septal pacemaking cells and eliminates theta in the hippocampus.

Using autoradiographic techniques, we demonstrated that RPO projects to SUM (Vertes, 1990; Vertes & Martin, 1988) and, confirming our retrograde findings (Vertes, 1988), showed that injections of the anterograde tracer PHA-L into SUM produced dense labeling in the medial and lateral septum as well as in the hippocampus (Vertes, 1992). Within the hippocampus, SUM fibers distribute selectively to the dentate gyrus and to CA2/CA3a of Ammon’s horn (Vertes, 1992; Vertes & McKenna, 2000). There are essentially no SUM projections to the CA1 region of Ammon’s horn. These patterns of projections are consistent with those described in other reports and across species (Borhegyi, Magloczky, Acsady, & Freund, 1998; Haglund, Swanson, & Kohler, 1984; Kiss, Csaki, Bokor, Shanabrough, & Leranth, 2000; Leranth & Kiss, 1996; Magloczky, Acsady, & Freund, 1994; Veazey, Amaral, & Cowan, 1982; Wyss, Swanson, & Cowan, 1979a).

In an initial study recording multiunit activity in anesthetized rats, Kirk and McNaughton (1991) identified a population of cells in the SUM that fired rhythmically with the hippocampal theta rhythm. They further showed that this activity did not depend on the MS/DBv; procaine injections in the MS/DBv that abolished
hippocampal theta did not alter the rhythmical firing of SUM neurons. In subsequent examinations (Kocsis & Vertes, 1994, 1997) of the activity of SUM cells as well as those in surrounding regions of the caudal diencephalon, we found that 29 of 170 neurons discharged rhythmically, synchronous with theta (Kocsis & Vertes, 1994). All 29 theta-related cells were localized to SUM or to the mammillary bodies, ventral to SUM; none of 141 neurons located outside of the SUM/MB fired synchronously with theta. Bland, Konopacki, Kirk, Oddie, and Dickson (1995) similarly reported that 16 of 16 SUM cells and 19 of 23 MB cells discharged rhythmically with theta—phasic theta-on cells in their terminology (Colom & Bland, 1987; Ford, Colom, & Bland, 1989).

Finally, the activation or suppression of SUM drives or blocks theta, respectively. Specifically, electrical stimulation or carbachol injections in SUM activate theta burst neurons of the septum and hippocampus and produce theta (Bland, Colom, & Ford, 1990; Bland et al., 1994; Bocian & Konopacki, 2004; Oddie et al., 1994; Smythe, Christie, Colom, Lawson, & Bland, 1991), whereas the reversible suppression of SUM with procaine injections in anesthetized rats disrupts the spontaneous as well as the RPO-elicited bursting discharge of septal pacemaking cells and eliminates hippocampal theta (Bland et al., 1994; Oddie et al., 1994). Procaine injections into SUM in awake (nonanesthetized) rats significantly reduces the frequency of theta but does not totally eliminate it (Kirk & McNaughton, 1993; Pan & McNaughton, 1997, 2002).

McNaughton and colleagues (Kirk & McNaughton, 1993; see also Gray & McNaughton, 2000; Kirk, 1998; Woodnorth, Kyd, Logan, Long, & McNaughton, 2003) have proposed that SUM codes the frequency of the theta rhythm and the medial septum the amplitude of theta. Specifically, they showed that procaine injections caudal to SUM (blocking RPO actions on SUM) or within SUM significantly reduced the frequency of theta, whereas procaine injections in the MS/DBv reduced the amplitude but not frequency of theta (Kirk & McNaughton, 1993). They concluded that the most parsimonious explanation of this result is that transduction of the intensity of reticular activation to the frequency of the resultant RSA (rhythmical slow activity or theta) takes place in the supramammillary region rather than in the septal area. It is likely that transduction occurs in the SuM itself. The frequency coded (i.e., phasic) information is then fed, probably via the MFB, to the MS/DBv. (Kirk & McNaughton, 1993, p. 520)

In addition to rhythmically firing neurons of SUM, cells of the posterior nucleus of the hypothalamus (PH) have been shown to discharge tonically with theta (Bland et al., 1995; Bland & Oddie, 1998). Specifically, Bland et al. (1995) reported that 43 of 54 neurons of PH fired at high tonic rates selectively during theta—their tonic theta-on cells. With respect to the possible PH influence on the hippocampus, PH does not project to the hippocampus (Vertes, Crane, Colom, & Bland, 1995) but distributes strongly to several structures with pronounced input to the hippocampus, prominently including the medial septum, RE of the thalamus (see below), and SUM. Bursting SUM neurons and the tonically firing PH cells may act synergistically to relay ascending reticular activity to the MS/DBv in the control of theta (Bland & Oddie, 1998; Vertes & Kocsis, 1997).

In summary, several lines of evidence indicate that the theta rhythm is controlled by a network of cells extending from the brain stem to the forebrain, that is, from RPO to the SUM to the septum/hippocampus. As depicted in Figure 1, during theta, tonically firing cells of the RPO activate putative glutamatergic neurons of SUM, which convert the steady barrage into a rhythmical pattern of discharge that is relayed to cholinergic and gamma-aminobutyric acid (GABA)-ergic pacemaking cells of the MS/DBv. Septal cholinergic neurons excite principal cells and interneurons of the hippocampus (Dutar, Bassant, Senut, & Lamour, 1995; Frotscher & Leranth, 1985; Leranth & Frotscher, 1989; Vertes & Kocsis, 1997), whereas septal GABAergic cells inhibit GABAergic interneurons of the hippocampus (Freund & Antal, 1988; Gulyas et al., 1991) in the generation of theta. Although septohippocampal cholinergic neurons fire rhythmically with theta, they may not rhythmically drive (or entrain) hippocampal neurons at theta frequencies. Rather, acetylcholine may exert “tonic” excitatory actions on hippocampal pyramidal cells, depolarizing them to threshold for the activation of intrinsic currents sufficient to produce membrane potential oscillations at theta frequencies (Vertes & Kocsis, 1997). This is supported by the demonstration that cholinomimetics produce a theta-like rhythm in the isolated hippocampal slice (Bland & Colom, 1993; Bland, Colom, Konopacki, & Roth, 1988; Konopacki, 1998; Konopacki, Maclver, Bland, & Roth, 1987).

Nontheta States of the Hippocampal EEG
(Hippocampal EEG Desynchronization):
Role of the Median Raphe Nucleus (MR)

The MR is a major serotonin-containing cell group of the midbrain with extensive projections to the forebrain (Aznar, Qian, & Knudsen, 2004; Leranth & Vertes, 1999; McKenna & Vertes, 2001; Morin & Meyer-Bernstein, 1999; Vertes, Fortin, & Crane, 1999; Vertes & Martin, 1988). An extensive body of evidence indicates that the MR is directly involved in the desynchronization of the
the hippocampal EEG (Assaf & Miller, 1978; Macadar et al., 1974; Vertes, 1981; Yamamoto, Watanabe, Oishi, & Ueki, 1979) and that MR lesions produced continuously running theta, independent of behavior (Maru, Takahashi, & Iwahara, 1979; Yamamoto et al., 1979). These effects reportedly involved serotonergic (5-HT) cells of MR. Assaf and Miller (1978) demonstrated that the disruptive effect of MR stimulation on septal pacemaking cells and the hippocampal EEG was blocked by pretreatment with the 5-HT synthesis inhibitor p-chlorophenylalanine, which produced a 60% to 80% depletion of forebrain serotonin. Yamamoto et al. (1979) reported that ongoing theta produced by MR lesions could be temporarily interrupted by intraperitoneal injections of the serotonin precursor L-5-hydroxytryptophan (L-5-HTP), and McNaughton et al. (1980) showed in behaving rats that the effectiveness of driving theta with septal stimulation was significantly enhanced following destruction of ascending serotonergic fibers.

More recently, we showed (Kinney, Kocsis, & Vertes, 1994, 1995, 1996; Vertes, Kinney, Kocsis, & Fortin, 1994) that injections of various substances into the MR in anesthetized rats that either suppressed 5-HT MR neurons (5-HT1A autoreceptor agonists or GABA agonists) or reduced excitatory drive to them (excitatory amino acid antagonists) produced theta at short latencies (1-2 minutes) and for long durations (20-80 minutes). In like manner, Varga, Sik, Freund, and Kocsis (2002) reported that GABAB receptors are selectively present on serotonergic neurons of MR and that infusions of the GABAB agonist, baclofen, into MR in anesthetized rats produced long-lasting theta. They concluded (Varga et al., 2002) that the effects of baclofen on theta “resulted from suppression of the serotonergic output from the median raphe.”

In examinations of the effects of manipulations of MR on the septum and hippocampus in awake rabbits, Vinogradova and colleagues (Kitchigina, Kudina, Kutyreva, & Vinogradova, 1999; Vinogradova, Kitchigina, Kudina, & Zenchenko, 1999) similarly showed that low-amplitude median raphe nucleus (MR) discharge at enhanced rates and activate GABAergic cells of the medial septum, which in turn inhibit GABAergic/cholinergic pacemaking cells of the medial septum in the desynchronization of the hippocampal EEG. Serotonergic neurons of the MR also project directly to the SUM and to the hippocampus and could also exert desynchronizing actions on the hippocampal EEG through these connections. See the text for further description of this circuitry. The dashed line signifies presently undetermined SUM glutamatergic projections to glutamatergic cells of the septum. Arrows (at the end of lines) indicate excitatory connections; straight lines indicate inhibitory connections. Ch = acetylcholine; G = GABA; Glu = glutamate; S = serotonin.

Figure 1: Schematic Diagram Showing the Major Interconnections of Ascending Systems Controlling Theta and Nontheta (Desynchronization) States of the Hippocampal Electroencephalogram.

NOTE: During theta, tonically firing cells of nucleus pontis oralis activate putative glutamatergic neurons of the supramammillary nucleus (SUM) which, in turn, convert this steady barrage into a rhythmic pattern of discharge that is relayed to cholinergic and gamma-aminobutyric acid (GABA)-ergic pacemaking cells of the medial septum. Medial septal GABAergic neurons connect with and inhibit GABAergic cells of the hippocampus, thereby exerting a disinhibitory action on pyramidal cells of the hippocampus. Medial septal GABAergic cells receive intra septal excitatory input from both septal cholinergic and glutamatergic neurons. Cholinergic septohippocampal pacemaking cells terminate on both interneurons and principal cells of the hippocampus. During states of hippocampal desynchronization (nontheta), a subset of serotonergic septal-projecting cells of the median raphe nucleus (MR) discharge at enhanced rates and activate GABAergic cells of the medial septum, which in turn inhibit GABAergic/cholinergic pacemaking cells of the medial septum in the desynchronization of the hippocampal EEG. Serotonergic neurons of the MR also project directly to the SUM and to the hippocampus and could also exert desynchronizing actions on the hippocampal EEG through these connections. See the text for further description of this circuitry. The dashed line signifies presently undetermined SUM glutamatergic projections to glutamatergic cells of the septum. Arrows (at the end of lines) indicate excitatory connections; straight lines indicate inhibitory connections. Ch = acetylcholine; G = GABA; Glu = glutamate; S = serotonin.

hippocampal EEG, or the blockade of theta. It was demonstrated early on that MR stimulation desynchronized
Figure 2: The Discharge Characteristics of a Theta-Off Neuron of the Median Raphe Nucleus.


NOTE: The cell showed an abrupt cessation of firing at the onset and for the duration of hippocampal theta elicited with either tail pinch (TP) (A) or with electrical stimulation of the tail (DC) (B). (C) Superimposed action potentials of the cell showing a wide spike of ~2 ms. (D) ISI histogram of the cell demonstrating a sharp peak at 110 ms, indicating that the cell fired at very regular rates during control (nontheta) conditions. (E) Autocorrelogram depicting the steady rate of discharge of the cell at ~9 Hz (peaks in E). (F) Cross-correlogram (spike-triggered averaging) showing that the cell did not discharge rhythmically synchronous theta as indicated by the flat unit-electroencephalogram cross-correlogram.
In addition to 5-HT cells, the MR contains significant numbers of GABAergic neurons (Jacobs & Azmitia, 1992; Mugnaini & Oertel, 1985; Maloney, Mainville, & Jones, 1999), which have been shown to contact and inhibit 5-HT MR cells (Forchetti & Meek, 1981; Nishikawa & Scatton, 1985a, 1985b). As discussed, injections of GABA, (Kinney et al., 1995) or GABA, agonists (Varga et al., 2002) into MR generate persistent theta. This suggests a GABAergic MR influence on 5-HT cells of MR in the modulation of the hippocampal EEG. In recent examinations of the discharge properties of MR neurons in anesthetized rats (Kocsis & Vertes, 1996; Viana Di Prisco, Albo, Vertes, & Kocsis, 2002), we identified two major populations of cells, putatively 5-HT and GABAergic neurons, with activity related to the hippocampal EEG. Specifically, we demonstrated that a very large percentage of MR cells showed changes in activity associated with changes in the hippocampal EEG (Viana Di Prisco et al., 2002). Approximately 80% (145/181) of MR neurons fired at increased or decreased rates of activity with theta and were termed theta-on and theta-off cells, respectively. These cells were further divided into slow (~1 Hz), moderate (5-11 Hz) and fast-firing (>12 Hz) theta-on or theta-off cells. The slow-firing theta-on and theta-off cells, as well as a subpopulation of the moderately firing cells, exhibited characteristics of classic 5-HT raphe neurons (Aghajanian, Foote, & Sheard, 1968, 1970; Jacobs, Heym, & Steinfels, 1984; Rasmussen, Heym, & Jacobs, 1984; Sprouse & Aghajanian, 1987; Jacobs & Azmitia, 1992) and were thought to be serotonergic cells. All fast-firing cells were theta-on cells; no fast-firing theta-off cells were observed. Fast-firing cells showed either tonic or phasic (rhythmic) increases in activity with theta.

The discharge profile of a moderately firing, putative serotonergic, theta-off cell is shown in Figure 2. As depicted, the cell discharged at very regular rates during control (nontheta) conditions (Figure 2A) and abruptly ceased firing with the onset, and essentially for the duration, of theta elicited with tail pinch (TP; Figure 2A) or by electrical stimulation of the tail (Figure 2B). For a few neurons tested, cells that were strongly inhibited during TP-elicited theta were also completely suppressed following the intravenous administration of the 5-HT, agonist, 8-OH-DPAT, further indicating that they were serotonergic neurons.

We suggested, then, that (a) the slow-firing cells (theta-on and theta-off) and a subset of the moderately discharging cells were serotonergic neurons and the phasic and tonic fast-firing cells were mainly GABAergic neurons, (b) the 5-HT theta-off (or desynchronization-on) cells were projection neurons and the 5-HT theta-on and GABAergic cells were primarily interneurons, and (c) these populations of cells mutually interact in the modulation of the hippocampal EEG (Viana Di Prisco et al., 2002). In effect, the activation of local 5-HT theta-on cells as well as the GABAergic theta-on cells would inhibit 5-HT theta-off projection cells to release or generate theta, whereas suppression of 5-HT theta-on and/or GABAergic theta-on activity would disinhibit 5-HT theta-off (desynchronization-on) cells resulting in a blockade of theta or a desynchronization of the hippocampal EEG (see Figure 1).

In one of the few studies examining the activity of MR cells in behaving animals, Jacobs and colleagues (Marrosu, Fornal, Metzler, & Jacobs, 1996) showed in awake cats that putative 5-HT cells of MR exhibited properties indicative of a role in the desynchronization of the hippocampal EEG. These cells fire at highest rates during automatic behaviors of waking and slow wave sleep (desynchronized states of the hippocampus) and at lowest rates during the exploration of waking and REM sleep (theta states; Jacobs & Azmitia, 1992; Marrosu et al., 1996).

Although not fully determined, the desynchronizing actions of MR on the hippocampus appear to be primarily mediated by the medial septum. The MR projects strongly to the medial septum (Aznar et al., 2004; Morin & Meyer-Bernstein, 1999; Vertes et al., 1999; Vertes & Martin, 1988), and MR fibers predominantly terminate on GABAergic cells of the MS/DBv (Leranth & Vertes, 1999, 2000), forming asymmetric (excitatory) connections with them. Alreja and colleagues (Alreja, 1996; Liu & Alreja, 1997) demonstrated that 5-HT excites local GABAergic cells of the MS/DBv, which in turn inhibit subsets of septal pacemaking GABAergic and cholinergic septohippocampal neurons, possibly in the control of the hippocampal EEG. Supporting this, Kinney et al. (1996) showed that injections of 8-OH-DPAT into MR (which inhibits 5-HT neurons) rhythmically activated septal pacemaking cells and generated theta.

The MR could also exert desynchronizing influences on the hippocampus via actions on other targets, such as the RPO, SUM, or directly on the hippocampus (Figure 1). With respect to MR-RPO (reticular) interactions in the control of theta, Vinogradova et al. (1999) proposed that a suppression of MR activity could result in the “elimination of the MR inhibitory influences on the reticular formation” and thereby “stimulate the generation of theta rhythm and increase of its frequency in the septo-hippocampal system” (p. 750).

In summary, two brain stem–originating systems exert pronounced (and opposing) actions on the electrical activity of the hippocampus, that is, hippocampal synchronizing (theta) and desynchronizing (non-theta) systems, originating from the RPO and MR, respectively. During theta, tonically firing cells of RPO activate neu-
rons of the SUM, which in turn convert this steady barrage into a rhythmical pattern of discharge that is relayed to pacemaking cells of the MS/DBv to generate theta. During states of hippocampal desynchronization, a subset of 5-HT, septal-projecting MR cells discharge at enhanced rates and activate local GABAergic cells of the MS/DBv, which in turn inhibit cholinergic/GABAergic pacemaking cells of the MS/DBv in the desynchronization of the hippocampal EEG (see Figure 1; Vertes & Kocsis, 1997; Bland & Oddie, 1998).

**THETA-RHYTHMIC SIGNALS EXITING THE HIPPOCAMPUS THROUGH STRUCTURES OF PAPEZ’S CIRCUIT AND POSSIBLE FUNCTIONAL SIGNIFICANCE**

As discussed, we described theta rhythmically firing neurons in SUM as well as in the mammillary bodies (MB), ventral to SUM (Kocsis & Vertes, 1994, 1997). Others studies have similarly demonstrated theta-rhythmic cells in MB (Bland et al., 1995; Kirk, Oddie, Konopacki, & Bland, 1996). Although SUM and MB cells fire rhythmically with theta, they bear different relationships to theta, that is, influencing theta (SUM) or influenced by it (MB). For instance, it has been shown that procaine injections in the MS/DBv (which abolish theta) disrupt the rhythmical discharge of MB cells but not that of SUM cells (Bland et al., 1995; Kirk et al., 1996; Kirk & McNaughton, 1991). This suggests that MB is part of a descending system driven from the septum/hippocampus, whereas SUM is a part of an ascending system generating theta. This is consistent with anatomical findings showing that MB receives major descending projections from the hippocampus via the postcommisural fornix (Amaral & Witter, 1995; Swanson & Cowan, 1977; Witter, Ostendorf, & Groenewegen, 1990) but does not project to the hippocampus, whereas the SUM receives few fibers from the hippocampus but is the source of dense projections to the septum and hippocampus (Borhegyi et al., 1998; Haglund et al., 1984; Kiss et al., 2000; Leranth & Kiss, 1996; Magloczky et al., 1994; Vertes, 1992; Vertes & McKenna, 2000).

The MB represents a major output of the hippocampus (Amaral & Witter, 1995) and are a principal component of Papez’s circuit, an anatomical circuit (a loop) beginning and ending in the hippocampus. As originally defined by Papez (1937), the projections of the circuit are hippocampal formation → mammillary bodies → anterior thalamus → cingulate cortex → parahippocampal gyrus → hippocampal formation. Although the circuit has been refined based on subsequent anatomical findings (Amaral & Witter, 1995; Shibata, 1992; van Groen & Wyss, 1995), the major links of the circuit unquestionably represent a prominent system of connections in the mammalian brain. Hence, the enduring nature of Papez’s circuit. Unlike, however, its persistence as anatomical entity, the proposed functional role for the circuit has been less resilient. The early notion that Papez’s circuit subserves emotional experience/expression (LeDoux, 1993) has been replaced by the proposal that it is primarily involved in mnemonic functions (Aggleton & Brown, 1999). Lesions of each of the major structures of Papez’s circuit have been shown to disrupt memory (Aggleton & Brown, 1999; Byatt & Dalrymple-Alford, 1996; Gabriel et al., 1995; Sutherland, Whishaw, & Kolb, 1988; Sziklas & Pertides, 1993, 1999; Tulving & Markowitsch, 1997; van Groen, Kadiš, & Wyss, 2002; Warburton, Baird, & Aggleton, 1997).

The findings that MB cells fire rhythmically with theta, coupled with the demonstration that MB projects massively to the anterior thalamus via the mammillothalamatic tract (Seki & Zho, 1984; Shibata, 1992), suggest that MB may exert a theta-rhythmic influence on the anterior thalamus, much like the hippocampus on MB. To assess this, we examined the activity of cells of the three divisions of the anterior thalamus (anteroventral [AV], anterodorsal [AD], anteromedial nuclei) with respect to the hippocampal EEG and found that neurons in all divisions fired rhythmically with theta (Vertes, Albo, & Viana Di Prisco, 2001; Albo, Viana Di Prisco, & Vertes, 2003), with the highest percentage in the AV nucleus.

We found (Vertes et al., 2001) that approximately 75% of AV neurons fired rhythmically with theta and the activity of about half of them (46%) was highly correlated with theta, as exemplified by the AV neuron of Figure 3. As depicted, with the onset of theta (elicited by tail pinch), the activity of the cell changed from an irregular pattern to a highly rhythmical pattern, synchronous with theta (Figure 3A). This change from nonrhythmical (control) to rhythmical (theta) activity is exemplified by the rhythmical peaks in the autocorrelogram (Figure 3B), unit-theta locked EEG oscillations (spike-triggered averaging) in the cross-correlogram (Figure 3C), and the pronounced coherence between unit discharge and the hippocampal EEG at theta frequency (about 3.3 Hz; Figure 3D). By comparison with the large percentage of theta-rhythmic neurons in AV (~75%), approximately 12% of AD cells and 6% of anteromedial nucleus cells were found to discharge rhythmically, strongly synchronous with theta (Albo et al., 2003).

As discussed, the MB distributes massively to the anterior thalamus and appear to exert a theta-rhythmic influence on the anterior thalamus, mainly on AV. The MB also projects strongly to, and receive pronounced projections from, the tegmental nuclei of the brain stem (the dorsal and ventral tegmental nuclei of Gudden; Allen & Hopkins, 1989, 1990; Hayakawa & Zyo, 1990, 1991), sug-
In a recent examination of the activity of cells of the ventral tegmental nucleus (VTg) and its rostral extension, the anterior tegmental nucleus (ATg), in anesthetized rats, we found that all cells of the VTg/ATg \((n = 37)\) fired rhythmically in bursts with theta (Kocsis, Viana Di Prisco, & Vertes, 2001). Furthermore, the discharge of VTg and ATg cells was highly coherent with the theta rhythm; that is, for segments of the record, coherences (spectral covariance) often exceeded 0.90. The theta-associated activity of VTg cells was often so intense that it could be readily identified as the electrode just approached the nucleus, that is, before single spikes could be clearly identified.

Figure 4 shows a strongly theta rhythmically firing VTg neuron (Kocsis et al., 2001). As depicted, the neuron discharged in rhythmic bursts with theta (Figure 4C), as demonstrated by rhythmical peaks in the autocorrelogram (Figure 4E) and a dominant rhythmic component at 3.7 Hz in the VTg-hippocampal cross-correlogram (Figure 4G). By contrast, during nontheta states (desynchronization), the neuron fired irregularly (Figure 4D) with no significant peaks in the autocorrelogram (Figure 4F) or in the spike-triggered average (Figure 4H).

Consistent with these findings, Bassant and Pindessous-Jazat (2001) demonstrated that the activity of VTg cells in behaving rats was highly correlated with theta during both waking and REM sleep. They further drew attention to the marked similarity of the rhythmic firing of VTg and MS/DBv cells, stating, “Interestingly, the characteristics of the rhythmic discharges in VTn are close to those observed in MS/DB, a region crucial for the generation of the hippocampal theta rhythm” (p. 810).

The activity of VTg is driven by MB projections, which are mainly excitatory (Hayakawa & Zyo, 1990), and return VTg to MB projections are predominantly inhibitory (GABAAergic; Hayakawa & Zyo, 1991), thus forming a recurrent excitatory-inhibitory network. This MB-VTg network exhibits a number of similarities with other excitatory-inhibitory recurrent systems known to generate rhythmic oscillations at low frequencies (Contreras & Steriade, 1996; Ritz & Sejnowski, 1997; Plenz & Kital, 1999). We suggest that VTg-MB connections may be an important subloop grafted onto Papez’s circuit, involved in maintaining theta rhythmical activity throughout Papez’s circuit.

**Theta-Rhythmic Cells and Head Direction (HD) Cells**

There is a remarkable correspondence in rats between structures containing theta-rhythmic neurons and those containing HD cells (Taube, 1998; Vann & Aggleton, 2004). HD cells fire selectively when a rat is facing or oriented in a particular direction (e.g., north-east) irrespective of its location in its environment (Taube, 1998). Both HD and theta-rhythmic cells have been described in the tegmental nuclei of Gudden, the MB, the anterior thalamus, posterior cingulate (retrosplenial) cortex, and the subiculum/hippocampus.

In addition (and importantly), theta and HD cells have been localized to separate subnuclei of structures (of Papez’s circuit) containing them. For example, HD cells are present in the dorsal tegmental nucleus (Bassett & Taube, 2001; Sharp, Tinkelman, & Cho, 2001), the lateral MB (Blair, Cho, & Sharp, 1998, 1999; Stackman & Taube, 1998), the AD nucleus of the thalamus (Blair et al., 1999; Blair, Lipscomb, & Sharp, 1997; Blair & Sharp, 1995; Goodridge & Taube, 1997; Taube & Muller, 1992).
Figure 4: Neuron of the Ventral Tegmental Nucleus of Gudden (VTg) That Fired Rhythmically in Bursts Synchronous With the Hippocampal Theta Rhythm.


NOTE: (A) Schematic representation of Papez’s circuit and anatomical interconnections of the tegmental nuclei (of Gudden) with Papez’s circuit. (B) Histological section localizing the recording site in VTg of the cell depicted below. (C, D) The discharge characteristics of a VTg neuron (lower traces) together with the simultaneously recorded hippocampal electroencephalogram (upper traces) showing a change from an irregular pattern of activity during nontheta states (D) to a rhythmic bursting pattern during theta (C) elicited with sensory stimulation. Autocorrelograms (E, F) and cross-correlograms (G, H) showing that the VTg neuron fired rhythmically, phased locked to the theta rhythm during theta (E, G) but not during control (nontheta) conditions (F, H). AT = anterior thalamus; DR = dorsal raphe nucleus; HF = hippocampal formation; MB = mammillary body; MR = median raphe nucleus.
1998; Zugaro, Tabuchi, Fouquier, Berthoz, & Wiener, 2001), the anterior retrosplenial cortex (Chen, Lin, Green, Barnes, & McNaughton, 1994; Cho & Sharp, 2001), and the postsubiculum (Golob, Wolk, & Taube, 1998; Taube & Muller, 1998; Taube, Muller, & Ranck, 1990). By contrast, theta cells are present in the VTg (Bassant & Poindeessou-Jazat, 2001; Kocsis et al., 2001), the medial MB (Bland et al., 1995; Kirk et al., 1996; Kocsis & Vertes, 1994, 1997), the AV nucleus of the thalamus (Albo et al., 2003; Vertes et al., 2001), the posterior retrosplenial cortex (Albo, Viana Di Prisco, Truccolo, Vertes, & Ding, 2001; Talk, Kang, & Gabriel, 2004), and the hippocampus/entorhinal cortex (EC; Alonso & Garcia-Austt, 1987; Brazhnik, Vinogradova, Stafekhina, & Kitchigina, 1993; Colom & Bland, 1987; Dickson, Kirk, Oddie, & Bland, 1995; Fox & Ranck, 1981; Stewart, Quirk, Barry, & Fox, 1992). Finally, the various subnuclei comprising these parallel, but segregated, theta and HD systems are themselves anatomically interconnected, with little crossover between systems.

Vann and Aggleton (2004) recently designated these two systems as the medial and lateral mammillary systems (and associated structures): the medial being theta and the lateral HD. As depicted in Figure 5 (from Vann & Aggleton, 2004), the medial system (theta) consists of VTg, the medial MB, AV, and the subiculum/EC; the lateral system (HD) consists of the dorsal tegmental nucleus, lateral MB, AD and the pre-, para-, and postsubiculum.

Vann and Aggleton (2004) suggested that the recent demonstration that the MB contains two (theta and HD) functionally segregated systems (or in their terms, two memory systems in one) may be a key to understanding the role of MB in memory, which has remained elusive despite the fact that “the mammillary bodies have been implicated in amnesia perhaps for longer than any other single brain region” (p. 35). Regarding these two systems and their possible interaction in memory processing, they stated,

At the same time it is assumed that the medial and lateral mammillary systems function in a synergistic way, as reflected by their common connections with the hippocampus, tegmentum and anterior thalamus. This cooperative activity raises the question of where the functions of these two systems might interact. Anatomically, the most plausible candidate regions are the retrosplenial cortex and the hippocampal formation, although this has not been formally examined. There is, in addition, the functional question of why head direction and theta might have a special relationship. The answer to this presumably lies in the hippocampus, as so many of the effects of mammillary body damage mimic those of hippocampal damage, but to a lesser degree. (p. 42)

The question of “why head direction and theta might have a special relationship” is an intriguing one and may involve the special properties of bursting neurons (Lisman, 1997). For instance, in a review comparing the characteristics of single spikes to bursts, Lisman (1997) concluded that, relative to single spikes, bursts represent a much more effective (or reliable) mode of communication between neurons. Specifically, Lisman pointed out that there is a very low probability that single presynaptic spikes can generate action potentials in postsynaptic cells (unreliable synapses), compared to a high probability that presynaptic bursts would drive postsynaptic neurons (reliable synapses). Hence, bursts convert unreliable to reliable synapses (Lisman, 1997). Although various factors undoubtedly contribute to the efficacy of bursts, presumably one of the most important is the steady accumulation of intracellular calcium in presynaptic terminals with successive spikes of bursts, eventually leading to the release of sufficient amounts of transmitter to fire postsynaptic cells (Tank, Regehr, & Delaney, 1995; Wu & Saggau, 1994).

A number of recent studies have shown that relay neurons of the thalamus fire tonically or in bursts, with differing characteristics in the two modes of discharge (Guido, Lu, & Sherman, 1992; Guido & Weyand, 1995; Ramcharan, Gnadt, & Sherman, 2000; Sherman, 1996; Weyand, Boudreaux, & Guido, 2001). For example, Guido and Weyand (1995) demonstrated in behaving cats that a large percentage (71%) of cells of the lateral geniculate nucleus of thalamus discharged in bursts to a gradient passed through the visual field and concluded that bursting “provides a form of visual amplification” serving to detect salient visual stimuli. In like manner,
Fanselow, Sameshima, Baccala, and Nicolelis (2001) reported that cells of the ventrobasal thalamus, when firing in bursts during whisker movements/twitches, are maximally sensitive to somatosensory stimulation in the periods immediately (120 ms) following the burst. They indicated that bursts “generate a period during which neurons are highly sensitive to incoming stimuli.” Finally, Swadlow and Gusev (2001) demonstrated in awake rabbits that ventrobasal thalamic neurons produce significantly stronger postsynaptic actions on cells of the somatosensory cortex when firing in bursts than tonically.

In a manner similar, then, to relay cells of the thalamus, the burst firing of neurons of Papez’s circuit at theta frequency could selectively modify the activity of postsynaptic targets, rendering them more responsive to other inputs. For example, the theta burst discharge of neurons of the AV nucleus of the thalamus could modify the activity of target cells of the hippocampus and/or the retrosplenial cortex, increasing their responsiveness to other inputs, such as from HD cells of the AD thalamus, thereby magnifying the influence of anterodorsal HD cells on hippocampal/retrosplenial neurons.

It would appear that directional information is very critical for a rat (and other species) when engaged in locomotor/exploratory behaviors (theta states) and less so during nonlocomotor activities such as grooming or consumatory acts (nontheta states). Accordingly, theta may serve as an important signal involved in the differential processing of HD activity under the two conditions (e.g., locomotion and grooming); that is, only when HD activity is coupled with theta-rhythmic discharge is HD activity processed and used to guide spatial behaviors.

In summary, recent evidence indicates that a theta-rhythmic signal exits the hippocampus and reverberates through structures of Papez’s circuit, possibly involved in memory processing functions of this circuit. Two parallel, and anatomically segregated, systems within Papez’s circuit have been identified: theta and HD circuits. The two systems may functionally interact at multiple levels of the circuit to process HD information used for spatial learning/navigation.

**THE NUCLEUS REUNIENS (RE) OF THE THALAMUS: A MAJOR SOURCE OF MULTIMODAL LIMBIC INFORMATION TO THE HIPPOCAMPUS**

Although the hippocampus receives a diverse array of information, there are few direct inputs to the hippocampus. Excluding monoaminergic afferents, few structures project directly to the hippocampus, essentially only the EC, medial septum, basal nucleus of amygdala, the SUM, and the RE of the thalamus (Witter & Amaral, 2004). Of these, the RE has been the least examined.

The RE lies ventrally on the midline, dorsal to the third ventricle and ventral to the rhomboid nucleus of the thalamus, and extends longitudinally, virtually throughout the thalamus (see Swanson, 1998). The RE is the largest of the midline nuclei of the thalamus. The RE is the major source of thalamic afferents to the hippocampus and parahippocampal structures (Bokor, Csaki, Kocsis, & Kiss, 2002; Dolleman-Van der Weel & Witter, 1996; Herkenham, 1978; Riley & Moore, 1981; Room & Groenewegen, 1986; Su & Bentivoglio, 1990; Wouterlood, 1991; Wouterlood, Saldana, & Witter, 1990; Wyss, Swanson, & Cowan, 1979b; Yanagihara, Niimi, & Ono, 1987). RE distributes densely to CA1 of Ammon’s horn, the ventral subiculum, and the medial EC, as well as moderately to the dorsal subiculum, the parasubiculum, and the lateral EC (Bokor et al., 2002; Su & Bentivoglio, 1990; Wouterlood, 1991; Wouterlood et al., 1990). There are essentially no RE projections to the dentate gyrus. RE fibers form asymmetric (excitatory) contacts predominantly on distal dendrites of pyramidal cells in stratum lacunosum-moleculare of CA1 (Wouterlood et al., 1990).

Based on the relationship of RE to the hippocampus, we were interested in sources of afferent projections to the RE. In an initial report (Vertes, 2002), we examined projections from the medial prefrontal cortex (mPFC) to the thalamus, with a concentration on RE. Injections of the anterograde tracer, PHA-L, were made in the four divisions of the mPFC (medial agranular, anterior cingulate, prelimbic cortex, and infralimbic cortex) and patterns of labeling determined. In brief, we showed that (a) the infralimbic (IL), prelimbic (PL), and anterior cingulate cortices distribute heavily and selectively to the mediodorsal thalamus, including the paratenial, paraventricular, interanteromedial, anteromedial, intermediodorsal, mediodorsal, reuniens, and central medial nuclei; (b) the medial agranular cortex distributes strongly to the rostral intralaminar nuclei (central lateral, paracentral, central medial nuclei) and to the ventromedial and ventrolateral nucleus of thalamus; and (c) importantly, all four divisions of the mPFC project densely (or massively) to the RE.

The pattern of distribution of infralimbic fibers to the thalamus is schematically illustrated in Figure 6. As depicted, labeling is restricted to the midline nuclei of the thalamus, and within the midline thalamus is very dense in the mediodorsal nucleus and RE. At the rostral thalamus (Figure 6A-6C), labeling spreads dorsoventrally throughout the midline, whereas at the caudal thalamus (Figure 6D-6G), labeling is essentially confined to the paraventricular and mediodorsal nuclei,
Several reports in various species have described prominent projections from the hippocampus to the prefrontal cortex (Carr & Sesack, 1996; Cavada, Llamas, & Reinoso-Suarez, 1983; Ferino, Thierry, & Glowinski, 1987; Irle & Markowitsch, 1982; Jay, Glowinski, & Thierry, 1989; Jay & Witter, 1991; Swanson, 1981; van Groen & Wyss, 1990). In rats, hippocampal projections to the mPFC arise from temporal aspects of CA1 and the subiculum and terminate in a fairly restricted region of the ventral mPFC, including the medial orbital area, IL, and PL (Jay et al., 1989; Jay & Witter, 1991). Despite well-documented hippocampal to mPFC projections, there are essentially no direct projections from the mPFC to the hippocampus (Beckstead, 1979; Buchanan, Thompson, Maxwell, & Powell, 1994; Hurley, Herbert, Moga, & Saper, 1991; Reep, Corwin, Hashimoto, & Watson, 1987; Room, Russchen, Groenewegen, & Lohman, 1985; Sesack, Deutch, Roth, & Bunney, 1989; Takagishi & Chiba, 1991).

In the absence of prefrontal projections to the hippocampus, the findings that the mPFC projects strongly to the RE (Vertes, 2002, 2004), coupled with the demonstration that RE is a major source of afferents to the hippocampus, suggests that RE is an important relay in the transfer of information from the mPFC to the hippocampus. This system of connections (mPFC-RE-hippocampus) would appear to be the major route from the prefrontal cortex to the hippocampus and accordingly would complete an important functional loop between the hippocampus and mPFC.

In a continuing analysis of RE, we recently examined other afferents to the RE, or the totality of inputs to the RE (McKenna & Vertes, 2004). Injections of the retrograde tracer Fluorogold were made into various regions of RE and patterns of retrogradely labeled cells determined. We showed that RE receives a very diverse and widely distributed set of afferent projections. Figure 8 schematically depicts patterns of projections to the rostromedial RE. As illustrated, the RE receives pronounced projections from several cortical and subcortical sites. They include (a) the orbitomedial (see also above), insular, ectorhinal, perirhinal, and retrosplenial cortices; (b) the CA1/subiculum of hippocampus; (c) the claustrum, lateral septum, substantia innominata, and lateral preoptic nucleus of the basal forebrain; (d) the medial nucleus of amygdala (MEA); (e) the paraventricular and lateral geniculate nuclei of thalamus; (f) the zona incerta; (g) the anterior, ventromedial, lateral, posterior, supramammillary, and dorsal premammillary nuclei of the hypothalamus; and (h) the ventral tegmental area, periaqueductual gray, medial and posterior pretectal nuclei, superior colliculus, precommissural nucleus, parabrachial nucleus, laterodorsal and pedunculopontine tegmental nuclei, nucleus incertus, and the dorsal and median raphe nuclei of the brainstem.
Figure 7: Darkfield Photomicrograph of a Transverse Section Through the Rostral Diencephalon Showing Patterns of Labeling at the Rostral Thalamus Produced by a PHA-L Injection in the Infralimbic Cortex.


NOTE: Note pronounced labeling in the paraventricular, mediodorsal (MD), and intermediodorsal nuclei, dorsally, and the nucleus reuniens (RE), ventrally. SM = stria medullaris Scale bar = 450 µm.
Figure 8: Series of Representative Rostrocaudally Aligned Schematic Transverse Sections (A-P) Depicting the Location of Retrogradely Labeled Cells in the Brain Produced by a Fluorogold Injection in the Rostromedial Part of Nucleus Reuniens (G).

SOURCE: Reprinted from McKenna and Vertes (2004), pp. 120-121, with permission of Wiley-Liss, Inc.

NOTE: Circles = 10 cells; triangles = 5 cells; stars = 2 cells. AC,d = anterior cingulate cortex, dorsal division; ACB = nucleus accumbens; AGm = medial agranular (prefrontal) cortex; AGl = lateral agranular (prefrontal) cortex; AHN = anterior hypothalamic nucleus, AL,d,p,v = agranular insular cortex, dorsal, posterior, ventral divisions; AM = antero medial nucleus of thalamus; APN = anterior pretectal nucleus; BST = bed nucleus of stria terminalis; CA1, CA3 = field CA1, CA3 of Ammon’s horn; CEA = central nucleus of amygdala; CLA = claustrum; COA = cortical nucleus of amygdala; COM = commissural nucleus of PAG; CP = caudate putamen; DBh = nucleus of the diagonal band, horizontal limb; DG = dentate gyrus of hippocampus; DMH = dorsomedial nucleus of hypothalamus; DR = dorsal raphe nucleus; EC = entorhinal cortex; ECT = ectorhinal cortex; EN = endopiriform nucleus; FF = fields of Forel; IC = inferior colliculus; IL = infralimbic cortex; IP = interpeduncular nucleus; LD = lateral dorsal nucleus of thalamus; LDT = laterodorsal tegmental nucleus; LG,d,v = lateral geniculate nucleus, dorsal, ventral divisions; LH = lateral habenula; LHyr = lateral hypothalamic area; LS = lateral septal nucleus; MA = magnocellular preoptic nucleus; MB = mammillary bodies; MD = mediodorsal nucleus of thalamus; MgRe = magnocellular reticular nucleus; MG5 = motor nucleus of trigeminal nerve; MPN = medial preoptic nucleus; MPO = medial preoptic area; MR = median raphe nucleus; MS = mesencephalic reticular formation; NTS = nucleus of solitary tract; N7 = facial nucleus; OC = occipital cortex; OT = olfactory tubercle; PAG = periaqueductal gray; PARA = parahippocampal gyrus; PBm = parabrachial nucleus, medial division; PCE = precentral nucleus of PAG; PERI = perirhinal cortex; PH = posterior nucleus of hypothalamus; PIR = piriform cortex; PL = prelimbic cortex; PN = nucleus of pons; PGC = nucleus paragigantocellularis; POST = postsubiculum of hippocampus; PPN = pedunculopontine tegmental nucleus; PRE = presubiculum of hippocampus; PV = paraventricular nucleus of thalamus; PVR = parvocellular reticular nucleus; RE = nucleus reuniens of thalamus; RM = raphe magnus; RPC = nucleus reticularis pontis
Figure 9 depicts patterns of retrograde labeling in the subiculum (of hippocampus) following the RE injection of Figure 8. As shown, the entire dorsoventral extent of the ventral subiculum (postsubiculum, dorsal/ventral subiculum) was densely labeled. These pronounced subicular-RE projections complement equally dense return RE projections to the hippocampus (Bokor et al., 2002; Dollemann-Van der Weel & Witter, 1996; Wouterlood, 1991; Wouterlood et al., 1990), indicating strong reciprocal connections between these structures. Figure 10 shows prominent cell labeling in the amygdala following a rostrolateral RE injection, mainly within the MEA and to a lesser extent in the anterior cortical and basomedial nuclei. An early report by Canteras, Simerly, and Swanson (1995) also described strong projections from the MEA to the RE, leading them to conclude that “another potentially significant way for the MEA to access the hippocampal formation is by way of inputs from the nucleus reuniens” (p. 238).

In summary, the RE receives pronounced projections from diverse regions of the brain involved in a host of functions. To our knowledge, no other nucleus of the thalamus, and certainly none outside of the midline thalamus, receives a comparable degree and diversity of inputs. Although RE receives projections from several structures of the brain, the output of RE is quite limited. RE essentially distributes only to the hippocampal formation, EC, and orbital/medial prefrontal cortices (Bokor et al., 2002; Conde, Maire-Lepoivre, Audinat, & Crepe, 1995; Herkenham, 1978; Ohtake & Yamada, 1989; Reep & Corwin, 1999; Reep, Corwin, & King, 1996; Risold, Thompson, & Swanson, 1997; Van der Werf, Witter, & Groenewegen, 2002; Vertes, Hoover, & Sherman, 2002; Vertes, McKenna, do Valle, Sherman, & Hoover, 2003; Wouterlood, 1991; Wouterlood et al., 1990; Zhang & Bertram, 2002). The RE appears to be a critical site for the convergence of information from various sources (mainly from limbic/limbic-related structures) and its transfer to the hippocampus and prefrontal cortex.

**RE Actions on the Hippocampus and mPFC**

Although it has been known for some time that the RE is a major input to the hippocampus (Herkenham, 1978), few studies have examined the physiological effects of the RE on the hippocampus. Two recent reports have shown, however, that the RE exerts significant actions at the CA1 of the hippocampus (Bertram & Zhang, 1999; Dollemann-Van der Weel, Lopes da Silva, & Witter, 1997).

Dollemann-Van der Weel et al. (1997) demonstrated that RE stimulation produced large negative-going field potentials at the stratum lacunosum-moleculare of CA1 of the hippocampus, indicative of prominent depolarizing actions on distal apical dendrites of CA1 pyramidal cells, as well as a marked facilitation of evoked responses at CA1 using a paired-pulse paradigm. They proposed that the RE may “exert a persistent influence on the state of pyramidal cell excitability,” depolarizing cells to close to threshold for activation by other excitatory inputs (p. 5684).

Consistent with this, Bertram and Zhang (1999) recently compared the effects of stimulation of the RE with stimulation of the CA3 region of the hippocampus on population responses (field excitatory postsynaptic potentials and spikes) at CA1 and reported that RE actions on CA1 were equivalent to, and in some cases considerably greater than, those of CA3 on CA1. They concluded that the RE projection to the hippocampus “allows for the direct and powerful excitation of the CA1 region. This thalamohippocampal connection bypasses the trisynaptic/commissural pathway that has been thought to be the exclusive excitatory drive to CA1” (p. 15).

As briefly discussed above, in addition to the hippocampus/EC, the RE also distributes strongly to the orbitomedial prefrontal cortex (see Vertes et al., 2003). In a manner shown for the hippocampus (Bertram & Zhang, 1999; Dollemann-Van der Weel et al., 1997), we recently demonstrated that RE stimulation produced marked excitatory actions on the mPFC. As depicted in Figure 11, RE stimulation gave rise to short-latency (~20 ms), large-amplitude (1-2 mV) evoked responses in the mPFC. The typical response consisted of a small positive deflection (P1) at about 7 ms, followed by a large negative deflection (N2) at 20 to 40 ms, and then a large positive wave at 60 to 80 ms. Although effects were observed throughout the dorsoventral extent of the mPFC with RE stimulation, they were most pronounced (i.e., largest amplitude) in the prelimbic and infralimbic cortices of the ventral mPFC (Figure 11).

The hippocampus and prefrontal cortex serve well-recognized roles in memory processing. In an interest-
Figure 9: Low- (A) and High-Magnification (B, C) Light-Field Photomicrographs Depicting Retrograde Cell Labeling in the Ventral Subicular Complex Produced by a Fluorogold Injection in the Rostromedial Part of the Nucleus Reuniens.


NOTE: As shown, pronounced numbers of labeled cells extended dorsal-ventrally throughout the subiculum within the postsubiculum and dorsal and ventral subiculum. (B, C) Clusters of labeled cells of the dorsal subiculum shown at high levels of magnification (see arrows). Scale bar for (A) = 325 µm; for (B) = 130 µm; for (C) = 65 µm.
ing variant on the role of the mPFC in memory (via interactions with the hippocampus), Buckner, Kelley, and Peterson (1999) suggested that the prefrontal cortex may promote memory formation without being directly involved in “intentional memorization.” According to the authors, the prefrontal cortex uses information in short-term (working) memory to deal with impending task demands, and its long-term storage may simply be a by-product of its use in meeting immediate demands. As such, the conversion from working memory to long-term stores involves the transfer of information from the mPFC to the hippocampus and adjacent structures of the temporal lobe. They stated,

One speculation would be that the critical cascade driving human memory formation occurs only when frontal activity provides information to medial temporal lobe structures. The medial temporal lobe may then function to bind together from frontal and other cortical regions to form lasting, recollectable memory traces. Thus, both regions would be critical to the conception of a memory, and lack of participation of either brain region would disrupt memory formation. (p. 313)

The demonstration that the RE is strongly reciprocally linked to the hippocampus and to the mPFC, and exerts pronounced excitatory actions on both structures, suggests that the RE may represent a critical interface between the hippocampus and orbitomedial prefrontal cortex in memory processing.

THE ROLE OF THE THETA RHYTHM OF THE HIPPOCAMPUS IN MEMORY

Although theta has been implicated in several functions including arousal (Green & Arduini, 1954) and recently sensorimotor integration (Bland & Colom, 1993; Bland & Oldie, 2001), the prevailing view is that theta serves a critical role in mnemonic functions of the hippocampus (N. Burgess, Maguire, & O’Keefe, 2002; Hasselmo, 2000; Hasselmo, Bodelon, & Wyble, 2002; Kahana, Seelig, & Masden, 2001; Kirk & Mackay, 2003; Vertes & Kocsis, 1997). In an early report, Winson (1978) described the important findings that small medial septal lesions that eliminated theta in the hippocampus produced severe spatial memory deficits in rats. Subsequent studies similarly reported that the loss of theta with reversible or irreversible lesions of the medial septum significantly altered performance on spatial (Leutgeb & Mizumori, 1999; M’Harzi & Jarrard, 1992; Mizumori, Perez, Alvarado, Barnes, & McNaughton, 1990) as well as nonspatial tasks (Asaka, Griffin, & Berry, 2002; Mizumori et al., 1990) in rats and rabbits.

In an early review (Vertes, 1986) we proposed that theta may potentiate memory in a manner comparable to the long-term changes produced by tetanic stimulation in long-term potentiation (LTP) experiments. Specifically, we stated that “theta rhythm, which involves the synchronous activation of large numbers of septohippocampal neurons, may act as a ‘natural tetanizer’ producing synaptic modifications at specific hippocampal sites supportive of long term changes at these sites” (p. 65). In effect, Vertes suggested that theta may potentiate the actions of other inputs to the hippo-
campus, possibly to encode information arriving via these afferents. This was supported by the early demonstration that septal stimulation, mimicking theta, significantly enhanced population responses in the hippocampus.

For instance, Krnjevic and Robert (1982) showed that single-pulse or tetanic stimulation of the medial septum significantly potentiated commissurally-elicited population spikes at CA1 and likened the process to what occurs naturally with theta. They stated,

"The fact that the septal facilitatory action is evoked most effectively by brief tetanic volleys at 50-100 Hz seems significant in view of previous observations that many septal units fire in 50-100 Hz bursts and that theta waves are especially readily evoked by septal stimulation in brief, high frequency trains. (p. 2181)"

In like manner, Buzsaki, Grastyan, Czopf, Kellenyi, and Prohaska (1981) described significantly larger amplitude population spikes at CA1 (to commissural stimulation) during theta-associated behaviors (e.g., running) than during nontheta associated behaviors (e.g., grooming and drinking) in freely moving rats and concluded that the medial septum, through its role in generating theta, “exerts a potent biasing effect on the efficacy of other afferents to the hippocampus” (p. 235).

Perhaps the strongest support, however, for the view that theta may act as "natural tetanizer" in the long-term modification of hippocampal activity is the demonstration that LTP is optimally elicited in the hippocampus with stimulation at theta frequency (for review, see Vertes & Kocsis, 1997). In an initial report, using the hippocampal slice preparation, Larson, Wong, and Lynch (1986) showed that LTP was most effectively induced in the CA1 area of rats by trains of stimulation that were separated by 200 ms (i.e., 5 Hz). Intervals shorter or longer than 200 ms produced significantly less, or no, potentiation. In a follow-up study, Staubli and Lynch (1987) showed that stimulation at theta fre-
quency also very effectively produced LTP at CA1 in the behaving rat, and it remained stable for 1 to 5 weeks or until the preparation deteriorated. The authors commented that it seemed remarkable that a brief period of stimulation (at theta frequency), which in total lasted about 300 ms, produced effects that persisted for several weeks but indicated that this would be expected of a process subserving memory. They stated that their findings “point to a possible link between the naturally occurring theta rhythm and the development of synaptic changes of the type needed for memory storage” (p. 233).

Diamond, Dunwiddie, and Rose (1988) subsequently examined various parameters of this effect in the hippocampal slice and behaving rat. Stimulation consisted of a single priming pulse followed 140 to 170 ms later by a high-frequency (100 Hz) burst ranging from 2 to 10 pulses. This was termed, and is now commonly referred to as, primed burst (PB) stimulation and the LTP elicited by it as primed burst potentiation. The authors showed that (a) priming intervals of 140 to 170 ms (i.e., between the priming pulse and bursts) produced LTP and shorter or longer intervals were ineffective; (b) 5 or 10 pulses that were not preceded by a priming pulse failed to elicit LTP; (c) significant PB potentiation was obtained with as few as 3 pulses (a single priming pulse followed 170 ms later by a 2-pulse burst); (d) PB potentiation could be elicited both homo- and heterosynaptically, that is, with the priming pulse and bursts delivered to the same or separate sets of afferents to CA1, respectively; (e) LTP elicited with PB and conventional stimulation parameters were not additive, suggesting common underlying mechanisms for the two forms of LTP; and (f) the magnitude and duration of PB potentiation at CA1 was virtually the same for the slice and awake preparation.

Rose and Dunwiddie (1986) pointed out that prior to the demonstration of PB potentiation, a major problem in viewing LTP as an endogenous substrate for memory was that the stimulation parameters commonly used to induce it (e.g., 100 Hz for 1 second) were very nonphysiological. This problem has seemingly been circumvented with the demonstration that LTP can be induced in the hippocampus with as few three to five pulses, when pulses are delivered at theta frequencies.

The findings that LTP can be optimally induced in the hippocampus with stimuli mimicking theta suggest a role for the naturally occurring theta rhythm in LTP/LTP-like effects. In this regard, it has been shown in the hippocampal slice (Huerta & Lisman, 1993, 1995, 1996) and intact preparation (Bramham & Srebro, 1989; Holscher, Anwyl, & Rowan, 1997; Pavlides, Greenstein, Grudman, & Winson, 1988) that stimulation delivered in the presence, but not in the absence, of theta generates LTP and that effects are most pronounced when stimulation is given on the positive phase of the theta rhythm (Holsher et al., 1997; Huerta & Lisman, 1993, 1995, 1996; Pavlides et al., 1988). Pavlides et al. (1988) showed in urethane anesthetized rats that PB stimulation of the perforant path delivered on the positive phase of the theta rhythm induced LTP, whereas that given on the negative phase of theta resulted in a decrease in population spike amplitudes or was without effect. Huerta and Lisman (1995, 1996) similarly reported that a single burst of four pulses at the peaks of carbachol-elicited theta in the hippocampal slice produced long-lasting LTP at CA1, whereas stimulation of the trough of theta produced a suppression (depotentiation) of previously potentiated synapses.

In accord with the foregoing, Kandel and colleagues (Bach, Hawkins, Osman, Kandel, & Mayford, 1995; Mayford et al., 1996; Mayford, Wang, Kandel, & Odell, 1995; Rotenberg, Mayford, Hawkins, Kandel, & Muller, 1996) recently examined several hippocampal-related functions in transgenic mice in which calcium-calmodulin-dependent kinase II (CaMKII) was rendered persistently active by replacing threonine286 with an aspartate group (CaMKII-Asp286). The genetically altered mice (CaMKII-Asp286) showed (a) a loss of LTP elicited with stimulation at theta frequency but not that elicited with high-frequency stimulation, (b) a disruption of place cell activity, and (c) severe deficits in spatial learning. Based on these findings, Kandel and associates (Bach et al., 1995) proposed that the endogenous theta rhythm may exert LTP-like effects, synaptically strengthening place cells leading to the formation of spatial maps necessary for spatial learning/memory. They stated,

There are, however, several reasons to believe that frequencies of about 5 Hz may be particularly important for spatial memory, because these frequencies stimulate endogenous firing patterns that seem important for spatial memory. When a rodent explores a new environment, it displays a 4-12 Hz theta rhythm in the hippocampus driven by cholinergic synaptic inputs from the medial septum. Cholinergic activation in turn leads to a depolarizing oscillation at the theta frequency in the membrane potential of the CA3 pyramidal neurons. At the peak of these depolarizations, the CA3 cells fire one or more action potentials that, in turn, might induce theta frequency LTP in the CA1 neurons. Thus, the learning impairment seen in CaMKII-Asp286 transgenic mice may be due to the lost capacity to form LTP in response to the synaptic activation patterns that occur during learning. (p. 913)

Finally, in line with the demonstration that LTP can be effectively elicited in the presence but not in the absence of theta, Berry and colleagues (Griffin, Asaka,
Darling, & Berry, 2004; Seager, Johnson, Chabot, Asaka, & Berry, 2002) recently reported that the rate of acquisition of a classically conditioned eye-blink response in rabbits was significantly accelerated when conditioning trials were given in the presence than in the absence of theta. Essentially, rabbits reached criteria responding in approximately half the time (or with 50% few trials) when trials were timed to occur with theta than without it. The researchers concluded that “naturally occurring theta activity plays a facilitatory role in the establishment of a neural representation of the CS-US contingency” (Griffin et al., 2004, p. 408).

**Theta and Human Memory**

Several recent studies have directly linked theta to memory-processing functions in humans (for review, see Basar, Schurmann, & Sakowitz, 2001; Bastiaansen & Hagoort, 2003; Klimesch, 1999). In an initial report, Klimesch (1996) described significant increases in the power of theta (4–7 Hz) during the encoding of words that were subsequently successfully recalled compared to those not recalled. In a follow-up study, they demonstrated equivalent increases in theta during the successful retrieval of lists of words, more pronounced in good than poor performers (Doppelmayr, Klimesch, Schwaiger, Aninger, & Winkler, 1998).

Similar changes in the percentage and/or power of theta have been described for the encoding/retrieval of visual (e.g., pictures; Klimesch et al., 2001), auditory (Krause, Sillanmaki, Haggqvist, & Heino, 2001), and tactile (Grunwald et al., 1999, 2001) information, as well as for tasks involving declarative memory, recognition memory, working memory, and spatial memory in humans (see below).

With respect to recognition memory, A. P. Burgess and Gruzelier (1997) described a greater than 2-fold increase in the power of theta during the presentation of a previously viewed (or repeated) list of words, compared to a new list of words. In an early report on working memory, Gevins, Smith, McEvoy, and Yu (1997) demonstrated significant increases in theta in the anterior cingulate cortex during performance of a working memory dual n-back task. Specifically, participants were presented with a short list of 1 of 12 letters in 1 of 12 locations on a video monitor and were required to match a probe letter with a letter n-back on the list (e.g., three back)—the letter or its location. Theta increased as a function of increased (working) memory load on the task, with additional changes occurring with improved performance on the task. These basic findings have been confirmed in several subsequent studies involving working memory tasks (Fell et al., 2003; Jensen & Tesche, 2002; Raghavachari et al., 2001; Sarnthein, Petsche, Rappelsberger, Shaw, & von Stein, 1998; Tesche & Karhu, 2000; Weiss, Muller, & Rappelsberger, 2000).

Recording EEG activity with depth (or subdural) electrodes in epileptic patients (as opposed to conventional scalp recordings), Kahana, Sekuler, Caplan, Kirschen, and Madsen (1999) demonstrated task-dependent changes in theta in various regions of the cortex during the navigation of virtual mazes. They showed that theta activity (a) was not continuously present but occurred during distinct well-defined episodes associated with the learning/recall of virtual mazes, (b) was visible in the raw EEG traces, and (c) was significantly more pronounced with the learning of complex 12-choice compared to simpler 6-choice mazes. They concluded that “it is likely that theta oscillations are important in human spatial navigation” (p. 783).

These investigators (Raghavachari et al., 2001) subsequently described similar findings using a nonspatial, verbal working memory task: the Sternberg task. Similar to the n-back task (see above), participants were presented with a list of one to four letters (consonants) and were required to determine whether a “probe” matched letters on the list. They showed that theta activity dramatically increased at the start of the trial, continued through the trial, and abruptly ended at the termination of the trial, a phenomena termed *gating*. They further reported that increases in theta were dependent on working memory components of the task and not on sensory or motor aspects of the task.

In contrast to (or in addition to) overall changes in levels of theta during memory-associated tasks, recent reports have described changes in degrees of coherence (spectral covariance) of theta between regions of the cortex (or between cortex and hippocampus) during working memory tasks. Sarnthein et al. (1998) reported strong theta coherence between the prefrontal and posterior association cortices during the acquisition of verbal and visuospatial working memory tasks. Participants were shown either a short sequence of (keyboard) characters or abstract line drawings and, following a 4-second delay, were required to reproduce them. Coherence was high during the 4-second working memory interval, leading the authors to conclude that “low frequency interactions between the prefrontal cortex and posterior association areas mediate working memory processes” (p. 7096).

In like manner, Fell et al. (2003) demonstrated that theta activity was highly coherent between the hippocampus and rhinal cortex during the encoding of successfully versus nonsuccessfully recalled words. The authors stated that this coupling of theta between the hippocampus and rhinal cortex “supports the hypothesis of a specific function of theta oscillations in declarative memory formation” (p. 1086).
Several lines of evidence indicate that the theta rhythm is critically involved in memory processing functions, as follows: (a) LTP in the hippocampus is optimally elicited with stimulation at theta frequency, (b) stimulation delivered in the presence but not in the absence of theta potentiates population responses in the hippocampus, (c) discrete medial septal lesions that abolish theta produce severe learning/memory deficits, (d) the loss of LTP with PB (or theta) stimulation in mutant mice is associated with a pronounced disruption of place cell activity and spatial memory, and (e) task-dependent increases in the percentage and/or power of theta have been described in the cortex and hippocampus of humans during performance of various episodic/declarative, recognition, spatial, and working memory tasks.

CONCLUSIONS

As reviewed above, theta promotes memory, and the question that begs answering is “by what mechanism(s)?” Insights into the process may be gained by reference to factors associated with LTP (see also above). In a recent review of LTP, Malenka and Nicoll (1999) described what they termed triggering mechanisms for LTP. According to the authors, LTP is triggered by the combined actions of high-frequency (e.g., tetanic stimulation) and low-frequency (normal synaptic) inputs to N-methyl-D-aspartate (NMDA) receptor–containing cells. The high-frequency (or strong) signal drives postsynaptic neurons to threshold for the opening of NMDA receptor channels, which become activated by the simultaneous release of glutamate from synaptic inputs to these neurons. This was referred to as the “pairing protocol.” As further pointed out, the opening of NMDA receptor channels results in a massive influx of calcium, which initiates a series of events that leads to a restructuring of the postsynaptic membrane and an eventual strengthening of connections between pre- and postsynaptic cells (Malenka & Nicoll, 1999).

By analogy, we suggest that the theta rhythm represents the strong depolarizing drive to the hippocampus, whereas other inputs (mainly cortical) constitute the...
information-carrying synaptic input to the hippocampus and, when coupled, produce lasting changes. Specifically, during theta-associated states, theta would drive large populations of hippocampal neurons to threshold for the activation of NMDA receptor channels, which, when coupled with the release of glutamate from other inputs to these cells, would result in the opening of NMDA channels and consequent cellular changes. Accordingly, events occurring coincident with theta would have greater (or selective) access to the hippocampus. In effect, theta would serve as a significance signal to the hippocampus; that is, information arriving with theta would be stored (at least temporarily) in the hippocampus, whereas information arriving in the absence of theta would not be encoded—or not to the same degree as that reaching the hippocampus concurrently with theta.

To conclude, the hippocampus receives two main types of input: theta from ascending brain stem-diencephalop septohippocampal systems (Figure 12) and information bearing mainly from thalamocortical/cortical systems (Figure 12). The temporal convergence of activity from these two systems would result in the encoding of information in the hippocampus mainly reaching it from the EC and RE (Figure 12).

REFERENCES
Bland, B. H., Oddie, S. D., Colom, L. V., & Vertes, R. P. (1994). Extrinsic modulation of medial septal cell discharges by the ascending...


Holscher, C., Amsel, R., & Rowan, M. J. (1997). Stimulation on the positive phase of hippocampal theta rhythm induces long-term potentiation that can be depotentiated by stimulation on the negative phase in area CA1 in vivo. *Journal of Neuroscience*, 17, 6470-6477.


Leranth, C., & Kiss, J. (1996). A population of supramammillary area calretinin neurons terminating on medial septal area cholinergic and lateral septal area calbindin-containing cells are aspartate/glutamatergic. *Journal of Neuroscience*, 16, 7699-7710.


