Exp Brain Res (2002) 145:383–394
DOI 10.1007/s00221-002-1123-8

RESEARCH ARTICLE

**Gonzalo Viana Di Prisco · Zimbul Albo · Robert P. Vertes · Bernat Kocsis**

# Discharge properties of neurons of the median raphe nucleus during hippocampal theta rhythm in the rat

Received: 20 August 2001 / Accepted: 2 April 2002 / Published online: 13 June 2002


**Abstract** The serotonin (5-HT)-containing median raphe nucleus has been shown to be critically involved in the control of desynchronized (non theta) states of the hippocampal electroencephalogram (EEG). We examined the activity of 181 cells of the median raphe nucleus in the urethane-anesthetized rat and found that approximately 80% (145/181) of them showed changes in activity associated with changes in the hippocampal EEG. These cells were subdivided into theta-on (68%) and theta-off (32%) based on increased or decreased rates of activity with theta, respectively. They were further classified as slow-firing (~1 Hz), moderate-firing (5–11 Hz), or fast-firing (>12 Hz) theta-on or theta-off cells. The slow-firing cells as well as a subset of moderate-firing theta-off cells displayed characteristics of "classic" serotonin-containing raphe neurons. All fast-firing neurons were theta-on cells and showed either tonic or phasic (rhythmical) increases in activity with theta. We propose that: (1) the slow-firing cells (on and off) as well as a subset of moderate-firing theta-off cells are serotonergic neurons; (2) the phasic and tonic fast-firing theta-on cells are GABAergic cells; and (3) these populations of cells mutually interact in the modulation of the hippocampal EEG. An activation of local serotonergic and GABAergic theta-on cells would inhibit 5-HT slow- or moderate-firing theta-off projection cells to release or generate theta, whereas the suppression of serotonergic- or GABAergic theta-on cells would disinhibit 5-HT theta-off cells, resulting in a blockade of theta or a desynchronization of the hippocampal EEG. A role for the median raphe nucleus in memory-associated functions of the hippocampus is discussed.

**Keywords** Serotonin · Rhythmically bursting neurons · Hippocampal EEG desynchronization · Theta on/off cells · Learning/memory · Rat

G. Viana Di Prisco · Z. Albo · R.P. Vertes (✉)
Center for Complex Systems and Brain Sciences,
Florida Atlantic University, Boca Raton, FL 33431, USA
e-mail: Vertes@walt.ccs.fau.edu
Tel.: +1-561-2972362
Fax: +1-561-2972363

G. Viana Di Prisco
Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL 60607, USA

B. Kocsis
Laboratory of Neurophysiology, Department of Psychiatry, Harvard Medical School, Boston, MA 02115, USA

## Introduction

The median raphe (MR) nucleus is a major serotonin (5-HT)-containing nucleus of the midbrain, with extensive projections to the forebrain (Vertes and Martin 1988; Halliday et al. 1995; Leranth and Vertes 1999; Morin and Meyer-Bernstein 1999; Vertes et al. 1999; McKenna and Vertes 2001). A considerable body of evidence indicates that the MR nucleus is directly involved in the control of the electroencephalographic (EEG) activity of the hippocampus, specifically states of hippocampal EEG desynchronization (for review: Vinogradova 1995; Vertes and Kocsis 1997; Leranth and Vertes 2000). It has been shown that: (1) MR stimulation disrupts the bursting discharge of septal pacemaking cells and desynchronizes the hippocampal EEG (Macadar et al. 1974; Assaf and Miller 1978; Vertes 1981; Kitchigina et al. 1999); (2) MR lesions produce continuous, ongoing theta (Maru et al. 1979; Yamamoto et al. 1979); and (3) injections of various pharmacological agents into MR that either suppress the activity of 5-HT MR cells or reduce excitatory drive to them generate persistent theta (Kinney et al. 1994, 1995, 1996; Vertes et al. 1994; Marrosu et al. 1996; Kitchigina et al. 1999; Vinogradova et al. 1999).

Based on their findings that the activation or suppression of MR desynchronizes or synchronizes (theta) the hippocampal EEG, respectively, Vinogradova and colleagues (Kitchigina et al. 1999) concluded that: "the MR nucleus can be regarded as a functional antagonist of the reticular formation, powerfully suppressing theta bursts of the medial septal area neurons and the hippocampal theta rhythm."

The discharge characteristics of 5-HT MR neurons across sleep/waking states is also consistent with their role in the desynchronization of the hippocampal EEG. MR 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 rapid eye movement (REM) sleep (theta states) (Jacobs and Azmitia 1992; Marrosu et al. 1996).

The MR contains not only significant numbers of 5-HT neurons but also a large population of GABAergic cells (Mugnaini and Oertel 1985; Jacobs and Azmitia 1992; Maloney et al. 1999) that have been shown to contact and inhibit 5-HT MR neurons (Forchetti and Meek 1981; Nishikawa and Scatton 1985a, 1985b). Injections of $GABA_A$ (Kinney et al. 1995) as well as $GABA_B$ (Varga et al. 2002) agonists into MR have been shown to generate long trains of theta, presumably through a direct suppression of 5-HT MR neurons (Varga et al. 2002).

In a previous examination of dorsal and MR neurons in the anesthetized rat (Kocsis and Vertes 1996), we identified a small population of MR cells that fired at high rates of activity (21–42 Hz), synchronously in bursts with the hippocampal theta rhythm. Based on their discharge characteristics, these cells were thought to be GABAergic neurons and to exert a rhythmical inhibitory influence on 5-HT MR cells in the control of the hippocampal EEG.

The aim of the present report was to comprehensively examine the discharge properties of cells of the MR nucleus with respect to states of hippocampal EEG in the urethane-anesthetized rat. We demonstrated that approximately 80% of MR neurons showed changes in activity associated with changes in the hippocampal EEG; that is, they fired at increased or decreased rates of activity during the presence of theta and were designated theta-on and theta-off cells, respectively. These cells were further subdivided into slow, moderate, and fast-firing (FF) theta-on and theta-off cells. We suggest that populations of slow-firing (SF) 5-HT theta-on cells and FF GABAergic theta-on cells modulate the discharge of SF 5-HT theta-off cells in the control of the hippocampal EEG. A preliminary report has been published previously (Viana Di Prisco et al. 1999).

## Materials and methods

Experiments were performed on 34 male Sprague-Dawley rats (Charles River, Wilmington, Mass.) weighing 350–400 g. The experiments were approved by the Florida Atlantic University Institutional Animal Care and Use Committee and conform to all federal regulations and National Institutes of Health guidelines for the care and use of laboratory animals. Rats were initially anesthetized with methoxyflurane or halothane anesthesia for the insertion of a catheter into the left femoral vein for subsequent delivery of urethane anesthesia. In eight animals a catheter was also inserted in the right femoral vein for drug administration. Thereafter, urethane was administered i.v. to maintain proper levels of anesthesia. Following induction of anesthesia, rats were prepared for recording of slow-wave activity in the hippocampus and unit activity in the MR nucleus. For hippocampal recording, two Teflon-coated, stainless steel twisted wires (125 μm) separated by 1 mm at the tip were stereotaxically implanted in the left dorsal hippocampus. The hippocampal EEG was recorded (bandpass 0.1–75 Hz or 100 Hz) either monopolarly, with respect to an indifferent screw electrode placed over the contralateral frontal cortex, or bipolarly. An additional screw electrode was implanted in the frontal bone and used as ground. For unit recording, a 2×2 mm rectangular opening was made over the region of the MR nucleus. Care was taken not to tear the dorsal vein wall. A tungsten microelectrode (5–10 MΩ) was lowered dorsoventrally through the opening into the MR and para-MR regions to record unit activity from these sites. Routinely 2–5 penetrations were made per rat. Bleeding was minimal and only observed when the electrodes were removed.

Unit recording and analysis techniques have previously been described in detail (Kocsis and Vertes 1992, 1994). In brief, unit activity was amplified and filtered (0.3 – 10 kHz), and spikes were isolated using a commercial window discriminator (FHC, Bowdoinham, Me.). Spikes together with the hippocampal EEG were acquired and sampled online at 14.3 kHz (70-μs sampling period) with a 12-bit AD converter and EGAA software of RC Electronics (Santa Barbara, Calif.) and saved to computer disk. For purposes of analysis, the data were digitally resampled at 476 Hz. Only single units showing a stable and reproducible waveform and a high signal-to-noise ratio (4:1 or more) were taken and subsequently analyzed. Every isolated unit that met criteria and was encountered at a depth of 6.5–10 mm was tested. Firing rates were measured during at least 10-s epochs both prior to (baseline) and during tail pinch-elicited (TP) theta. The cycle was repeated 3–4 times and rates were averaged over the cycles. Based on firing rates during spontaneous or sensory-induced theta, units were divided into theta-related or non theta-related cells, depending on whether they did or did not exhibit a more than 20% change in activity with theta. Theta related-cells were further classified as theta-on and theta-off cells based on whether they increased or decreased in discharge with theta (Colom and Bland 1987; Ford et al. 1989). Finally, these two populations of cells were divided into SF (~1 Hz), moderate-firing (5–11 Hz), or FF (>12 Hz) theta-on and theta-off cells. Mean firing rates and standard errors (SEM) were calculated for all conditions. Differences in rates of firing between control and theta states for all

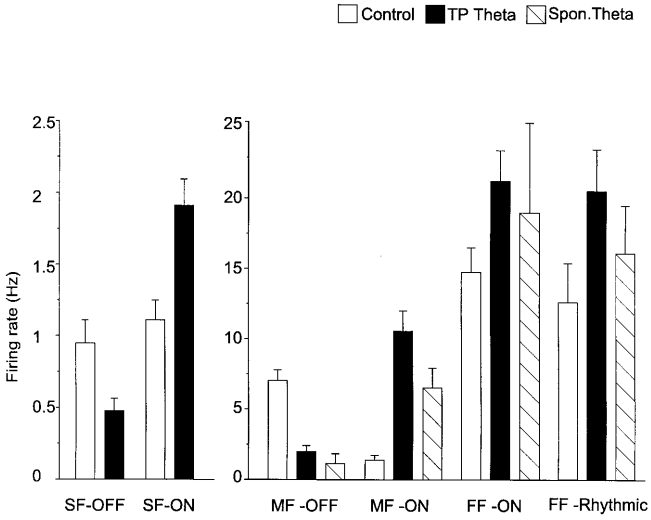


**Fig. 1** *Left side*: Mean discharge rates of slow-firing (*SF*) theta-on and theta-off cells of the median raphe (*MR*) nucleus during control conditions (non theta) and during theta induced with tail pinch (*TP*). *Right side*: Mean discharge rates of medium-firing (*MF*) theta-on and theta-off cells as well as fast-firing (*FF*) tonic and phasic theta-on cells during control conditions, during spontaneous theta and during TP-elicited theta. Differences in rates of discharge between control and theta were statistically significant for each group by the paired *t*-test ($P<0.002$)

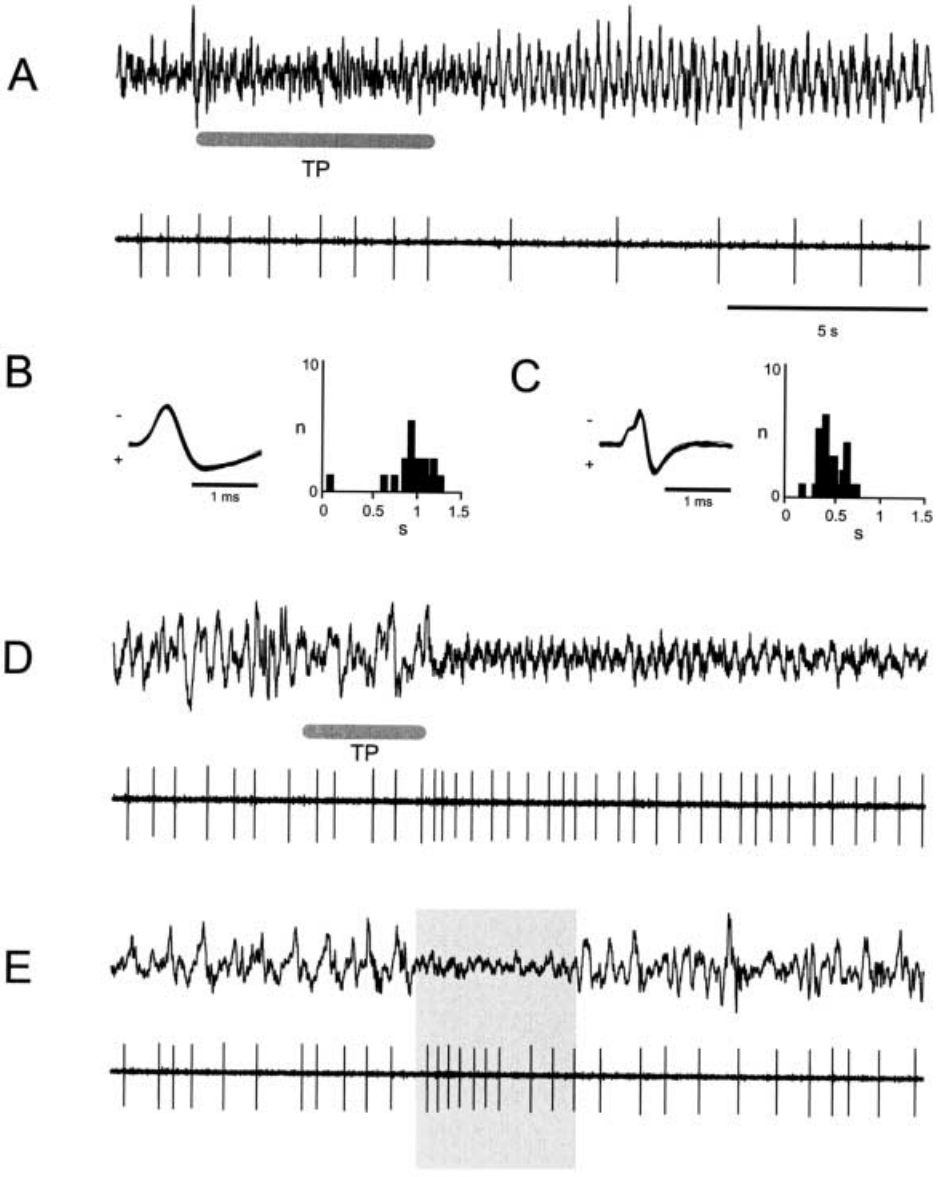


**Fig. 2** **A** The discharge characteristics of a slow-firing (*SF*) cell of the median raphe nucleus that decreased in discharge from control (*left side*) to theta induced by tail pinch (*TP*, *horizontal gray bar*). **B** Superimposed action potentials of the theta-off cell (**A**) showing a wide spike (~2 ms) and interspike interval histogram (*ISIH*) demonstrating the slow rate of firing of the cell (~1.2 Hz; 0.8-s peak in ISIH) during control conditions. **C** Superimposed action potentials of the SF theta-on cell of **D, E**, showing a wide spike (~2 ms) and ISIH demonstrating the enhanced firing of the cell to 2.4 Hz (0.4-s peak in ISIH) in the 10-s period following TP-elicited theta. **D, E** The discharge characteristics of the SF theta-on cell of **C** showing increased rates of firing during both TP-elicited (**D**) and spontaneous theta (*gray rectangle* in **E**)

groups were statistically analyzed using the paired *t*-test and differences in firing rates across groups were analyzed using a 1-way ANOVA followed by post hoc pairwise comparisons using Tukey's test.

Interspike interval histograms (ISIHs) and perievent histograms (PSHs) were calculated from the standard pulse train. Autocorrelograms, unit-EEG cross-correlograms, as well as autospectra and unit-EEG coherences, were calculated to assess rhythmicity and synchrony with hippocampal theta. Based on previous criteria (Kocsis and Vertes 1994), cells with coherences of more than 0.4 were considered to be theta rhythmically firing neurons.

Microelectrode locations were determined by histological analysis of electrolytic lesions. At the end of recording sessions, rats were perfused (10% formalin) and their brains removed and stored. Fifty-micron sections were taken with a freezing microtome and stained with cresyl violet.

## Results

We examined the activity of 181 neurons of the MR nucleus, the paramedian region surrounding MR (PMR), or the interface region between the dorsal and median raphe (DRI) during three states of the hippocampal EEG: (1) large-amplitude, irregular activity (control); (2) spontaneous theta activity; and (3) sensory-elicited theta activity (TP). Approximately 80% (145/181) of cells were "theta-related" neurons; that is, they responded differently ($P<0.05$) in the presence and in the absence of theta. These neurons were divided into two groups: those showing increased rates of discharge with theta (theta-on cells), and those showing decreased rates of discharge with theta (theta-off cells). Theta-on cells represented 68% (98/145) of the theta-related neurons; theta-off cells represented 32% (47/145) of these neurons. These two

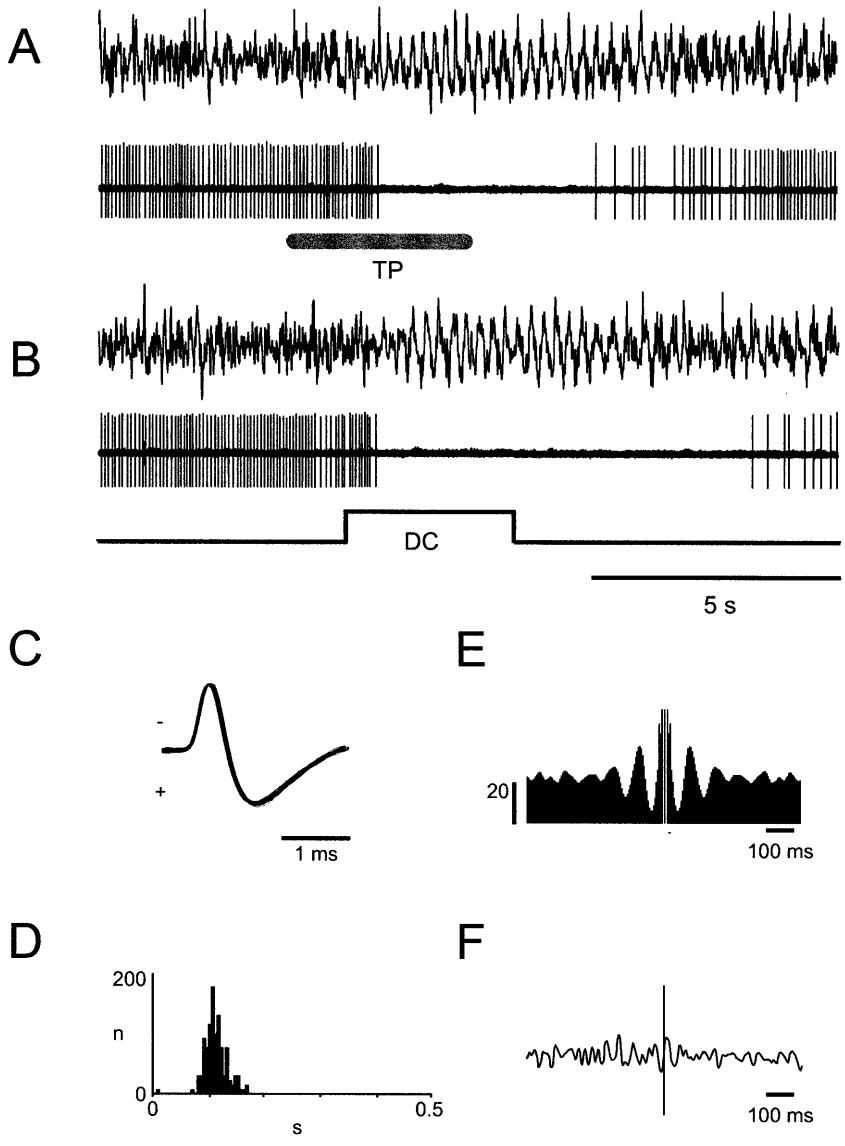


**Fig. 3 A, B** The discharge characteristics of a moderately firing (*MR*) cell that showed an abrupt cessation of firing at the onset and for the duration of theta elicited with either TP (**A**) or with electrical stimulation (*DC*) of the tail (**B**). **C** Superimposed APs of this moderately firing 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 (non theta) 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 activity of the cell was not correlated with the hippocampal EEG as demonstrated by the flat unit-EEG cross-correlogram

populations were further subdivided into SF (~1 Hz; 34%), moderately firing (MF; 5–11 Hz; 37%), and FF (>12 Hz; 29%) theta-on or theta-off cells (Fig. 1). There was a significant difference in baseline rates of discharge across groups ($F_{5, 180}$=26.7, $P$<0.0001).

## Slow-firing theta-on and theta-off cells

About one-third of MR cells (49/145; 34%) exhibited characteristics of "classic" serotonin-containing neurons of the raphe (Aghajanian et al. 1968); that is, wide spikes (2.0±0.49 ms) and a slow (~1 Hz), regular pattern of discharge (Figs. 1, 2).

Approximately 29% (14/49) of SF cells were theta-off cells and as a population decreased in discharge from baseline rates of 1±0.2 Hz (range 0.2–2.3 Hz) to 0.5±0.1 Hz (range 0.1–1.2 Hz) ($P$<0.002) with theta (Fig. 1). Figure 2A depicts a SF theta-off cell that showed a pronounced slowing of activity with TP-elicited theta. The cell had a wide spike (2 ms; Fig. 2B) and fired at baseline (non theta) rates of ~1.2 Hz (0.8 s peak in ISI histogram).

The majority of SF cells (35/49; 71%) were "theta-on" cells and as a group increased in firing from mean baseline rates of 1.1±0.1 Hz (range 0.1 – 2.8) to 1.8±0.2 Hz (range 0.4–4.6) ($P$<0.0001) with theta (Fig. 1). Figure 2C–E depicts a SF theta-on cell showing enhanced-firing during both TP (Fig. 2D) and spontaneous (Fig. 2E) theta. The cell had a wide action potential (AP; 2 ms; Fig. 2C) and fired at rates of about 2 Hz (0.5 s peak in ISI histogram) in the 10-s period following TP-induced theta.

It was generally the case for both SF theta-on and theta-off cells that maximal changes in discharge (i.e., increases or decreases) occurred at the beginning of the transition from non theta to theta states and gradually tapered thereafter (see Fig. 2A, D). Twenty-three SF cells

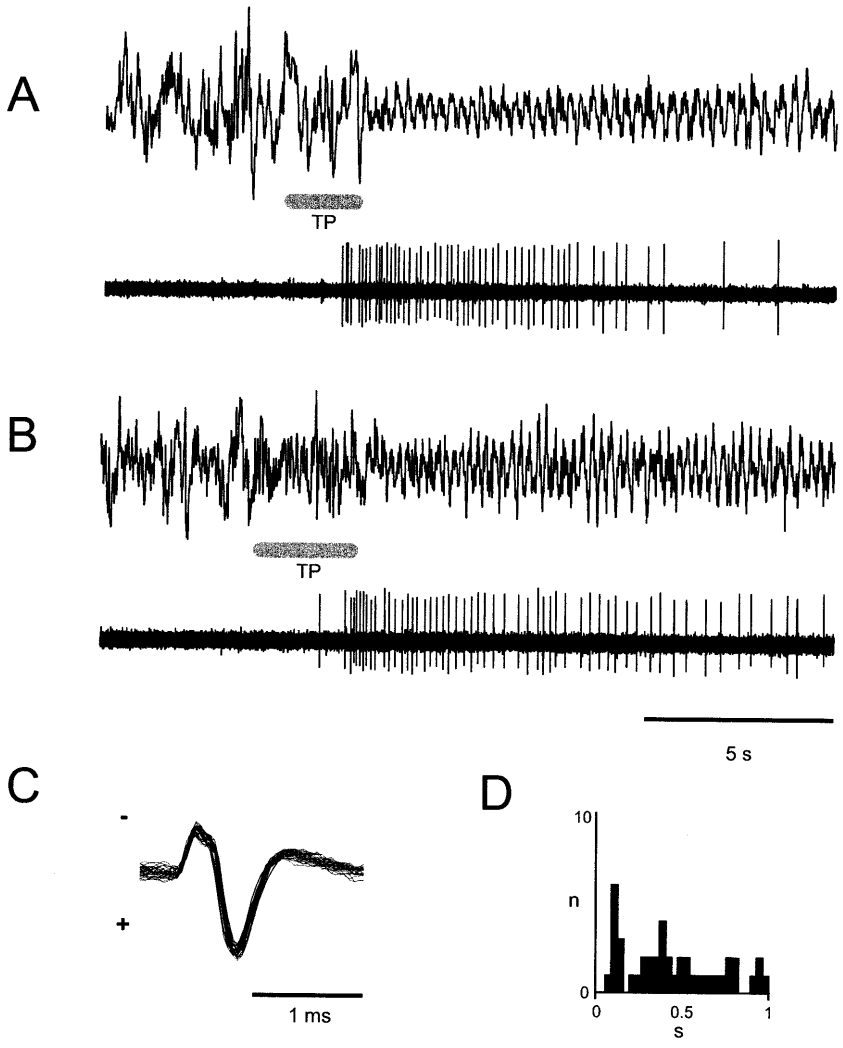


**Fig. 4 A, B** The discharge characteristics of two moderately firing (*MR*) cells that showed significant increases in discharge from control at the onset and essentially for the duration of theta elicited with TP. **C** Superimposed APs of the cell in **A** showing a spike width of 0.8 ms. **D** ISI histogram of the cell in **A** demonstrating enhanced firing to ~10 Hz (100-ms peak in ISIH) in the 5-s period following TP-elicited theta

were located in MR, 7 in the PMR region, and 19 in DRI (Fig. 8).

## Moderately firing theta-off cells

Approximately 22% (32/145) of theta-related cells were MF theta-off neurons. These cells decreased in firing from mean baseline rates of 7.0±0.8 Hz (range 2.7–15.9 Hz) to rates of 2.0±0.4 Hz (range 0.0–8.1 Hz) ($P$<0.0001) with theta. MF theta-off cells represented a heterogenous population of neurons as indicated by marked variability in their firing during both non theta and theta conditions (Fig. 1). An interesting subgroup of MF theta-off cells (8/32) exhibited regular patterns of discharge similar to that seen with classic SF neurons, the main difference being that they fired at higher baseline rates than did SF neurons. Figure 3 depicts a MF cell of this type. As shown, the cell discharged at very regular rates during control conditions and virtually ceased firing with theta elicited with TP (Fig. 3A) or with electrical stimulation of the tail (Fig. 3B). The regular firing of the cell is demonstrated by the sharp peak in the ISI histogram at 110 ms (Fig. 3D), as well as by peaks in the autocorrelation function at about 9 Hz (Fig. 3E). The cell had a spike width of ~2 ms (Fig. 3C) and showed no rhythmical activity with theta as demonstrated by the flat unit-EEG cross-correlogram (Fig. 3F). The locations of MF theta-off cells are depicted in Fig. 8.

## Moderately firing theta-on cells

Fifteen percent (21/145 cells) of theta-related MR neurons were MF theta-on cells (Fig. 1). The majority of MF theta-on cells fired at exceedingly low rates or were virtually silent during control conditions. For example, MF theta-on cells fired at mean baseline (control) rates at 0.3 Hz, which is less than that of SF cells (~1 Hz; Fig. 1).

MF theta-on cells showed a 20- to 30-fold increased rate of discharge with theta; that is, an increase from 1.3±0.3 to 10.5±1.5 Hz (range 2.2–23.7 Hz) ($P$<0.0001) with TP-elicited theta and from 1.3±0.3 to 6.5±0.7 Hz (range 4.2–11.4 Hz) ($P$<0.01) with spontaneous theta

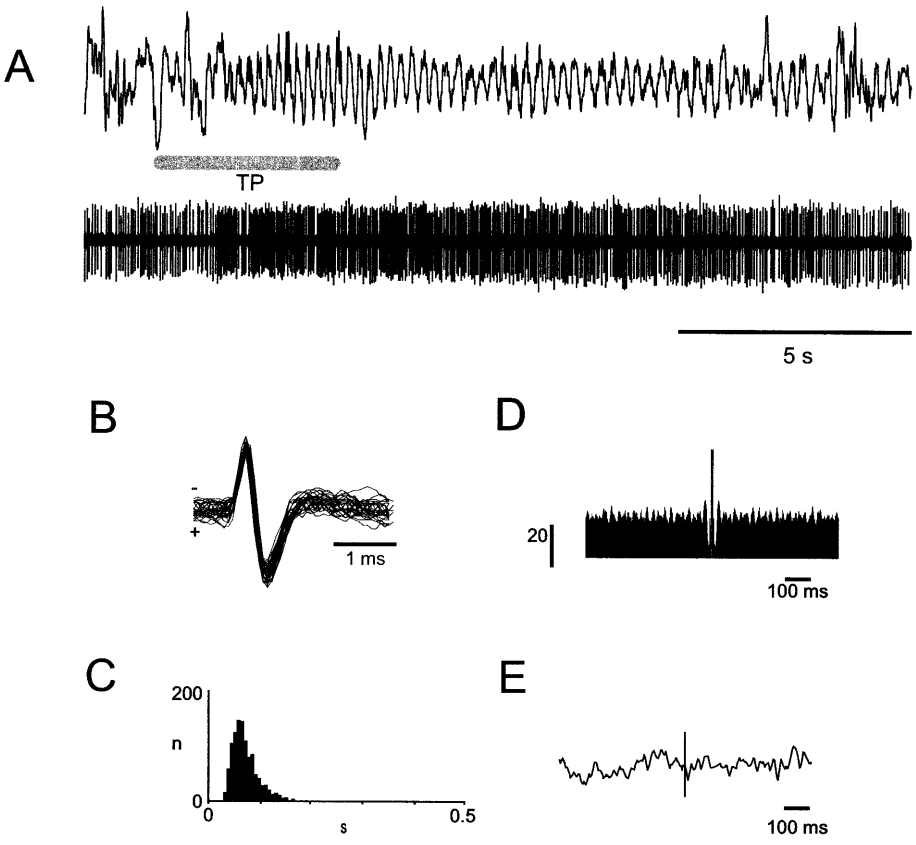


**Fig. 5** **A** The discharge characteristics of a fast-firing MR cell showing a pronounced increase in discharge from control (approximately doubling) during theta elicited with TP. **B** Superimposed APs of the cell showing a narrow spike width of ~1 ms. **C** ISI histogram for the cell showing a peak at ~60 ms, indicating that the cell fired at high and regular rates during theta. **D, E** Autocorrelogram and cross-correlogram showing the lack of rhythmical discharge of the cell at theta frequencies (absence of peaks in the autocorrelogram at those frequencies) and a lack of correlation between unit and EEG signals (flat unit-EEG cross-correlogram)

(Fig. 1). Two MF theta-on cells are depicted in Fig. 4A, B. Both cells were essentially silent during control conditions and showed pronounced increases in discharge to about 10 Hz (Fig. 4D) with the onset of theta and continued to fire at this approximate rate for another 5–10 s (Fig. 4). The spike width of the top unit (Fig. 4A) was of 0.8 ms (Fig. 4C), and the cell fired at ~10 Hz (100 ms peak in ISIH; Fig. 4D) for about 5 s following the onset of theta and slowed thereafter. MF theta-on and theta-off cells were located in close proximity and could often be simultaneously recorded from the same electrode site (Fig. 8).

## Fast-firing tonic theta-on cells

Approximately 17% (25/145) of theta-related MR cells were FF tonic theta-on cells. These cells fired at high baseline rates (14.8±1.8 Hz; range 4–38.4 Hz) and showed further increases in rate with either TP-elicited (21.3±2.2 Hz; range 7.6–48.7 Hz) ($P<0.0001$) or spontaneous (19±3.5 Hz; range 9.4–50.3 Hz) ($P<0.02$) theta. Figure 5 depicts a tonic FF theta-on cell. As shown (Fig. 5A, C), the cell fired at a high spontaneous rate (~28 Hz), which virtually doubled with the onset and for the approximate duration of theta. The cell had a short-duration AP (1 ms; Fig. 5B) and showed no rhythmical discharge with theta, as indicated by the lack of peaks in the autocorrelogram (Fig. 5D), as well as by the flat unit-EEG cross-correlogram (Fig. 5E). Tonic FF cells were primarily located in the paramedian region of MR (Fig. 8).

## Fast-firing rhythmic theta-on cells

Approximately 12% (18/145) of theta-related neurons were FF phasic theta-on cells; that is, they not only showed pronounced increases in rates of discharge with theta, but also fired rhythmically, synchronously with theta. Phasic FF cells discharged at baseline rates of 12.7±2.8 Hz (range 1.9–37.3 Hz) that increased to 20.7±3.1 Hz (range 6.2–50.3 Hz) ($P<0.002$) with TP-elicited theta and to 16.2±2.7 Hz (range 4.1–42.6 Hz) ($P<0.01$) with spontaneous theta. Figure 6 depicts one such cell. The cell fired arhythmically in the absence of theta (control) and rhythmically and at increased rates with TP-elicited theta (Fig. 6A). The rhythmical discharge of the cell is shown by: (1) the second peak in the ISI histogram at approximately 200 ms (5 Hz; Fig. 6C); (2) rhythmical peaks at theta frequency in the autocorrelogram (Fig. 6D); (3) unit-EEG locked oscillations in the cross-correlogram (Fig. 6E); (4) peaks in the unit and EEG autospectra at approximately 5 Hz (Fig. 6F); and (5) pronounced coherence between unit and EEG signals at theta frequency (Fig. 6F). Rhythmic FF cells fired at higher rates and showed greater coherences with theta during TP-elicited than during spontaneous theta; that is,

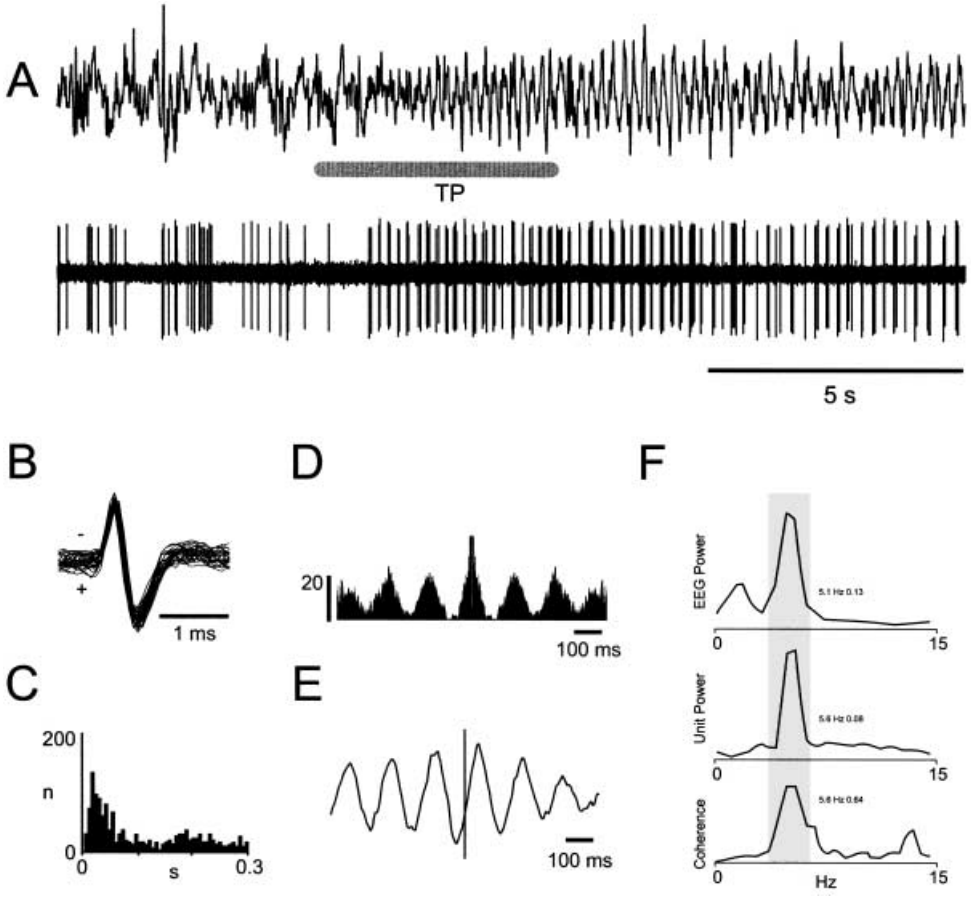


**Fig. 6** **A** The discharge characteristics of a fast-firing MR cell that increased in rate of discharge and fired rhythmically to TP-elicited theta. **B** Superimposed APs of the cell showing a narrow spike width of ~1 ms. **C** ISI histogram showing clustering at two intervals (~20 ms and ~200 ms), reflecting inter- and intraburst frequencies during theta. **D, E** Autocorrelogram and cross-correlogram depicting the rhythmical discharge of the cell (**D**) locked to theta (**E**) during theta conditions. **F** Spectral and coherence plots showing peaks in the EEG and unit signals at theta frequency (~5.6 Hz) and significant coherence (0.64) between EEG and unit signals at theta frequency

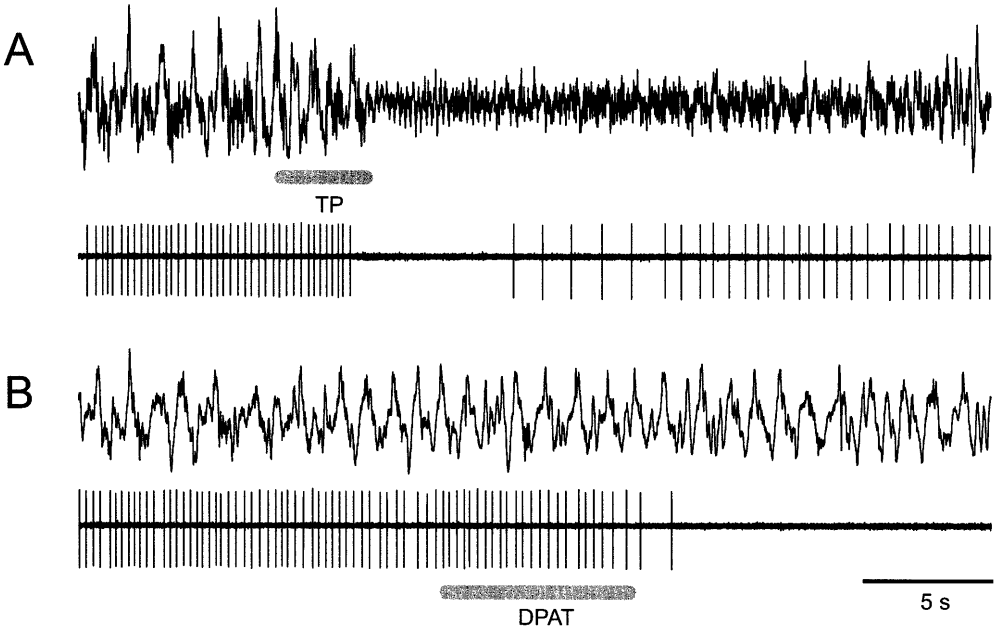


**Fig. 7** The discharge characteristics of a moderately firing (*MR*) cell that showed a pronounced reduction in discharge during theta elicited with TP (**A**) and a complete suppression of activity to the i.v. administration of 8-hydroxy-2-(di-*n*-propylamino)tetralin (DPAT in **B**)

rates and coherences of 20.7 Hz and 0.67 with TP theta compared with 16.2 Hz and 0.56 with spontaneous theta.

## Experiments with 8-hydroxy-2-(di-*n*-propylamino)tetralin

We examined the effects of systemic injections of the 5-$HT_{1A}$ agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-

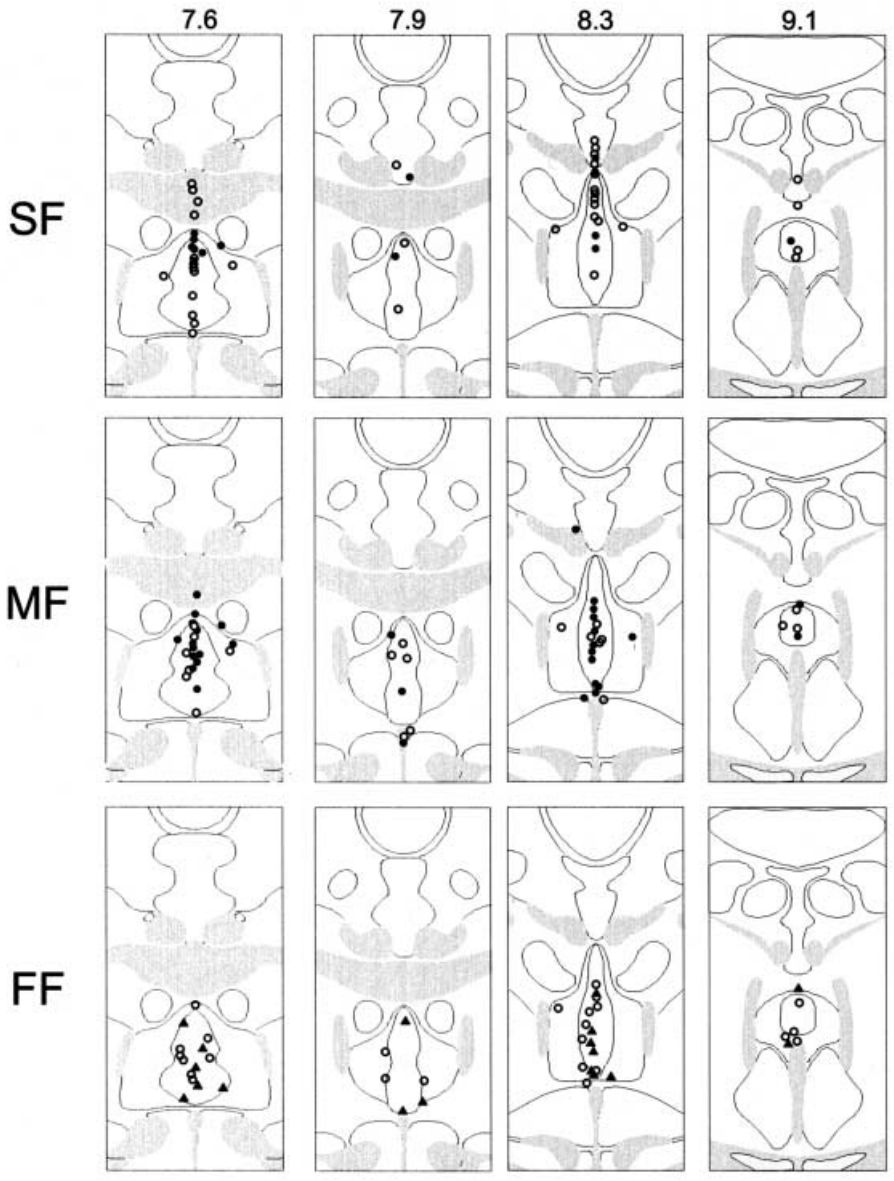


**Fig. 8** Schematic representation of the locations of slow (*SF*), moderate (MF), and fast-firing (*FF*) cells in the median and paramedian raphe nucleus, as well as the interface between the dorsal and median raphe nuclei at four levels of the brainstem (7.6 mm, 7.9 mm, 8.3 mm, and 9.1 mm caudal to bregma). *Open circles*, theta-on cells; *solid circles*, theta-off cells; and *triangles*, theta rhythmic cells. (Schematic sections adapted from Swanson 1998)

OH-DPAT), on some SF and MF theta-off cells. 5-HT agonists inhibit 5-HT raphe neurons through autoreceptor mechanisms (Sprouse and Aghajanian 1987; Jacobs and Azmitia 1992; Fornal 1994a, 1994b). As expected, the two SF (presumed 5-HT) cells that were examined were inhibited by 8-OH-DPAT. In addition, we found that two of three MF theta-off cells were also inhibited by 8-OH-DPAT – as illustrated for one MF cell in Fig. 7. As depicted, the activity of the cell was strongly inhibited during TP-elicited theta (Fig. 7A) and completely suppressed following the administration of 8-OH-DPAT (Fig. 7B). These findings suggest that a subset of MF theta-off cells are serotonergic neurons.

Figure 8 depicts the locations of SF, MF and FF cells in the MR, paramedian raphe nucleus, and interface region between the MR and the dorsal raphe nucleus at four levels of the brainstem. As shown, SF, MF and FF theta-on and theta-off cells were found at all levels of the MR. FF theta rhythmic neurons showed a slight rostral predominance.

## Discussion

We showed that a large percentage of cells of the MR nucleus exhibit changes in activity associated with the changes from desynchronized to synchronized (theta rhythm) states of the hippocampal EEG. Specifically, about 80% (145/181) of MR cells fired at increased or decreased rates of activity in the presence of theta; that is, theta-on and theta-off cells (Colom and Bland 1987; Ford et al. 1989; Bland and Colom 1993). These cells were further divided into slow (~1 Hz), moderate (5–11 Hz), and FF (>12 Hz) theta-on or theta-off cells. The SF theta-

on and theta-off cells displayed characteristics of "classic" 5-HT raphe neurons (Aghajanian et al. 1968, 1970; Jacobs et al. 1984; Rasmussen et al. 1984; Sprouse and Aghajanian 1987; Jacobs and Azmitia 1992) and were thought to be serotonergic cells. All FF cells were theta-on cells; no FF theta-off cells were observed. FF cells showed either tonic or phasic (rhythmical) increases in activity with theta.

## Role of MR neurons in the desynchronization of the hippocampal EEG: implications of present findings

### *Slow-firing and moderately firing theta-off neurons*

We demonstrated that 34% (49/145) of theta-related MR cells were SF neurons and that 14 of 49 were theta-off cells. SF theta-off cells fired at mean baseline rates of 1.0 Hz that slowed to 0.5 Hz with theta. We also identified a significant population (32/145 or 22%) of MF (5–11 Hz) theta-off cells. Some of these cells also fired at very regular rates and a few were strongly suppressed with the 5-$HT_{1A}$ agonist 8-OH-DPAT, suggesting that they may be serotonergic cells. The discharge characteristics of putative serotonergic SF and MF theta-off cells suggests that they may serve a direct role in the modulation of the hippocampal EEG; that is, when active they suppress theta or desynchronize the hippocampal EEG and when inhibited they release theta.

### *Slow-firing theta-on cells*

We showed that 35 of 145 theta-related MR cells were SF theta-on cells. A larger percentage of SF cells were theta-on (71%) than theta-off (29%) cells. SF theta-on cells, like SF theta-off cells, displayed properties of "classic" 5-HT neurons and were presumed to be serotonergic cells.

Combining unit recording with antidromic techniques, Crunelli and Segal (1985) reported that 112 of 355 (31%) MR neurons exhibited characteristics of 5-HT neurons and about half of them (59 of 112 cells) could be antidromically driven from the septum or hippocampus. The latter suggests that the MR contains approximately equal numbers of 5-HT projection and non projection neurons. Accordingly, it seems possible that a significant percentage of the presently identified SF theta-on cells may be local, non projection 5-HT neurons, which may inhibit 5-HT projection cells (SF theta-off cells), and/or other MR neurons, in the modulation of the hippocampal EEG. In this regard, it is well documented that serotonin (or 5-HT agonists) exerts a suppressive effect on serotonergic raphe neurons (Sprouse and Aghajanian 1987; Jacobs and Azmitia 1992; Pan et al. 1993; Fornal et al. 1994a, 1994b).

It has also been shown recently (Liu et al. 2000) that 5-HT raphe cells indirectly inhibit serotonergic neurons, via GABAergic cells. For instance, Liu et al. (2000) showed that the application of 5-HT in the slice increased the frequency and amplitude of inhibitory postsynaptic currents (IPSCs) on serotonergic neurons of the dorsal raphe nucleus (DR), and importantly the IPSCs were abolished by the $GABA_A$ antagonist bicuculline. They argued for a negative feedback loop, whereby 5-HT cells excite local GABAergic neurons which, in turn, inhibit other 5-HT neurons of DR. A similar mechanism may be present in the MR.

### *Fast-firing tonic and rhythmical theta-on cells*

We identified two populations of FF (12–22 Hz) MR neurons: tonic and rhythmical FF theta-on cells. Specifically, 17% (25 of 145) of cells discharged at high tonic rates with theta, while 12% (18 of 145) fired at enhanced rates and rhythmically with theta.

Although it has not been conclusively established, it is likely that a significant percentage of FF cells are GABAergic neurons. The MR contains a large population of GABAergic cells (Mugnaini and Oertel 1985; Jacobs and Azmitia 1992; Maloney et al. 1999) which reportedly contact and modulate the activity of serotonergic MR neurons (Forchetti and Meek 1981; Nishikawa and Scatton 1985a; Liu et al. 2000). MR injections of GABA agonists significantly reduce 5-HT markers in MR projection areas, particularly in the septum and hippocampus (Forchetti and Meek 1981; Nishikawa and Scatton 1985b; Wirtshafter et al. 1988), and injections of GABA antagonists promote the release of 5-HT in the raphe (Tao et al. 1996).

We propose that the activation of putative GABAergic FF theta-on cells would inhibit 5-HT theta-off cells to release theta, whereas GABAergic suppression would disinhibit 5-HT theta-off cells in the desynchronization of the hippocampal EEG. Supporting this possibility, Kinney et al. (1995) showed that microinjections of the $GABA_A$ agonist muscimol into MR generated theta at short latencies (30–60 s) and for long durations (>30 min), while Varga et al. (2002) recently demonstrated that virtually all 5-HT cells of MR contain $GABA_B$ receptors, and injections of $GABA_B$ agonists into MR significantly increased amounts of theta in the hippocampus.

### *Conclusions*

We suggest that: (1) slow (theta-on and theta-off) cells and a subset of the MF theta-off cells are serotonergic neurons; (2) phasic and tonic FF theta-on cells are GABAergic cells; and (3) these populations of cells mutually interact in the modulation of the hippocampal EEG. In effect, activation of local SF 5-HT theta-on cells as well as FF GABAergic theta-on cells would inhibit MR projection 5-HT theta-off cells to release or generate theta, whereas suppression of 5-HT SF theta-on and GABAergic FF theta-on activity would disinhibit 5-HT SF theta-off cells resulting in a blockade of theta or a desynchronization of the hippocampal EEG.

## Functional significance of desynchronized states of the hippocampal EEG

In addition to its effects on the hippocampal EEG, the MR serves a well-documented role in the control of locomotion. MR stimulation suppresses locomotor behavior (Graeff et al. 1980; Peck and Vanderwolf 1991), while various manipulations that reversibly or irreversibly inhibit the activity of MR cells produce locomotion (Sainati and Lorens 1982; Paris and Lorens 1987; Wirtshafter and McWilliams 1987; Hillegaart and Hjorth 1989; Wirtshafter et al. 1986, 1989, 1993; Hillegaart 1990; Shim et al. 1997). The demonstration that the MR affects both locomotion and the hippocampal EEG suggests common MR mechanisms controlling both functions. In this regard, Sinnamon et al. (2000) recently demonstrated the important findings that injections of GABA (or GABA agonists) into MR enhanced hypothalamic-induced stepping behavior (locomotion) and increased the power of theta in the 4-to 5-Hz band.

The possible dual MR control of locomotion and states of the hippocampal EEG may have important functional consequences. It is obviously critical for a rat (or other species) when moving through its environment to commit relevant aspects of it to memory, hence a coupling of motor behavior with theta to encode information when locomoting. On the other hand, there may be less of a demand to commit information to memory when an animal is stationary or engaged in automatic behaviors such as grooming or consumatory acts, and hence the absence of theta during these conditions.

An accumulating body of evidence indicates that the theta rhythm is critically involved in memory-processing functions of the hippocampus (for review, see Vertes and Kocsis 1997). If, as indicated, theta serves an important role in LTP/memory, it would seem that a 5-HT MR-mediated disruption of theta (hippocampal desynchronization) might suppress LTP and memory. Consistent with this possibility, several reports have shown that serotonergic agents block LTP and that 5-HT antagonists (mainly 5-$HT_3$ antagonists) enhance LTP and/or memory (Corradetti et al. 1992; Staubli and Otaky 1994; Staubli and Xu 1995; Buhot 1997). Ascending 5-HT MR fibers, by disrupting theta (or desynchronizing the hippocampal EEG), may block or temporarily suspend mnemonic processes in the hippocampus. MR may be an important part of a system of connections that directs the hippocampus to essentially disregard insignificant environmental events.

In conclusion, the MR nucleus is known to serve a direct role in the control of the hippocampal EEG. We found that the activity of a very large percentage of MR cells was modulated with respect to the hippocampal EEG and propose that these various MR neurons interact to regulate states of hippocampal EEG synchronization (theta) and desynchronization.

**Acknowledgements** This work was supported by NIH grant NS35883 and NIMH grant MH01476 to R.P.V., and by NIMH grant MH62525 and a William F. Milton Fund grant to B.K.

## References

Aghajanian GK, Foote WE, Sheard MH (1968) Lysergic acid diethylamide sensitive neuronal units in the midbrain raphe. Science 161:706–708

Aghajanian GK, Foote WE, Sheard MH (1970) Action of psychogenic drugs on single midbrain neurons. J Pharmacol Exp Ther 171:178–187

Assaf SY, Miller JJ (1978) The role of a raphe serotonin system in the control of septal unit activity and hippocampal desynchronization. Neuroscience 3:539–550

Bland BH, Colom LV (1993) Extrinsic and intrinsic properties underlying oscillation and synchrony in limbic cortex. Prog Neurobiol 41:157–208

Buhot MC (1997) Serotonin receptors in cognitive behaviors. Curr Opin Neurobiol 7:243–254

Colom LV, Bland BH (1987) State-dependent spike train dynamics of hippocampal formation neurons: evidence for theta-on and theta-off cells. Brain Res 422:277–286

Corradetti R, Ballerini L, Pugliese AM, Pepeu G (1992) Serotonin blocks the long-term potentiation induced by primed burst stimulation in the CA1 region of rat hippocampal slices. Neuroscience 46:511–518

Crunelli V, Segal M (1985) An electrophysiological study of neurones in the rat median raphe and their projections to the septum and hippocampus. Neuroscience 15:47–60

Forchetti CM, Meek JL (1981) Evidence for a tonic GABAergic control of serotonin neurons in the median raphe nucleus. Brain Res 206:208–212

Ford R, Colom LV, Bland BH (1989) The classification of medial septum-diagonal band cells as theta-on or theta-off in relation to hippocampal EEG states. Brain Res 493:269–282

Fornal CA, Litto WJ, Metzler CW, Marrosu F, Tada K, Jacobs BL (1994a) Single-unit responses of serotonergic dorsal raphe neurons to 5-$HT_{1A}$ agonist and antagonist drug administration in behaving cats. J Pharmacol Exp Ther 270:1345–1358

Fornal CA, Marrosu F, Metzler CW, Tada K, Jacobs BL (1994b) Effects of the putative 5-hydroxytryptamine$_{1A}$ antagonists BMY 7378, NAN 190 and (–)-propranolol on serotonergic dorsal raphe unit activity in behaving cats. J Pharmacol Exp Ther 270:1359–1366

Graeff FG, Quintero S, Gray JA (1980) Median raphe stimulation, hippocampal theta rhythm and threat-induced behavioral inhibition. Physiol Behav 25:253–261

Halliday G, Harding A, Paxinos G (1995) Serotonin and tachykinin systems. In: Paxinos G (ed) The rat nervous system, 2nd edn. Academic, New York, pp 929–974

Hillegaart V (1990) Effects of local application of 5-HT and 8-OH-DPAT into the dorsal and median raphe nuclei on motor activity in the rat. Physiol Behav 48:143–148

Hillegaart V, Hjorth S (1989) Median raphe, but not dorsal raphe, application of the 5-HT1A agonist 8-OH-DPAT stimulates rat motor activity. Eur J Pharmacol 160:303–307

Jacobs BL, Azmitia EC (1992) Structure and function of the brain serotonin system. Physiol Rev 72:165–229

Jacobs BL, Heym J, Steinfels GF (1984) Physiological and behavioral analysis of raphe unit activity. In: Iverson LL, Iversen SD, Snyder SH (eds) Handbook of psychopharmacology. Plenum, New York, pp 343–395

Kinney GG, Kocsis B, Vertes RP (1994) Injections of excitatory amino acid antagonists into the median raphe nucleus produce hippocampal theta rhythm in the urethane anesthetized rat. Brain Res 654:96–104

Kinney GG, Kocsis B, Vertes RP (1995) Injections of muscimol into the median raphe nucleus produce hippocampal theta rhythm in the rat. Psychopharmacology 120:244–248

Kinney GG, Kocsis B, Vertes RP (1996) Medial septal unit-firing characteristics following injections of 8-OH-DPAT into the median raphe nucleus. Brain Res 708:116–122

Kitchigina VF, Kudina TA, Kutyreva EV, Vinogradova OS (1999) Neuronal activity of the septal pacemaker of theta rhythm under the influence of stimulation and blockade of the median raphe nucleus in the awake rabbit. Neuroscience 94:453–463

Kocsis B, Vertes RP (1992) Dorsal raphe neurons: synchronous discharge with the theta rhythm of the hippocampus in the freely behaving rat. J Neurophysiol 68:1463–1467

Kocsis B, Vertes RP (1994) Characterization of neurons of the supramammillary nucleus and mammillary body that discharge rhythmically with the hippocampal theta rhythm in the rat. J Neurosci 14:7040–7052

Kocsis B, Vertes RP (1996) Midbrain raphe cell-firing and hippocampal theta rhythm in urethane-anesthetized rats. Neuroreport 7:2867–2872

Leranth C, Vertes RP (1999) Median raphe serotonergic innervation of medial septum/diagonal band of Broca (MSDB) parvalbumin-containing neurons: possible involvement of the MSDB in the desynchronization of the hippocampal EEG. J Comp Neurol 410:586–598

Leranth C, Vertes RP (2000) Neuronal networks that control the septal pacemaker system: synaptic interconnections between the septal complex, hippocampus, supramammillary area, and median raphe. In: Numan R (ed) The behavioral neuroscience of the septal region. Springer, New York, pp 15–47

Liu R, Jolas T, Aghajanian G (2000) Serotonin 5-$HT_2$ receptors activate local GABA inhibitory inputs to serotonergic neurons of the dorsal raphe nucleus. Brain Res 873:34–45

Macadar AW, Chalupa LM, Lindsley DB (1974) Differentiation of brain stem loci which affect hippocampal and neocortical electrical activity. Exp Neurol 43:499–514

Maloney KJ, Mainville L, Jones BE (1999) Differential c-Fos expression in cholinergic, monoaminergic, and GABAergic cell groups of the pontomesencephalic tegmentum after paradoxical sleep deprivation and recovery. J Neurosci 19:3057–3072

Marrosu F, Fornal CA, Metzler CW, Jacobs BL (1996) 5-$HT_{1A}$ agonists induce hippocampal theta activity in freely moving cats: role of presynaptic 5-$HT_{1A}$ receptors. Brain Res 739:192–200

Maru E, Takahashi LK, Iwahara S (1979) Effects of median raphe nucleus lesions on hippocampal EEG in the freely moving rat. Brain Res 163:223–234

McKenna JT, Vertes RP (2001) Collateral projections from the median raphe nucleus to the medial septum and hippocampus. Brain Res Bull 54:619–630

Morin LP, Meyer-Bernstein EL (1999) The ascending serotonergic system in the hamster: comparison with projections of the dorsal and median raphe nuclei. Neuroscience 91:81–105

Mugnaini E, Oertel WH (1985) An atlas of the distribution of GABAergic neurons and terminals in the rat CNS as revealed by GAD immunohistochemistry. In: Bjorklund A, Hokfelt T (eds) Handbook of chemical neuroanatomy: GABA and neuropeptides in the CNS. Elsevier, New York, pp 436–608

Nishikawa T, Scatton B (1985a) Inhibitory influence of GABA on central serotonergic transmission. Involvement of the habenulo-raphe pathways in the GABAergic inhibition of ascending cerebral serotonergic neurons. Brain Res 331:81–90

Nishikawa T, Scatton B (1985b) Inhibitory influence of GABA on central serotonergic transmission. Raphe nuclei as the neuroanatomical site of the GABAergic inhibition of cerebral serotonergic neurons. Brain Res 331:91–103

Pan ZZ, Wessendorf MW, Williams JT (1993) Modulation by serotonin of the neurons in rat nucleus raphe magnus in vitro. Neuroscience 54:421–429

Paris JM, Lorens SA (1987) Intra-median raphe infusions of muscimol and the substance P analogue DiMe-C7 produce hyperactivity: role of serotonin neurons. Behav Brain Res 26:139–151

Peck BK, Vanderwolf CH (1991) Effects of raphe stimulation on hippocampal and neocortical activity and behavior. Brain Res 568:244–252

Rasmussen K, Heym J, Jacobs BL (1984) Activity of serotonin-containing neurons in nucleus centralis superior of freely moving cats. Exp Neurol 83:302–317

Sainati SM, Lorens SA (1982) Intra-raphe muscimol induced hyperactivity depends on ascending serotonergic projections. Pharmacol Biochem Behav 17:973–986

Shim I, Javaid J, Wirtshafter D (1997) Dissociation of hippocampal serotonin release and locomotor activity following pharmacological manipulations of the median raphe nucleus. Behav Brain Res 89:191–198

Sinnamon HM, Jassen AK, Ilch C (2000) Hippocampal theta activity and facilitated locomotor stepping produced by GABA injections in the midbrain raphe region. Behav Brain Res 107:93–103

Sprouse JS, Aghajanian GK (1987) Electrophysiological responses of serotonergic dorsal raphe neurons to 5-$HT_{1A}$ and 5-$HT_{1B}$ agonists. Synapse 1:3–9

Staubli U, Otaky N (1994) Serotonin controls the magnitude of LTP induced by theta bursts via an action on NMDA-receptor-mediated responses. Brain Res 643:10–16

Staubli U, Xu F (1995) Effects of 5-HT3 receptor antagonism on hippocampal theta rhythm, memory, and LTP induction in the freely moving rat. J Neurosci 15:2445–2452

Swanson LW (1998) Brain maps: structure of the rat brain. Elsevier, New York

Tao R, Ma Z, Auerbach SB (1996) Differential regulation of 5-hydroxytryptamine release by $GABA_A$ and $GABA_B$ receptors in midbrain raphe nuclei and forebrain of rats. Br J Pharmacol 119:1375–1384

Varga V, Sik A, Freund TF, Kocsis B (2002) $GABA_B$ receptors in the median raphe nucleus: distribution and role in the serotonergic control of hippocampal activity. Neuroscience 109:119–132.

Vertes RP (1981) An analysis of ascending brain stem systems involved in hippocampal synchronization and desynchronization. J Neurophysiol 46:1140–1159

Vertes RP, Kocsis B (1997) Brainstem-diencephalo-septohippocampal systems controlling the theta rhythm of the hippocampus. Neuroscience 81:893–926

Vertes RP, Martin GF (1988) An autoradiographic analysis of ascending projections from the pontine and mesencephalic reticular formation and median raphe nucleus in the rat. J Comp Neurol 275:511–541

Vertes RP, Kinney GG, Kocsis B, Fortin WJ (1994) Pharmacological suppression of the median raphe nucleus with serotonin-1A agonists, 8-OH-DPAT and buspirone, produces hippocampal theta rhythm in the rat. Neuroscience 60:441–451

Vertes RP, Fortin WJ, Crane AM (1999) Projections of the median raphe nucleus in the rat. J Comp Neurol 407:555–582

Viana Di Prisco G, Albo Z, Vertes RP (1999) Patterns of neuronal activity in the median raphe nucleus during hippocampal theta rhythm in the anesthetized rat. Soc Neurosci Abstr 25:119

Vinogradova OS (1995) Expression, control and probable functional significance of the neuronal theta-rhythm. Prog Neurobiol 45:523–583

Vinogradova OS, Kitchigina VF, Kudina TA, Zenchenko KI (1999) Spontaneous activity and sensory responses of hippocampal neurons during persistent theta-rhythm evoked by median raphe nucleus blockade in the rabbit. Neuroscience 94:745–753

Wirtshafter D, McWilliams C (1987) Suppression of locomotor activity produced by acute injections of kainic acid into the median raphe nucleus. Brain Res 408:349–352

Wirtshafter D, Montana W, Asin KE (1986) Behavioral and biochemical studies of the substrates of median raphe lesion induced hyperactivity. Physiol Behav 37:213–219

Wirtshafter D, Klitenick, MA, Asin KE (1988) Is dopamine involved in the hyperactivity produced by injections of muscimol into the median raphe nucleus? Pharmacol Biochem Behav 30:577–583

Wirtshafter D, Trifunovic R, Krebs JC (1989) Behavioral and biochemical evidence for a functional role of excitatory amino acids in the median raphe nucleus. Brain Res 483:225–234

Wirtshafter D, Stratford TR, Pitzer MR (1993) Studies on the behavioral activation produced by stimulation of GABA-B receptors in the median raphe nucleus. Behav Brain Res 59:83–93

Yamamoto T, Watanabe S, Oishi R, Ueki S (1979) Effects of midbrain raphe stimulation and lesion on EEG activity in rats. Brain Res Bull 4:491–495