GABAergic Control of the Ascending Input From the Median Raphe Nucleus to the Limbic System

Shaomin Li, Viktor Varga, Attila Sik, and Bernat Kocsis.

GABAergic control of the ascending input from the median raphe nucleus to the limbic system. J Neurophysiol 94: 2561–2574, 2005. First published June 8, 2005; doi:10.1152/jn.00379.2005. The median raphe nucleus (MRN) is the primary source of serotonergic afferents to the limbic system that are generally considered to suppress hippocampal theta oscillations. GABA receptors are expressed in the MRN by serotonergic and nonserotonergic cells, including GABAergic and glutamatergic neurons. This study investigated the mechanisms by which the fluctuating GABA tone in the MRN leads to induction or suppression of hippocampal theta rhythm. We found that MRN application of the GABA_A agonist muscimol (0.05–1.0 mM) or GABA_B agonist baclofen (0.2 mM) by reverse microdialysis had strong theta promoting effects. The GABA_A antagonist bicuculline infused in low concentrations (0.1, 0.2 mM) eliminated theta rhythm. A short period of theta activity of higher than normal frequency preceded hippocampal desynchronization in 46% of rats. Bicuculline in larger concentrations (0.5, 1.0, 2.0 mM) resulted in a biphasic response of an initial short (<10 min) hippocampal desynchronization followed by stable theta rhythm that lasted as long as the infusion continued. The frequency and amplitude of theta waves were higher than in control recordings and the oscillations showed a conspicuous intermittent character. Hippocampal theta rhythm elicited by MRN administration of bicuculline could be completely (0.5 mM bicuculline) or partially (1.0 mM bicuculline) blocked by simultaneous infusion of the GABA_B antagonist CGP35348. Our findings suggest that the GABAergic network may have two opposing functions in the MRN: relieving the theta-generators from serotonergic inhibition and regulating the activity of a theta-promoting circuitry by the fluctuating GABA tone. The two mechanisms may be involved in different functions.

INTRODUCTION

The role of the hippocampus in learning and memory is well established, and the importance of subcortical networks controlling the electrical activity of the hippocampus has been recognized for decades. Hippocampal network activity exhibits two behavior-dependent patterns associated with different states of hippocampal information processing: large-amplitude irregular activity with sharp waves and theta rhythm (Buzsáki et al. 1983; O’Keefe and Nadel 1978; Vanderwolf 1969). The ascending serotonergic system has a fundamental role in switching between the two states of hippocampal activity (Vertes and Kocsis 1997). The main source of the serotonergic input to the hippocampus is the median raphe nucleus (MRN) (Freund et al. 1990). Stimulation of the MRN blocks theta rhythm by desynchronizing the hippocampal electroencephalogram (EEG) (Vertes 1981), whereas lesioning or pharmacological suppression of the MRN produces long-lasting, uninterrupted theta activity in the hippocampus (Assaf and Miller 1978; Vertes et al. 1994). The latter effect can be elicited by the selective blockade of 5-HT neurons in the MRN (Vertes et al. 1994).

Besides the serotonergic population a large number of GABAergic neurons can be found in the midbrain raphe nuclei, including both dorsal (DRN) and MRN, which innervate serotonergic (5-HT) cells (Forchetti and Meek 1981; Maloney et al. 1999; Tao et al. 1996; Varga et al. 2001; Wang et al. 1992). Several lines of evidence indicate that GABAergic mechanisms participate in the behavioral state-dependent regulation of 5-HT cell firing (Gervasoni et al. 2000). Excitatory pathways from limbic cortical and diencephalic structures target GABAergic neurons in the raphe nuclei; and forebrain regions exert inhibitory influence on 5-HT cells via local GABAergic neurons (Ferraro et al. 1996; Varga et al. 2001, 2003). These cells fire at higher frequency (Allers and Sharp 2003) and, in some cases, phase-locked to the hippocampal theta rhythm (Kocsis and Vertes 1992, 1996; Viana Di Prisco et al. 2002).

Both GABA_A and GABA_B receptors (GABA-Rs) are expressed in the MRN, the latter almost exclusively by 5-HT neurons (Gao et al. 1993; Serrats et al. 2003; Varga et al. 2002). Local application of GABA_A and GABA_B agonists in the MRN reduces the 5-HT tone in forebrain regions including the hippocampus (Forchetti and Meek 1981; Shim et al. 1997; Tao et al. 1996). Furthermore, intra-raphe injection of the GABA_A antagonist bicuculline results in an elevation of forebrain 5-HT level; this suggests that GABA_A-Rs are tonically active in both DRN (Tao et al. 1996) and MRN (Forchetti and Meek 1981). GABA_B-R antagonist failed to increase forebrain 5-HT level unless it was coadministered with GABA_A-R antagonist (Tao et al. 1996).

Serotonergic neurons play a prominent role in brain state regulation (Hobson et al. 1975). The activity of serotonergic neurons of the DRN changes according to vigilance states; i.e., their average firing rate decreases from waking to slow wave sleep to rapid-eye-movement (REM) sleep (McGinty and Harper 1976). Recent c-fos studies indicated that the MRN have a similar pattern of discharge (Maloney et al. 1999; Yamuy et al. 1995). It has been demonstrated that during REM sleep when 5-HT neurons are virtually silent, extracellular GABA level increased in the DRN (Nitz and Siegel 1997) and GABAergic cells were activated in both DRN and MRN (Maloney et al. 1999). The suppressed firing of 5-HT neurons could be restored to waking level by blocking GABA_A-Rs.

Under urethan anesthesia, hippocampal activity spontaneously alternates between slow theta (4–5 Hz) and non-theta patterns, whereas faster theta oscillations (~8 Hz) can be elicited for relatively short periods by sensory stimulation. It was reported that in anesthetized rats, a transition from non-theta to a theta state in the hippocampus could be induced by either GABA_A-R or GABA_B-R activation in the MRN and that the effect of the GABA_A agonist was mediated by 5-HT neurons (Kinney et al. 1995; Varga et al. 2002). Unexpectedly, the blockade of GABA_A-Rs by a high dose of GABA_A antagonist bicuculline produced the same effect as GABA_A-R activation i.e., long-lasting theta in the hippocampus (Thinschmidt et al. 1995).

The aim of this study was to unravel the specific role of GABA_A and GABA_B-Rs in the regulation of MRN-mediated state transitions of the hippocampal EEG. We hypothesized that fluctuations in a GABA tone in the MRN modulates the ascending raphe-limbic input by maintaining a delicate balance of activity of several types of local and projecting MRN neurons involved in the regulation of theta and non-theta states in the hippocampus. There are at least three neuronal targets of GABA in the MRN the activation of which would have an impact on hippocampal EEG: 5-HT cells (GABA_A-R and GABA_B-Rs), local GABA interneurons (GABA_A-Rs), and ascending glutamatergic neurons (GABA_A-R). In principle, GABA in the MRN may act either to decrease or increase the ascending 5-HT output through direct inhibition of 5-HT cells or by suppressing local GABAergic interneurons, respectively. The hippocampal theta generators would be suppressed or activated, accordingly.

For investigating the effect of GABA tone in the MRN on the occurrence and characteristics of spontaneous, i.e., state-dependent, theta oscillations we chose the in vivo microdialysis technique because it allows maintaining stable drug concentrations in the MRN for extended periods of time. Assuming that the intensities of GABA action at different concentrations on the three MRN targets are uneven, we hypothesized that after altering the level of sustained MRN GABA tone, the changes in hippocampal EEG pattern would not be limited to gradual shifts in the theta parameters. Instead, a switch from a stable theta to a lasting non-theta state would be expected if the primary target of GABA at a certain concentration shifted from the receptors on 5-HT cells to the receptors on interneurons. Thus the present experiments were designed to answer the following questions. First we tested the effect of direct suppression of the MRN output by GABA_A-R and GABA_B-R agonists to confirm earlier findings (Kinney et al. 1995; Varga et al. 2002) in the experimental settings of this study. Second, we tested whether different levels of GABA_A-R antagonists would lead to opposite patterns of hippocampal EEG. Third, we tested the hypothesis that complete GABA blockade i.e., including GABA_A- GABA_B-Rs will desynchronize hippocampal EEG even when GABA_A-Rs in other MRN neurons are blocked, too (e.g., at high bicuculline concentrations). Forth, because GABA was shown acting on glutamatergic neurons in the raphe (Tao and Auerbach 2003) and there is a massive glutamatergic input from the MRN to the limbic theta oscillators (Kiss et al. 2002), we also hypothesized that GABA-R blockade will activate this pathway. The character of theta rhythm would be different however, as glutamatergic activation is involved in generation of fast theta episodes rather than lasting state-dependent, slow theta oscillations.

**METHODS**

Experiments were performed on 67 male Sprague–Dawley rats (220–420 g, Charles River Laboratories) treated in accordance with National Institutes of Health guidelines. All procedures were approved by the Institutional Animal Care and Use Committees of Harvard Medical School and Beth Israel Deaconess Medical Center. All effort was made to minimize both the suffering and the number of animals used. Rats were allowed food and water ad libitum prior to the beginning of the experiments.

**Electrophysiological recordings**

Surgery and electrophysiological recordings were performed under urethan anesthesia (1.2–1.5 g/kg ip). Hippocampal field activity was recorded with insulated stainless steel electrodes positioned in the dorsal hippocampi on both sides. With the rats mounted in a stereotaxic frame, two pairs of twisted wires (diameter: 125 μm) separated by 1 mm at their tips were implanted (AP: −3.7, L: ±2.2, H: −3.5), one in the CA1 region and the other below the hippocampal fissure, verified by the out-of-phase rhythmicity in the two recordings, and fixed with dental cement. Hippocampal EEG was amplified, filtered (0.1–70 Hz) and stored on a computer (sampling rate: 256/s; Daq/216B, IOTech, Cleveland, OH). The traces of hippocampal EEG along with their spectra and the power within the theta range (2–8 Hz) was continuously monitored during the experiment (DasyLab).

**Drugs and drug administration by microdialysis**

The following drugs were applied (see Table 1): Muscimol, bicuculline, gabazine, baclofen, CGP35348, and atropine from Sigma (St. Louis, MO). Atropine was injected intraperitoneally in a dose of 50 mg/kg. All other drugs, administered intracerebrally were dissolved in artificial cerebrospinal fluid [ACSF; containing (in mM) 147 NaCl, 2.7 KCl, 1.2 CaCl_2_, and 0.85 MgCl_2_; pH = 7.4 CMA/Microdialysis AB, Solna, Sweden]. For drug administration, concentric microdialysis probes mounted in a guide cannula were used (cuprophane membrane with an outside diameter of 0.24 mm and length of 1 or 3 mm, molecular wt. Cutoff: 6 kDa; CMA/Microdialysis AB). The probes were perfused (perfusion rate: 80 μl/h; sp101 syringe pump, WPI, Sarasota, FL) with ACSF. They were placed in the brain stem in the sagital plane under a 24° angle in a caudorostral direction so the dialysis membrane was entirely in the MRN (Fig. 1). The dialysis probe was lowered in 0.5 mm steps made every 2–5 min for a total of ~1 h to reach the target and was left in its final position for one additional hour before starting experiments. The probe was lowered at a slow speed to reduce tissue damage. After halting the probe in the target area, we waited an hour to allow consolidation of the surrounding tissue. After this “recovery period,” no overt changes can be detected as indicated by stable amino acid and nucleoside levels from the end of this period (Robinson and Juscitte 1991).

**TABLE 1. Drugs and concentrations used**

<table>
<thead>
<tr>
<th>Agonists, mM</th>
<th>GABA_A-R</th>
<th>GABA_B-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscimol</td>
<td>0.05, 0.1, 0.5, 1.0</td>
<td>Baclofen* 2</td>
</tr>
<tr>
<td>Bicuculline</td>
<td>0.01, 0.1, 0.2, 0.5, 1.0, 2.0</td>
<td>CGP35348† 2.5, 5, 18, 50</td>
</tr>
<tr>
<td>Gabazine</td>
<td>0.04, 0.5</td>
<td></td>
</tr>
</tbody>
</table>

*Baclofen in other concentrations were used in (Varga et al. 2002).
†CGP35348 was only used in combination with bicuculline for single use and combination with baclofen see (Varga et al. 2002).
Data analysis

Power spectra were calculated using Fast Fourier Transform on 4-s windows as described previously in detail (Kocsis and Vertes 1994). The spectral peaks corresponding to theta oscillations (frequency between 3 and 10 Hz, power \( \times 4 \) times larger than background) were identified and subjected to the following statistical analyses: peak frequency and power of EEG segments recorded during control and drug administrations (time windows of equal lengths) were compared using two-way ANOVA with drug concentrations and time points (before and 20–30 and 60 min after drug administration) as classification variables with post hoc Bonferroni comparisons where significance was found. Differences were considered significant if \( P < 0.05 \). Values are expressed as means \( \pm \) SE.

Histology

At the end of experiments the rats were deeply anesthetized and perfused through the aortic arch with 0.9% NaCl followed by a fixative solution containing 4% paraformaldehyde (Sigma-Aldrich), and 15% (V/V%) saturated picric acid (Sigma-Aldrich) in 0.1 M phosphate buffer (PB, pH 7.4, 0.1M). After perfusion, brains were removed, coronal blocks were dissected containing the raphe nuclei, then 60-µm thin sections were cut from the blocks with a vibrotome, (Leica, VT 1000S); washed extensively in phosphate buffer (pH 7.4, 0.1 M) containing 0.1% Triton X (Sigma-Aldrich). Sections were incubated in 4',6-diamidino-2-phenylindole (DAPI, 300 nM, Molecular Probes) for 10 min in the dark, washed, mounted, and coverslipped using Moviol antifade medium. Specimens were examined using fluorescent light microscopes (Olympus Provis AX70) and photographs were taken by a Spot digital camera.

There are numerous methods to unspecifically stain cells’ nuclei that are used for identifying brain areas. Conventional histochemical procedures involve air-drying of sections, whereas fluorescent labeling, like DAPI, use hydrated sections. We have shown previously, that air-dried sections shrink \(~75%\) in \( z \) direction (and \(~25%\) in \( x \) and \( y \) axes). Using hydrated tissue prevents this problem (Pyapali et al. 1998). Because the collapse of the tissue makes the probe localization inaccurate (i.e., difficult to find the extreme tip of the probe’s track) or in some cases even impossible, we used the DAPI labeling that is a widely used and accepted method. As the figure illustrates the location of the probe is very well visible in hydrated DAPI-labeled sections.

RESULTS

Effect of GABA-R agonist administration in the MRN on hippocampal activity

Strong, lasting activation of hippocampal theta rhythm after injection of GABA\(_A\) or GABA\(_B\) agonists in the median raphe nucleus was reported previously (Kinney et al. 1995; Varga et al. 2002). Similar experiments in the present study were thus performed primarily to verify and extend these findings with two major modifications in the experimental procedure. First, drugs in this study were injected using the reverse microdialysis technique, which, unlike pressure injection used earlier (Kinney et al. 1995), allowed administration of muscimol by maintaining prolonged stable concentrations in the target tissue (see METHODS). Second, unlike in previous studies (Varga et al. 2002), the microdialysis probe was inserted in a way that allowed avoiding the DRN (see METHODS) and to manipulate GABAergic mechanisms only in the median raphe with primary projection to the hippocampus and related limbic structures (Fig. 1).

Figure 2 shows two representative experiments in which the GABA\(_A\) agonist, muscimol, or GABA\(_B\) agonist, baclofen, was administered into the MRN for 1 h. In control recordings,
hippocampal EEG alternated between two patterns of activity, theta rhythm and irregular nonrhythmic activity. The average time spent in the theta state was 40–60% for different groups with large individual variations (Fig. 4A; note large standard errors). After switching the infusion from ACSF to muscimol, theta rhythm became dominant (theta percentage ~95%, note small standard errors, Fig. 4A) and ran uninterrupted for 30–40 min. After ~40 min, theta completely disappeared as reported previously (Kinney et al. 1995) and did not return even after infusion of ACSF for ≤3 h. Concentrations of muscimol >0.05 mM (0.05, 0.1, 0.5, and 1.0 mM; n = 2, 2, 5, and 5, respectively) induced similar changes in the hippocampus; lasting theta occurred in all experiments. The same effect was also observed in five rats in which 1.0 mM muscimol was perfused for a shorter period of time (15 min). Muscimol administered in a concentration of 0.001 mM was ineffective. Muscimol was also ineffective when infused in the adjacent reticular formation (0.5 mM, n = 3, Fig. 5A) (see also in Kinney et al. 1995).

Similarly, the GABA_A-R agonist baclofen (0.2 mM, n = 6) elicited continuous hippocampal theta rhythm (Fig. 2, Table 2, Fig. 4A), which outlasted the 1 h infusion and after which the usual, alternating theta/non-theta EEG pattern returned. These findings were consistent with those reported earlier (Varga et al. 2002), indicating that baclofen can modulate theta activity via mechanisms in the MRN even without the involvement of DRN (see location of the microdialysis probe in Fig. 1). Similar to muscimol, continuous baclofen administration can also lead to hippocampal desynchronization after 40–60 min but only in concentrations >0.5 mM that were not tested in this study (Varga et al. 2002).

Hippocampal activity after GABA_A-R blockade of MRN neurons

GABA_A-R blockade was achieved by either one of two different antagonists, bicuculline (BIC) and gabazine, administered in the MRN using the reverse microdialysis technique for 1 h. After the probe was placed in the MRN, hippocampal EEG was monitored ≥1 h before the perfusion was switched from ACSF to BIC or gabazine. One or two concentrations were used in each rat with ≥1 h wash out between two drug perfusions.

Bicuculline was applied in concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 mM. A typical example of these experiments is shown in Fig. 3. In control recordings, i.e., during ACSF perfusion, hippocampal EEG irregularly switched between theta rhythm with high concentration of the signal power within a narrow range around 4 Hz (see red zones in Fig. 3C) and non-theta periods when the EEG power was distributed over a wide frequency band with relatively low peaks below 2 Hz (Fig. 3, B and C, top). Ten minutes after switching to 0.2 mM BIC the amplitude of theta abruptly decreased and a few minutes later completely disappeared (Fig. 3C, middle) and only returned 20 min after

![FIG. 2. Effect of injections of GABA receptor agonists in the median raphe nucleus. A: sample hippocampal EEG recordings during control non-theta (a) and control theta epochs (b), during muscimol (0.1 mM) injection (c), and 20 min after perfusion with muscimol halted (d). B: power spectra of hippocampal EEG (4-s segments) during perfusion of GABA_A agonist (a, muscimol, 0.1 mM, red line) and GABA_A agonist (b, baclofen, 0.2 mM, red line). Black lines show control ACSF perfusion. C: time–frequency contour plot of hippocampal EEG power spectra before and during drug administration (a, muscimol 0.1 mM and b, baclofen 0.2 mM). White arrows indicate start of drug perfusion. Calibration: 2 mV, 1 s (A).](JNeurophysiol.94.10.2005.2564.1.png)
The end of infusion (Fig. 3). Higher concentrations exerted a different effect. Theta suppression switching back to ACSF (not shown). BIC administration in differences from experiment to experiment (Fig. 4).

Depending on the concentration, BIC either elicited lasting theta suppression or a biphasic response composed of a short period of desynchronized activity followed by lasting theta facilitation. These effects were extremely robust and independent of hippocampal activity before drug administration. The latency and duration of hippocampal desynchronization are summarized in Table 3. BIC had no effect when it was given in 0.05 mM concentration (n = 3). After 0.1 mM BIC injection, theta disappeared in 50% of experiments (3 of 6 rats) with latencies between 15 and 35 min (average: 25.0 min) and did not return until perfusion switched back to ACSF. During administration of 0.2 mM BIC, theta was eliminated in most (8 of 10) experiments at a shorter average latency of 11.2 min. On average, percent theta occurrence dropped from 43.3 to 61.2% in the last 20 min of control was between 43.3 and 61.2%

The frequency of theta elicited by infusion of 0.5 or 1.0 mM BIC in the same rat only lasted 7 min after which theta returned with gradually increasing amplitude and continued until the end of infusion (Fig. 3C, bottom).

The effect of GABA_A-R blockade was tested in 48 rats. In control recordings, hippocampal EEG alternated between theta and non-theta, irregularly. The average proportion of theta segments in the last 20 min of control was between 43.3 and 61.2% in different dose groups with large SDs (19.7–36.2%) due to differences from experiment to experiment (Fig. 4A). Depending on the concentration, BIC either elicited lasting theta suppression or a biphasic response composed of a short period of desynchronized activity followed by lasting theta facilitation. These effects were extremely robust and independent of hippocampal activity before drug administration. The latency and duration of hippocampal desynchronization are summarized in Table 3. BIC had no effect when it was given in 0.05 mM concentration (n = 3). After 0.1 mM BIC injection, theta disappeared in 50% of experiments (3 of 6 rats) with latencies between 15 and 35 min (average: 25.0 min) and did not return until perfusion switched back to ACSF. During administration of 0.2 mM BIC, theta was eliminated in most (8 of 10) experiments at a shorter average latency of 11.2 min. On average, percent theta occurrence dropped from 43.3 to 61.2% in the last 20 min of control was between 43.3 and 61.2%

The frequency of theta elicited by infusion of 0.5 or 1.0 mM BIC in the same rat only lasted 7 min after which theta returned with gradually increasing amplitude and continued until the end of infusion (Fig. 3C, bottom).

### Table 2. Theta peak frequency and peak power

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Concentrations</th>
<th>n</th>
<th>Theta Peak Frequency, Hz</th>
<th>Theta Peak Power (% of RMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>20–30 min</td>
</tr>
<tr>
<td>Muscimol</td>
<td>0.05–0.1 mM</td>
<td>4</td>
<td>3.97 ± 0.06</td>
<td>3.79 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>0.5 mM</td>
<td>5</td>
<td>4.10 ± 0.14</td>
<td>4.10 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>1.0 mM</td>
<td>5</td>
<td>4.15 ± 0.05</td>
<td>4.10 ± 0.08</td>
</tr>
<tr>
<td>Baclofen</td>
<td>0.2 mM</td>
<td>6</td>
<td>3.74 ± 0.11</td>
<td>3.70 ± 0.08</td>
</tr>
<tr>
<td>Bicuculline</td>
<td>0.1 mM</td>
<td>3*</td>
<td>4.07 ± 0.16</td>
<td>4.15 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>0.2 mM</td>
<td>2*</td>
<td>4.03 ± 0.36</td>
<td>4.03 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>0.5 mM</td>
<td>9</td>
<td>4.01 ± 0.09</td>
<td>4.01 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>1.0 mM</td>
<td>15</td>
<td>3.82 ± 0.09</td>
<td>3.95 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>2.0 mM</td>
<td>5</td>
<td>3.66 ± 0.27</td>
<td>5.08 ± 0.41</td>
</tr>
<tr>
<td>SR95531 (gabazine)</td>
<td>0.04 mM</td>
<td>5</td>
<td>3.89 ± 0.19</td>
<td>No theta</td>
</tr>
<tr>
<td></td>
<td>0.5 mM</td>
<td>4</td>
<td>3.91 ± 0.15</td>
<td>4.58 ± 0.21</td>
</tr>
<tr>
<td>CGP55438+bicuculline</td>
<td>0.5 mM BIC</td>
<td>2</td>
<td>3.91 ± 0.24</td>
<td>No theta</td>
</tr>
<tr>
<td></td>
<td>1.0 mM BIC</td>
<td>8</td>
<td>3.88 ± 0.11</td>
<td>3.66 ± 0.15</td>
</tr>
</tbody>
</table>

Values are means ± SE. ANOVA: significant effect of drug on frequency and power in groups of bicuculline (BIC) and of CGP+BIC Means comparison using Bonferroni test: significant differences between control and 20 vs. 60 min theta frequency and control vs. 20 and 60 min power. *only experiments with theta were included in statistical analysis. †Significantly different from the effect of 1.0 mM BIC (t-test < 0.05).

The changes in hippocampal EEG were also tested after injection of another GABA_A antagonist, gabazine. This test was necessary because, the salts of bicuculline, i.e., the water-soluble forms of BIC used in pharmacological experiments, in addition to GABA_A-Rs, exert a direct effect on Ca^{2+} activated K^+ channels, and the functional consequences of the resulting blockade of afterhyperpolarization of the target neurons were reported to be similar to that of their disinhibition (Seutin and Johnson 1999). Gabazine was infused in concentrations of 0.04 and 0.5 mM in five rats using the same experimental protocol. The results were similar to those observed after BIC administration (Tables 2 and 3). In low concentration, gabazine lead to hippocampal desynchronization at latency of 10 ± 1.8 min. Increase in the concentration to 0.5 mM changed the response to lasting theta, which followed a short (11.7 ± 1.0 min) period of desynchronized EEG. The effect was reversible and could be repeated after 1 h wash with ACSF.

In nine rats, the microdialysis probe was placed outside the MRN (2 mm lateral from midline, tilt varied between 24 and 70°) and BIC was administered in 0.1 or 0.5 mM concentrations. Theta suppression was not observed in any of these experiments. Strong, lasting theta oscillation was elicited in most experiments (Fig. 5B). The dynamics of this rhythm was different from that after MRN injection; there was a rapid rise in theta frequency from the onset reaching 6–8 Hz; this is similar to the theta frequency elicited by electrical stimulation of the reticular formation (Green and Arduini 1954; Kocsis and Li 2004; Petsche et al. 1962; Vertes 1981).

Characterization of the theta pattern elicited by GABA_A-R blockade in the MRN

The frequency of theta elicited by infusion of 0.5 or 1.0 mM BIC into the MRN was generally maintained near the control level (~4
Hz, Table 2) with a steady slow rise (Fig. 6B), resulting in a mild but significant theta acceleration by the end of the 60-min injections (Table 2 and Fig. 4B). 2.0 mM BIC or 0.5 mM of gabazine elicited a larger (25%) increase in theta at shorter latencies (8.4 ± 1.2 min).

In addition to this “late” theta acceleration, in many experiments, there was a rapid “early” increase in frequency that appeared right after the start of drug administration but tended to last only a few minutes before the onset of hippocampal desynchronization (Fig. 6A). Early theta acceleration could develop more easily at low concentrations of BIC when theta suppression arrived at longer latencies, i.e., in 1 of 3 experiments with 0.1, and in 5 of 10 experiments with 0.2 mM BIC infusion, and only in three and four rats (<30%) with 0.5 and 1.0 mM, respectively. In this latter group, however elevated theta frequency was observed immediately after the initial non-theta phase, in another five rats (see Fig. 3C for example).

The amplitude of theta oscillations were generally reduced as indicated by a significant decrease in the relative power at theta frequency (spectral peak) during perfusion of BIC in concentrations higher than 0.2 mM (2-way ANOVA; source: BIC main effect, F = 4.35844, df = 2, P = 0.016; Table 2 and Fig. 4B). Furthermore, the amplitude was much more variable during drug administration than in control recordings and the rhythm in most cases exhibited a remarkable intermittent character. Figure 7 shows two examples in which theta was strongly modulated either by regularly occurring disruptions of the oscillations or by periodic modulation of its amplitude. The frequency of the intermittency was <2 Hz and in many experiments was strong enough to generate a spectral peak comparable to the peak at theta frequency (Fig. 7C).

Another form of intermittent hippocampal oscillations introduced by BIC administration in the MRN in concentrations of ≥1.0 mM was represented by well separated theta bursts lasting ~1 s each and periodically repeated every 2–7 s. The intraburst frequency of these oscillations (6–8 Hz) was considerably higher than the regular theta rhythm (~4 Hz). The bursts seemed independent of the on-going slow hippocampal EEG as they could develop either on the background of theta (Fig. 8A) or non-theta (B) activity.

**Effect of combined GABA<sub>A</sub>- and GABA<sub>B</sub>-R blockade of MRN neurons on hippocampal activity**

It was demonstrated earlier that serotonergic neurons in the MRN, in addition to GABA<sub>A</sub>-Rs express GABA<sub>B</sub>-Rs, on their
administration of the GABA\textsubscript{B}-R antagonist in proportions 1:5, 1:18, when the concentration of BIC was increased to 1.0 mM, coadministration of 2.5 mM CGP35348 (elicited by 0.5 mM BIC was completely eliminated by coadministration of bicuculline and baclofen, and BIC injections. Note large decrease in SE after muscimol, baclofen, and BIC injections. B: theta frequency before, during (20 min), and at the end (60 min) of infusion of GABA antagonists. C: theta power before, during (20 min), and at the end (60 min) of infusion of GABA antagonists. Error bars mark SE.

somata and dendrites. Thus to achieve total GABA-R blockade, a mixture of BIC and GABA\textsubscript{B}-R antagonist CGP35348 was infused in the MRN in 10 rats in various proportions. Theta rhythm elicited by 0.5 mM BIC was completely eliminated by coadministration of 2.5 mM CGP35348 (n = 2; see example in Fig. 9A). When the concentration of BIC was increased to 1.0 mM, coadministration of the GABA\textsubscript{B}-R agonist in proportions 1:5, 1:18, or 1:50 was effective in blocking theta rhythm during the first 40 min in four of eight experiments. In three of the four remaining experiments, lasting theta appeared 12, 22, or 27 min after the start of infusion and, in one rat, theta and non-theta alternated (this is shown in Fig. 9B). The group average of the duration of theta suppression was still significantly higher (25.1 ± 5.5 min) than during administration of BIC alone (Table 3). In addition, the frequency of theta rhythm was also reduced and was significantly different from that during control or BIC. Late theta acceleration was similar to that after administration of BIC alone (Table 2 and Fig. 4C) whereas early theta acceleration was not observed in any of these experiments.

DISCUSSION

This study concerned the role of GABAergic mechanisms in MRN in the control of hippocampal activity. MRN is the primary source of serotonergic afferents to the limbic system that are generally considered to have a suppressing effect on hippocampal theta oscillations (Vertes and Kocsis 1997). Thus GABAergic inhibition or disinhibition of 5-HT neurons in the MRN is expected to facilitate or inhibit, respectively, the theta rhythm. GABA-Rs are also expressed, however, on other types of MRN neurons, including local GABAergic interneurons and possibly long-projecting GABAergic (Jankowski and Sesack 2002; Puig et al. 2004) and glutamatergic cells (Tao and Auerbach 2003). The action of GABAergic substances at these sites can be opposite to those on 5-HT neurons which might explain earlier findings of similar actions of muscimol (Kinney et al. 1995) and BIC (Thinschmidt et al. 1995) injected into the same structure. The present report offers several important observations to clarify this issue. First, we confirmed and extended previous findings (Kinney et al. 1995; Varga et al. 2002) regarding the theta-promoting effects of MRN application of the GABA\textsubscript{A} agonist muscimol and GABA\textsubscript{B} agonist baclofen. Second, we demonstrated that the GABA\textsubscript{A} antagonist bicuculline infused in low concentrations (0.1, 0.2 mM) into the MRN eliminated theta rhythm. Lasting hippocampal desynchronization followed a short period of theta activity of higher than normal frequency in 46% of rats. Third, we found that MRN administration of bicuculline in larger concentrations (0.5, 1.0, and 2.0 mM) resulted in a biphasic response. In these experiments, the period of hippocampal desynchronization was shorter than 10 min and was followed by lasting theta rhythm as long as the perfusion continued. The frequency and amplitude of theta waves were significantly higher than in control recordings and the oscillations showed a conspicuous intermittent character. Fourth, hippocampal theta rhythm elicited by MRN administration of bicuculline could be completely (BIC in 0.5 mM) or partially (BIC in 1.0 mM) blocked by simultaneous infusion of GABA\textsubscript{B} antagonist CGP35348.

**Advantages and limitations of the methodology**

Administration of drugs using reverse microdialysis has several advantages over pressure microinjections. After microinjection, the drug concentration at the cannula, at the moment of injection is relatively high; this represents a powerful drive for diffusion. The drug diffuses to a certain distance and gets eliminated by different mechanisms. The affected area first rapidly expands and then shrinks at a lower pace. The concentration is therefore never stable at any point in the structure, at any time, and the affected

**TABLE 3.** Latency and duration of suppression of hippocampal theta elicited by GABA\textsubscript{A} receptor blockade in the MRN

<table>
<thead>
<tr>
<th>Concentration</th>
<th>n</th>
<th>Latency of Theta Suppression</th>
<th>Duration, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIC 0.1 mM</td>
<td>3 of 6</td>
<td>25.0 ± 5.0</td>
<td>&gt;50 ± 0.0</td>
</tr>
<tr>
<td>BIC 0.2 mM</td>
<td>9 of 10</td>
<td>11.2 ± 2.0</td>
<td>&gt;50 ± 0.0</td>
</tr>
<tr>
<td>BIC 0.5 mM</td>
<td>9 of 11</td>
<td>4.3 ± 1.5</td>
<td>9.7 ± 1.0</td>
</tr>
<tr>
<td>BIC 1.0 mM</td>
<td>15 of 15</td>
<td>3.3 ± 1.0</td>
<td>7.5 ± 2.8</td>
</tr>
<tr>
<td>BIC 2.0 mM</td>
<td>5 of 5</td>
<td>2.6 ± 0.2</td>
<td>5.8 ± 1.1</td>
</tr>
<tr>
<td>GABA\textsubscript{B} 0.04 mM</td>
<td>5 of 5</td>
<td>10.0 ± 1.8</td>
<td>&gt;50 ± 0.0</td>
</tr>
<tr>
<td>GABA\textsubscript{B} 0.5 mM</td>
<td>4 of 4</td>
<td>4.2 ± 0.7</td>
<td>7.5 ± 1.2</td>
</tr>
<tr>
<td>BIC + CGP 35348</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIC + CGP 0.5 mM</td>
<td>2</td>
<td>8.0 ± 2.0</td>
<td>&gt;50 ± 0.0</td>
</tr>
<tr>
<td>BIC + CGP 1.0 mM</td>
<td>8</td>
<td>2.7 ± 1.5</td>
<td>25.1 ± 5.5</td>
</tr>
</tbody>
</table>

n is the number of experiments in which theta suppression occurred. *Proportion of concentrations BIC:CGP = 1:5. †proportion of concentrations BIC:CGP = 1:5 (n = 2), 1:18 (n = 4), 1:50 (n = 2).
area constantly changes. Microdialysis can deliver the same amount of drug stretched out in time. The concentration in the tissue starts from low values and rises continuously until it reaches equilibrium. From that moment, it stays relatively stable at any location for as long as the experiment continues. The affected area also remains steady. From this there are two important practical conclusions: that the latencies are longer i.e., the latency is not the time necessary for diffusion (as in microinjection) rather the time necessary to equilibrate the concentrations in different probe and tissue compartments and that the distance of the diffusion is much more limited due to the lack of the initial big pressure inherent for microinjection. Modeling and experimental investigations (Boehnke and Rasmusson 2001; Dykstra et al. 1992; Robinson and Juscice 1991) have shown that once the equilibrium is established the distance of the diffusion and thus the affected area no longer increases—no matter how long the dialysis lasts.

Although the specifics regarding the effective spread of bicuculline in the brain tissue are not known, the diffusion of neuroactive compounds are limited by a number of factors, including binding to receptors and removal into the microcirculation. Earlier dialysis studies indicated, for example, that bicuculline, muscimol, or baclofen infused in the MRN did not diffuse as far as the nearby DRN and vice versa (Tao and Auerbach 2003; Tao et al. 1996). Furthermore, mathematical modeling and in vivo measurements of various substances (e.g., sucrose, mannitol) indicated that the concentration outside of a 1-mm radius around the probe was very low (Dykstra et al. 1992; Höistad et al. 2002). Yet in another study, significant physiological effect of lidocaine and tetrodotoxin could be detected as far as 1.5–2 mm after 15- to 20-min dialysis perfusion (Boehnke and Rasmusson 2001). Although this issue remains unresolved, the possibility of the build-up of an effective concentration of bicuculline outside the MRN cannot be excluded. It could have played a role in promoting theta when applied in high concentrations (1.0 mM and higher), especially during the second half of the 1-h perfusion period. It could also contribute to the steady increase in theta frequency in the second half of the perfusion; “late” theta acceleration usually started after 40 min (see Figs. 3B, 4B, and 8B).

**GABAergic suppression of 5-HT neurons drives hippocampal theta rhythm**

There is ample evidence indicating that the 5-HT input originating in the MRN suppresses theta bursts in the medial septum and theta rhythm in the hippocampus (Vertes and Kocsis 1997). Electrical stimulation of the MRN desynchronizes the hippocampal EEG in freely behaving as well as in anesthetized rodents (Assaf and Miller 1978; Kitchigina et al. 1999; Macadar et al. 1974; Vertes 1981; Yamamoto et al. 1979), whereas MRN lesions (Kitchigina et al. 1999; Maru et al. 1979; Vinogradova et al. 1999; Yamamoto et al. 1979), 5-HT depletion (Mushiake et al. 1988), or selective inhibition of MRN serotonergic cells through 5-HT1A autoreceptors (Vertes et al. 1994) produce continuous theta rhythm. The median raphe contains a large population of GABAergic interneurons (Jacobs and Azmitia 1992; Maloney et al. 1999; Mugnaini and Oertel 1985) and receives GABAergic input from numerous distant regions from the forebrain to the medulla (Gervasoni et al. 2000). The schematics in Fig. 10 summarizes existing data on the complex interactions between GABAergic and serotonergic neuron populations. GABAergic neurons are excited by locally released 5-HT via 5-HT2-Rs and, in turn, inhibit serotonergic neurons via GABA receptors (Bowery et al. 1987; Chu et al. 1990; Gao et al. 1993; Serrats et al. 2003; Varga et al. 2002). 5-HT neuron firing was suppressed by stimulation of GABA (Gallager and Aghajanian 1976) and GABA-Rs (Bowery et al. 1987; Chu et al. 1990; Gao et al. 1993; Serrats et al. 2003; Varga et al. 2002).
hippocampus (Forchetti and Meek 1981; Wirtshafter et al. 1987b). Thus the strong theta rhythm observed after injection of GABA-R agonists (Kinney et al. 1995; Varga et al. 2002) was most likely due to direct inhibition of the ascending serotonergic pathway. In this study, we found that both muscimol and baclofen were effective in eliciting theta when injected in the MRN in small concentrations. Continuous hippocampal theta was observed in all experiments during infusion of muscimol in concentrations as low as 50 $\mu$M while the threshold for baclofen was found higher, at 200 $\mu$M (Varga et al. 2002). Considering a significant, i.e., $\approx 90\%$ (Dykstra et al. 1992), drop in drug concentrations across the dialysis membrane, these findings were in agreement with results of direct examination of serotonergic neurons in raphe slices. In vitro, 10 $\mu$M muscimol effectively decreased local serotonin release (Bagdy et al. 2000) and the firing rate (Gallager and Aghajanian 1976). Baclofen induced marked hyperpolarization of 5-HT neurons and completely inhibited their firing at concentrations between 10 and 50 $\mu$M (Innis and Aghajanian 1988, 1987).

After $\sim 40$ min of muscimol application, theta activity disappeared and did not recover until the end of the experiments. This delayed effect of muscimol can be due either to local mechanisms or to suppression of theta-inducing circuitry outside the median raphe nucleus, or both. In the first case, prolonged administration of the agonist can induce adaptive changes in GABA$_A$-R function. Desensitization followed by the upregulation of agonist binding sites has recently been reported (Pericic et al. 2003). These changes can be subunit specific affecting various GABA$_A$-R subunits to different extent. Thus prolonged GABA$_A$-R activation can result in a disinhibition of the MRN neuronal network leading to an increased serotonergic outflow and desynchronization of the hippocampal EEG. The return of theta can take several hours beyond the length of the experiments.

**Effect of GABA-R antagonism in the MRN on hippocampal theta rhythm**

Depending on the concentration and the length of exposure, perfusion of GABA$_A$-R antagonist BIC in the MRN consis-
FIG. 7. Modulation of theta amplitude during infusion of GABA_A receptor blocker in the MRN. A: EEG traces before (ctrl) and during BIC infusion. Traces in b were passed through a digital high pass filter (Butterworth, order 10) to eliminate slow baseline fluctuations. B: strong amplitude modulation in another experiment with slow theta (theta frequency: 4.2 Hz, modulation frequency: 1.8 Hz). C: time-frequency contour plot of hippocampal EEG. Note increase in theta frequency from 5 Hz in control to 5.8 Hz 15–20 min after start of drug administration. A low-frequency component (~1.1 Hz) also appeared in the power spectra about the same time.

FIG. 8. Fast theta bursts in the hippocampus during infusion of GABA_A receptor blocker in the MRN. A: time-frequency contour plot shows three frequency components in an experiment at the end of the 1-h-long injection of 1.0 mM BIC. Note a fast ~8-Hz component in addition to theta (4–5 Hz) and delta (1–2 Hz) peaks. Right: traces show a segment of the original EEG (a; 1–70 Hz); and its low-pass (b; <5 Hz) and high-pass filtered (c; >5 Hz) components. B: regular ~4-Hz theta rhythm during control (left) and fast theta bursts (~6 Hz, right) in a different rat receiving 0.5 mM BIC. Contour plot shows regular (~4 Hz) theta in control; a–c as above.
tently elicited an orderly combination of the following three patterns of hippocampal activity.

**LASTING HIPPOCAMPAL DESYNCHRONIZATION.** Lasting hippocampal desynchronization appeared at decreasing latencies as the BIC concentration increased. Anatomical distribution of GABA<sub>A</sub>-Rs suggests that in the 5-HT-GABA network of the raphe, BIC can lead to activation of both neuron populations resulting in a rapid elevation in both GABA and 5-HT levels (see Fig. 10). This balance, however, can eventually shift to either GABA or 5-HT dominance. Elevation of local serotonin concentration in MRN will, through 5-HT<sub>2A</sub>-R activation, further enhance GABA tone, which now can reach extrasynaptic GABA<sub>B</sub>-Rs on 5-HT cells and together with 5-HT<sub>1A</sub>-mediated autoregulation suppresses serotonergic cells. This can in turn lead to disfacilitation of GABA neurons, an ensuing disinhibition of 5-HT, and so on. The source of asymmetry in this feedback system is the postsynaptic GABA<sub>B</sub>-Rs, which in the raphe are primarily expressed by 5-HT neurons (Varga et al. 2002) and might thus play a significant role in switching between states depending on the level of local GABA tone. Due to their extrasynaptic location, these receptors have a higher threshold (Scanziani 2000) and can be activated by fast or synchronized firing of GABAergic interneurons as found in unanesthetized rats during REM sleep (Kocsis and Vertes 1992; Maloney et al. 2000; Nitz and Siegel 1997; Torterolo et al. 2000). Accordingly, in the present experiments lasting desynchronization of hippocampal EEG only appeared at moderate (0.1–0.2 mM) concentrations of BIC disinhibiting both 5-HT and GABA neurons. Suppression of hippocampal theta rhythm indicates that at this level the elevated GABA input to serotonergic cells was subthreshold for GABA<sub>B</sub>-Rs resulting in a net increase in 5-HT output. When the probe was outside the MRN suppression of theta was not observed.

**SUSTAINED SLOW (4–5 Hz) THETA RHYTHM** Sustained slow (4–5 Hz) theta rhythm observed during high concentration BIC administration can be the result of two, synergistically acting mechanisms. First, it could be elicited by GABA<sub>B</sub>-mediated blockade of 5-HT cells as discussed earlier (Fig. 10). The role of GABA<sub>B</sub>-receptors in the suppression of serotonergic activity was strongly supported by the observation that combined blockade of GABA<sub>A</sub>- and GABA<sub>B</sub>-Rs eliminated or attenuated this type of hippocampal theta activity (see Fig. 9). A second mechanism that may have played an increasing role toward the end of the 1-h-long infusion in many experiments was a lateral diffusion of BIC into adjacent areas surrounding the MRN also known as the theta induction zone (Green and Arduini 1954; Kocsis and Li 2004; Vertes 1981). In control experiments when the probe was located in or near this area, theta rhythm was elicited by 0.5 mM BIC. and the frequency of this rhythm was fast rising from the onset of infusion (Fig. 5B). The distance of diffusion from the probe is very limited but for certain drugs can be >1 mm (Boehnke and Rasmusson 2001). A cylinder with a radius of 1–2 mm around the probe membrane partially overlaps with the area of the theta induction zone (see Fig. 1). Activation of extra-raphe mechanisms would also explain the late acceleration of theta oscillations which does not appear
even after total blockade of 5-HT cells (Kinney et al. 1995; Varga et al. 2002; Vertes et al. 1994).

**FAST (6–8 Hz) INTERMITTENT THETA OSCILLATIONS.** Immediately after the onset of BIC infusion in low concentrations, a transient, short-lasting, fast theta rhythm dominated the hippocampal EEG. Its frequency significantly surpassed the maximum observed during nonselective or selective suppression of the serotonergic output of the MRN (e.g., Fig. 2) (see also Kinney et al. 1995; Varga et al. 2002; Vertes et al. 1994). This indicates that the initial transient fast theta during the injection of BIC in <0.5 mM and the short theta bursts that appeared at later stages on the background of either stationary theta or non-theta EEG could be primarily caused by the activation of nonserotonergic, theta-inducing circuits within the MRN (Fig. 10, right). MRN has a massive nonserotonergic projection to the limbic system including glutamatergic neurons targeting the supramammillary nucleus (SUM) (Kiss et al. 2002). This latter is directly involved in theta generation (Kirk and McNaughton 1991; Kocsis and Vertes 1994), in particular during episodes of theta acceleration (Kaminski and Kocsis 1992). Furthermore, changes in glutamate concentration in the MRN also suggested that the mechanisms involved in the MRN control of lasting slow theta rhythm and those driving fast theta episodes were different. In a microdialysis study, Varga et al. (1998) found that spontaneous slow theta was coupled with a decrease in MRN glutamate levels in keeping with the concept of theta due to disfacilitation of 5-HT neurons, whereas the opposite was true for fast theta episodes elicited by sensory stimulation. Nonserotonergic MRN efferents and their GABAergic control have also been implicated in behavioral regulation (Tao and Auerbach 2003; Wirtshafter et al. 1987a, 1989).

GABAergic control of serotonergic and nonserotonergic elements of the raphe

In principle, the GABA$_A$-R antagonist BIC could influence two pathways ascending from MRN that have opposite effects on hippocampal theta rhythm. The major serotonergic MRN input desynchronizes hippocampal EEG, whereas the recently described glutamatergic input to SUM (Kiss et al. 2002) most likely drives theta rhythm. In addition, the two pathways also differ in the function and the dynamics of the regulation they provide. 5-HT is a state-controlling modulatory input i.e., its suppression alone is sufficient to switch the hippocampus from desynchronization back to the theta state (4–5 Hz under urethan). In contrast, the MRN glutamatergic neurons along with the reticular input, SUM, and others are rapid-action elements generating fast theta oscillations (6–8 Hz). GABA$_A$-Rs are expressed by serotonergic and nonserotonergic cells that include GABAergic and glutamatergic neurons, whereas postsynaptic GABA$_B$-Rs in the MRN are predominantly located on 5-HT neurons (Serrats et al. 2003; Varga et al. 2002). 5-HT cells are also known to be embedded in multiple feedback loops through 5-HT$_{1A}$ autoreceptors and
through 5-HT₂ activation of local GABAergic neurons (Glass et al. 2004; Liu et al. 2000). Thus BIC could act as a theta-suppressing factor through direct effect on 5-HT cells as well as a pro-theta agent by acting on GABAergic or glutamatergic units.

Figure 10 shows a simplified wiring model of GABAergic regulation of the serotonergic output of the MRN: during non-theta states the MRN output is dominated by serotonergic activity. At the onset of theta, incoming excitatory drive increases GABAergic tone and suppresses serotonergic cells via GABA_A and GABA_B receptors thus releasing the theta-generating circuitry from serotonergic inhibition. The MRN 5-HT-GABA system is stabilized by feedback through 5-HT₁a autoreceptors and 5-HT₂ receptors on GABAergic neurons (Liu et al. 2000). The recruitment of GABA_B receptors requires increased GABAergic tone (Nitz and Siegel 1997) and synchronized firing of GABAergic cells (Kocsis and Vertes 1992). Besides the inhibition of serotonergic neurons putative glutamatergic, theta-promoting circuits are activated. The latter neurons can also be the targets of GABAergic inhibition.

Thus the GABAergic network may have two seemingly opposing functions in the MRN: relieving the theta-generators from serotonergic inhibition and regulating the activity in the theta-promoting circuitry by the fluctuating GABAergic tone. The resulting hippocampal oscillations would, however, show different characteristics i.e., slow and on-going theta rhythm due to 5-HT withdrawal and fast intermittent theta bursts due to glutamatergic activation or disinhibition. The two mechanisms may be involved in different functions, and they may show species differences in their development and activation. In the rat, REM sleep is characterized by on-going theta rhythm in the hippocampus that, however, is not stationary and includes rapid bursts of high-frequency theta oscillations. It was shown that these episodes of fast theta are not distributed randomly but follow a sequence predictable by the animal’s previous learning experience (Louie and Wilson 2001). In human, REM sleep-associated hippocampal theta appears in the form of short (1-2s) theta bursts of 4–7 Hz repeated regularly every ~6 s on the background of non-theta activity (Cantero et al. 2003), and similar theta bursts in waking were shown to be related to spatial learning (Kahana et al. 1999).

Under urethan anesthesia spontaneous theta is usually slow; waking were shown to be related to spatial learning (Louie and Wilson 2001). In human, REM sleep associated theta is demonstrated an extended capacity of intrinsic raphe circuits even in those seen in REM sleep, by changing the MRN GABA tone.

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Present address of V. Varga: Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest.

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