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Glutamatergic Synaptic Transmission Participates in Generating the Hippocampal EEG

L. Stan Leung[1,2*] and Bixia Shen[1]

ABSTRACT: The participation of ionotropic glutamatergic synapses in the generation of hippocampal electroencephalography (EEG) of behaving rats has not been systematically studied. In this study, field potentials in hippocampal CA1 were recorded following injection of N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonists, or vehicle control, either into the lateral ventricles or directly into the hippocampus or the medial septum. Intraventricular (i.c.v.) AMPA receptor antagonist 6,7-dinitroquinoxaline-2,3,dione (DNQX, 5–10 μg) decreased the commissural evoked potential and the amplitude of the hippocampal EEG, including the theta rhythm. Theta frequency was decreased by 10 μg, but not 5 μg DNQX i.c.v. Unilateral intrahippocampal injection of DNQX (5 μg) only decreased the amplitude, but not the frequency, of the theta rhythm near the site of injection, without affecting theta amplitude or frequency at the opposite hippocampus. Other than theta, the large irregular activity (with a delta frequency peak at 1–2 Hz) and gamma EEG (30–100 Hz) were also decreased by i.c.v. and intrahippocampal injections of DNQX. Intrahippocampal injection of NMDA receptor antagonist D-2-amino-5-phosphonovaleric acid (D-APV, 2.5 μg) decreased the amplitude of the theta rhythm and, less consistently, the gamma EEG. The frequency of the theta rhythm and the peak of the commissural evoked potential were not significantly affected by intrahippocampal D-APV injection. Medial septal injections of D-APV or D,L-APV (2.24 μg in 0.4 μl), but not DNQX (10 μg in 0.4 μl), decreased the amplitude of the hippocampal theta significantly, but theta frequency was not significantly affected. It is concluded that both NMDA and AMPA receptors in the hippocampus are involved in generating the amplitude of the hippocampal EEG of theta and gamma frequencies, while NMDA receptors in the medial septum are involved in controlling the amplitude of theta and gamma EEG in the hippocampus. Excitatory glutamatergic synaptic currents, activated by afferents from the entorhinal cortex and CA3, are suggested to participate in hippocampal EEG activities. © **2004 Wiley-Liss, Inc.**

KEY WORDS: theta rhythm; gamma rhythm; medial septum; NMDA receptors; AMPA receptors

INTRODUCTION

The hippocampal theta rhythm is one of the most regular brain rhythms in many laboratory animals. Early studies by Green and associates (Green, 1964) suggested that the hippocampal theta rhythm was associated with arousal. The theta rhythm is believed to be important in mediating the behavioral functions of the hippocampus, such as voluntary movements (Vanderwolf, 1969), sensorimotor integration (Bland, 1986), spatial navigation (O'Keefe and Reece, 1993; Buzsaki, 2002), synaptic plasticity (Larson et al., 1986; Diamond et al., 1988), and spatial memory (Winson, 1978).

The generation of theta rhythm may be separated into two components—a temporal (frequency pacemaker) and a spatial (current generator) component. The medial septum and diagonal band of Broca (DB) have been recognized as participating in the rhythmic generation, while the neurons of the hippocampus, in particular the pyramidal cells, generate the extracellular currents and potentials. In the classic model, Petsche and colleagues (Petsche et al., 1962; Stumpf, 1965) proposed that the septal/DB neurons convert the tonic brainstem/subcortical activity into rhythmic firing at the theta frequency. Theta-frequency firing of the medial septal/DB neurons was shown to persist after spreading depression in the hippocampus, but the hippocampal theta disappeared after septal lesion (reviewed by Stumpf, 1965). More recently, it has been suggested that the isolated hippocampus in vitro may show an intrinsic theta rhythm after cholinergic activation (Konopacki et al., 1987; MacVicar and Tse, 1989; Williams and Kauer, 1997; Fischer et al., 2002; Fellous and Sjenowski, 2000), and single hippocampal neurons may show membrane potential oscillations (Leung and Yim, 1991). In our view, the medial septum serves as the master clock to coordinate the theta rhythms in different brain regions, including the hippocampus and the entorhinal cortex (Vanderwolf and Leung, 1983; Stewart and Fox, 1990; Bland and Colom, 1993; Vertes and Kocsis, 1997).

There are two main ways that glutamatergic receptors may participate in the generation of the theta rhythm. First, among other inputs, the medial septum receives glutamatergic and nonglutamatergic inputs from the supramammillary area (SUM) (Leranth and Kiss, 1996; Kiss et al., 2000). Thus, our first hypothesis is that the frequency and amplitude of the theta rhythm are controlled by glutamatergic synapses in the medial septum. Second, the entorhinal cortex has been proposed to generate part of the hippocampal theta rhythm (Vanderwolf and Leung, 1983; Bragin et al., 1995; Heynen and Bilkey, 1994), probably through distal dendritic glutamatergic excitation in CA1 (Leung, 1984c) and the den-

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tate gyrus (Buzsaki et al., 1983). Thus, our second hypothesis is that glutamatergic blockade decreases the amplitude of the hippocampal theta rhythm. In addition to the theta rhythm, large irregular slow activity and gamma rhythm (30–100 Hz) are modulated by the behavior of the rat (Vanderwolf, 1969, 1988; Leung et al., 1982; Bland, 1986; Bragin et al., 1995; Leung, 1998). The dependence of these activities on ionotropic glutamatergic receptors was also investigated in this study.

Hippocampal electroencephalography (EEG) in response to ionotropic glutamatergic blockade has not been studied systematically in the behaving rat. Leung and Desborough (1988) found that intraventricular (i.c.v.) N-methyl-D-aspartate (NMDA) receptor antagonist APV reduced the amplitude, but not the frequency, of the theta rhythm in the walking rat. In an abstract, Horvarth et al. (1988) also reported that NMDA receptor antagonist abolished one component of the theta rhythm, presumably the atropine-resistant component (Buzsaki, 2002). In this report, hippocampal theta rhythm was studied by spectral analysis following injection of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist in the lateral ventricle, and AMPA and NMDA antagonists locally in the hippocampus and medial septum.

MATERIALS AND METHODS

Thirty-five adult male Long-Evans rats were used. Under sodium pentobarbital anesthesia, the skull was adjusted to fit bregma and lambda in a horizontal plane. Bipolar electrode pairs were placed in the left and right hippocampus with coordinates posterior (P) and lateral (L) to bregma of P3.3, L ± 1.7 to P4.6, L ± 2.8. Each electrode was a 125-μm wire insulated with Teflon, except at the cut end. The deep (ventral) electrode was targeted at stratum radiatum or stratum lacunosum-moleculare of CA1 in the dorsal hippocampus, using electrophysiological criteria during the implantation procedure (Leung, 1984a). The surface electrode (targeted at the ventricular surface of the hippocampus) was placed 0.5–1 mm dorsal to the deep electrode. Jeweler's screws, placed in the skull over the cerebellum and the frontal cortex, served as reference and ground electrodes, respectively. Guide cannulae (23-gauge, 0.6-mm outer diameter) were placed (1) bilaterally over the lateral ventricle (Leung and Desborough, 1988) at P0.8, L1.4 and 2.4 mm ventral (V) to the skull surface (V-2.4); (2) bi- or unilaterally into the dorsal hippocampus (P3.5, L ± 2.7, V-1.2); or (3) at the midline (single cannula) over the medial septal area at A0.7, L0, V-4. In the group intended for hippocampal injections, the guide cannula was glued to the bipolar recording electrodes, with the deep electrode 2 mm ventral to the cannula tip. The guide cannula was occluded by a pin, except at the time of injection.

Rats were given 7 days for recovery from surgery. The hippocampal EEG, monopolarly referred to a screw in the skull over the cerebellum, was recorded on a polygraph and by a microcomputer during one of two behavioral states: (1) awake immobility—when the rat held its head up against gravity, with no discernible movement of the head or body; and (2) walking—when the rat walked and reared spontaneously; in cases when the rat did not walk spontaneously, walking was induced by turning the cage or by "wheel-barreling" the rat across the floor surface. Commissural average evoked potential (AEP) was recorded during awake-immobility on the side of drug injection (if the injection was unilateral), and it was evoked by stimulation at a stimulating electrode located at the stratum oriens or stratum radiatum of the opposite CA1. Stimulus intensity was typically 2 to 3 times the response threshold, and 6 sweeps were averaged. EEG and AEPs were recorded periodically for 5–120 min before and 5–180 min after drug/vehicle injection.

During drug/vehicle injection, the rat was held manually and a 30-gauge inner cannula was inserted 2 mm ventral to the guide cannula to reach the target area. The volume injected intracerebroventricularly (i.c.v.) and intracerebrally (directly into the brain) was ≤3 μl and ≤0.4 μl, respectively, and the injection was done by a Hamilton syringe over a time course of >30 s.

Drugs used were (1) competitive non-NMDA receptor antagonist, 6,7-dinitroquinoxaline-2,3,dione (DNQX; molecular weight (MW) 252.14) (Muller et al., 1988) or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; MW 232.16); and (2) competitive NMDA receptor antagonist, 2-amino-5-phosphonovaleric acid (APV), as either D,L-APV or D-APV (MW 191.73). All drugs were purchased from Research Biochemicals (now Sigma, St. Louis, MO). DNQX and CNQX were dissolved in dimethylsulfoxide (DMSO), and APV was dissolved in 0.9% saline. Control vehicle injections (DMSO or saline) were made in a quasi-random fashion, such that about equal number of rats received vehicle and drug injection first. The effective i.c.v. dose was estimated by assuming that the drug diffused in a volume of cerebrospinal fluid of ~1.5 ml, and thus 10 μg DNQX i.c.v. would give a 26 μM concentration, an effective dose in vitro (Muller et al., 1988). The concentration of an intracerebral injection was determined empirically. As determined by autoradiography (Martin, 1991) or the behavioral effects after intracerebral injections (Ma et al., 2002), the spread of an injected 0.4–1-μl volume (of muscimol and lidocaine) in 30 min was estimated to be 1–2-mm radius. Typically, we found that the effective amount of CNQX/DNQX and APV injected intracerebrally was within the range of 10–22 nmoles, similar or lower than reports of previous injections in the medial septum (Puma et al., 1996; Puma and Bizot, 1999). CNQX, in the amount of 5 μg (22 nmoles), did not completely abolish the hippocampal AEP recorded at an electrode ~0.5 mm away from the center of the injection (see Results and Fig. 4), suggesting that the dose did not saturate all the AMPA receptors in the vicinity of the electrode. DNQX 5 μg (13 nmoles) injected intracerebrally was more effective in attenuating the AEP (see Fig. 3), and thus a range of AMPA receptor blockade was inferred indirectly by the different degree of suppression of the hippocampal AEP.

EEG signals were sampled at 200 Hz. Power, phase, and coherence spectra of the EEG signals, >30 s in duration and with >60 degrees of freedom, were derived from fast Fourier transform (FFT) as described elsewhere (Leung et al., 1982; Leung, 1985). Power spectra were plotted in arbitrary logarithmic units, with the same calibration in each rat. In most EEGs, after 12-bit analog-to-digital conversion (ADC), the spectra had 0.195-Hz resolution and 2.15-Hz bandwidth, and a power peak of 4.8 log units was

equivalent to a sinusoidal (theta-frequency) wave of 0.5-mV peak-to-peak amplitude. Other EEG signals subjected to 8-bit ADC had 0.78-Hz resolution and 2.34-Hz bandwidth; a power peak of 3.6 log units was equivalent to a sinusoidal wave of 0.5-mV peak-to-peak amplitude. An increase of 1 logarithmic power unit indicated a 10-fold increase in the squared amplitude of the EEG at the particular frequency. Coherence was given as the Fisher z-transform value (Leung et al., 1982), which is equal to $0.5^* \ln(1 + c)/\ln(1 - c)$ where c is linear coherence. Dorsoventral coherence means the coherence between deep and surface electrodes on one side. Phase of the EEG was estimated at the deep electrode (apical dendrites), using the EEG of the surface electrode as the reference. Theta phase was reported at the frequency of the peak theta power. In this study, placement of the deep electrode varied from the proximal stratum radiatum to the molecular layer in CA1. However, some but not all of the rats in each group showed a 90–170° theta phase that was sensitive to drugs and corresponded to a stratum radiatum electrode placement (Leung, 1984b; Leung et al., 1994). At the end of the experiment, each rat was sacrificed and perfused with formalin. The brain was sectioned using a freezing microtome, and sections were stained using thionin.

RESULTS

Intraventricular Injection of DNQX

Injection of DNQX (5–10 μg in 1–2 μl i.c.v) resulted in a large decrease in the commissural average evoked potential (AEP) in the hippocampus, starting 5–10 min (Fig. 1D). By 10–20 min after i.c.v. 10 μg DNQX, the peak of the AEP decreased to 28 ± 6 % (N = 8) of the baseline peak, as compared to 93 ± 4 % (N = 7) of the baseline following i.c.v. vehicle (DMSO) injection (Table 1). The results are reported for 10–20 min after injection, unless otherwise noted. Depression of the AEP was maximal at 10–30 min after DNQX, after which it slowly recovered (Fig. 1D2). Since the commissural AEP consisted mainly of population excitatory postsynaptic potentials (Kaibara and Leung, 1993), its suppression indicates the functional blockade of AMPA receptors, as had been shown in vitro (Muller et al., 1988).

Similar to the decline of the AEP, the amplitude of the hippocampal EEG, including the theta rhythm, was decreased by i.c.v. 10 μg DNQX, starting 5–10 min after injection (Fig. 1B,D) and lasting >1 h. Power spectral analysis confirmed a decrease in theta power in CA1 at electrodes dorsal and ventral to the CA1 cell layer (Fig. 2C). The average decrease in theta power during walking at 10–20 min after DNQX was highly significant ($P < 0.01$, Wilcoxon) and measured 0.52 ± 0.06 (N = 8) and 0.59 ± 0.06 (N = 7) log units at electrodes dorsal and ventral to the CA1 cell layer, respectively. The corresponding decrease in the mean theta amplitudes was 53% and 51% of the control walking theta amplitude, respectively, at the dorsal and ventral electrode. The peak frequency of theta was also reduced in each of the eight rats given 10 μg DNQX i.c.v. (Fig. 2C), from 7.9 ± 0.1 Hz during baseline to 6.2 ± 0.3 Hz (N = 8) after DNQX. The dorsoventral coherence at the theta frequency was significantly reduced by DNQX (Fig. 2C; Table 1). Theta phase was not consistently affected by 10 μg DNQX (Table 1), although in the example shown, the theta phase showed a decrease (arrow in Fig. 2C3), along with theta frequency, after DNQX.

The decrease of theta power and frequency was observed at a time (5–10 min) after 10 μg DNQX i.c.v. injection when a rat was still capable of voluntary movements. At about 10–30 min after i.c.v. injection, when DNQX had maximal effects, rearing and righting were difficult, although movements and struggling could still be induced and the rat still responded to tail pinch. In behaving rats in which rectal temperature was recorded, the decrease of theta amplitude and frequency was found after i.c.v. DNQX without a decrease in the core temperature of the rat.

A lower dose of DNQX (5 μg i.c.v.) induced a small depression of the commissural AEP (mean 56%) and theta power, and no significant change in theta coherence and frequency (Figs. 1D and 2B; Table 1). Behavioral effects were also small after 5 μg i.c.v. DNQX. Vehicle (DMSO) injection induced no significant change in behavior, theta frequency, theta power, or dorsoventral theta coherence (Table 1).

Local Injection of DNQX into the Hippocampus and Medial Septum

To show the possible sites of action of the AMPA receptor antagonist, local injections were made in the medial septum and the hippocampus. Injection of 10 μg (19.8 nmoles) of DNQX (in 0.4 μl) into the medial septum had no significant effect on the hippocampal EEG or the commissural hippocampal AEP (Table 1; EEG and spectra not shown).

In contrast to medial septal injections, bilateral injections of the hippocampus with DNQX (5 μg in 0.3 μl) resulted in a strong depression of the hippocampal EEG and > 90% reduction of the commissural AEPs (Fig. 3B; Table 1). Rats were capable of walking and rearing after injection of DNQX into the hippocampus, although they tended to remain immobile on their own. The maximal depression of AEP and EEG occurred at 10–30 min after local injection. The hippocampal theta power at either dorsal or ventral electrodes was significantly reduced after DNQX local injections ($P < 0.05$, Wilcoxon), as compared to after vehicle (DMSO) injections. The dorsoventral coherence at the theta frequency was not significantly reduced after DNQX, and the peak frequency of theta was not consistently reduced in the four rats tested.

The AMPA receptor antagonist was also injected into one hippocampus. Seven rats were injected with CNQX (5 μg in 0.4 μl) into one hippocampus. DNQX had the same effect as CNQX, as verified by injection of DNQX (5 μg) into one hippocampus (two rats). CNQX decreased the power of the hippocampal theta on the side of injection (Fig. 4B), but it did not significantly affect theta power on the side opposite to the injection site (Fig. 4C; Table 1). Voluntary movements were not obviously affected after unilateral injection of CNQX. Hippocampal AEPs were depressed ~50% on the side of CNQX injection (Fig. 4A; Table 1). As a group, the decrease in hippocampal theta power on the injected side during walking was 0.47 ± 0.12 (N = 6) and 0.46 ± 0.12 (N = 7) log

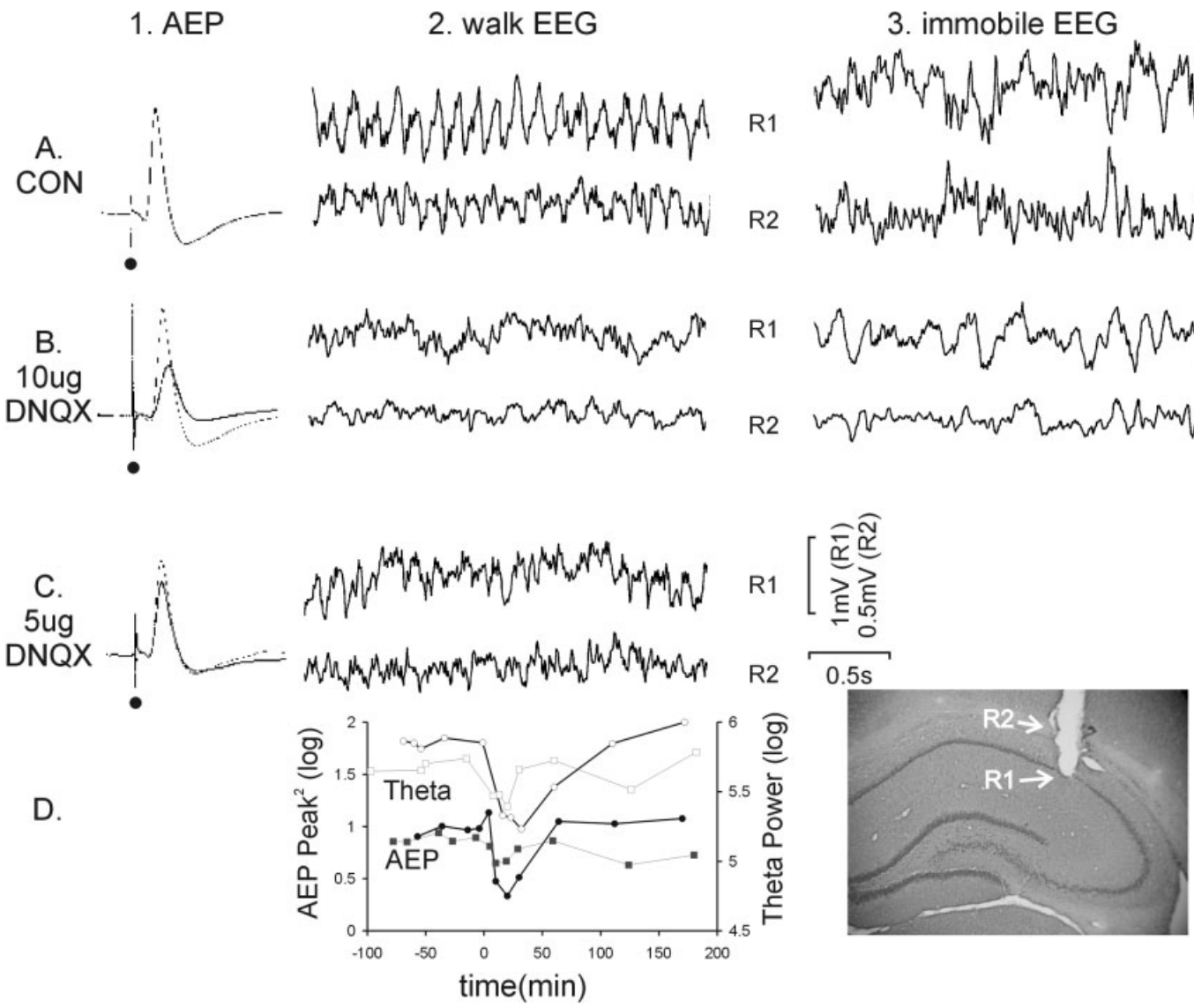

FIGURE 1. Intraventricular (i.c.v.) injection of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist 6,7-dinitroquinoxaline-2,3,dione (DNQX) decreased the power of the hippocampal electroencephalogram (EEG). Column 1, hippocampal commissural average evoked potential (AEP) following stimulation of the contralateral CA1. Column 2, hippocampal EEG during walking at recording electrodes R1 and R2. Column 3, hippocampal EEG during awake immobility. A: AEP and EEG baseline before drug injection. B: After 10 μg DNQX i.c.v. B1: AEP after DNQX (thick trace) overlaid with baseline AEP before injection (light trace). B2: Walking EEG, including theta, was greatly decreased. B3: Immobile EEG showed slow waves and loss of high-frequency components. C: After 5 μg DNQX i.c.v. C1: AEP after DNQX (thick trace) overlaid with baseline AEP (light trace). C2: EEG during walking appears normal (baseline EEG before injection not shown); immobile EEG after 5 μg DNQX was not available. D2: time course of change of AEP peak and hippocampal theta power. The logarithm of the squared AEP peak amplitude (during immobility) and logarithmic theta power (during walking) are plotted versus time, with DNQX injected at time 0. Circles, 10 μg DNQX; squares, 5 μg DNQX; filled symbols, AEP power; open symbols, theta power. D3: Histological section (Nissl stained) shows R1 location at proximal stratum radiatum of CA1, and R2 at corpus callosum immediately dorsal to the CA1 alvear surface. Rat 405.

units, at electrodes dorsal and ventral to the CA1 cell layer, respectively (Table 1). The power decrease at either electrode was significant ($P < 0.05$, Wilcoxon). The dorsoventral coherence at the theta frequency was generally reduced by CNQX on the injected side (Table 1), but it was only marginally nonsignificant ($P = 0.07$; the control group was given DMSO injections). The frequency of theta was not altered significantly by hippocampal CNQX injections. Control injections of DMSO unilaterally into the hippocampus resulted in only small changes in the hippocampal EEG and AEP as compared to the baseline before injection (Table 1).

Effects of AMPA Antagonists on the Residue Hippocampal EEG

The hippocampal EEG spectrum without the theta peaks (including harmonics) has been called the residue spectrum

TABLE 1.

Summary of theta power, frequency, and coherence changes after DNQX/CNQX or vehicle (DMSO) injections intraventricularly (i.c.v.) or locally into the medial septum (SEP) or hippocampus (HPC). For HPC ipsi (ipsilateral) injection, HPC contr (contralateral) side was not injected. In the "Both HPC DNQX" group, injections were made on both right and left sides. "AEP peak" indicates the peak amplitude of the hippocampal commissural average evoked potential (AEP) after injection, expressed as a percent of the baseline before injection. Dorsal and ventral power indicated theta peak power at the electrode dorsal and ventral, respectively, to the CA1 cell layer. Power is in logarithmic unit, and coherence is the Fisher z-transform. Negative values indicate smaller power, phase, coherence or frequency after, as compared to before injection. Number in bracket shows the number of rats. Electrodes not terminating in the ventral location were excluded, together with the dorsoventral phase and coherence measures.

	AEP peak	Dorsal power	Ventral power	Phase	Coherence	Frequency (Hz)
icv 10 μg DNQX	28 ± 6% (8)*	−0.52 ± 0.07 (8)*	−0.59 ± 0.06 (7)*	−15 ± 17° (7)	−0.5 ± 0.15 (7)*	−1.8 ± 0.3 (8)*
icv 5 μg DNQX	56 ± 16% (3)*	−0.26 ± 0.05 (3)	−0.31 ± 0.06 (3)*	−11 ± 12° (3)	−0.12 ± 0.16 (3)	−0.4 ± 0.2 (3)
icv vehicle DMSO	93 ± 4% (7)	−0.02 ± 0.07 (7)	−0.04 ± 0.02 (6)	−3 ± 3° (6)	−0.05 ± 0.09 (7)	−0.1 ± 0.1 (7)
SEP DNQX	88 ± 5% (6)	−0.2 ± 0.1 (6)	−0.09 ± 0.03 (6)	−8 ± 8° (6)	−0.07 ± 0.19 (6)	−0.3 ± 0.1 (6)
SEP DMSO	97 ± 11% (6)	−0.02 ± 0.09 (6)	−0.1 ± 0.04 (6)	−9 ± 6° (6)	−0.12 ± 0.15 (6)	−0.0 ± 0.2 (6)
HPC ipsi CNQX	48 ± 9% (5)*	−0.47 ± 0.12 (6)*	−0.46 ± 0.12 (7)*	−1 ± 17° (6)	−0.25 ± 0.09 (6)	−0.4 ± 0.1 (7)
HPC contr CNQX	NA	−0.08 ± 0.08 (5)	−0.04 ± 0.11 (4)	−7 ± 4° (4)	−0.1 ± 0.1 (4)	0.2 ± 0.2 (5)
HPC ipsi DMSO	100 ± 6% (5)	−0.16 ± 0.08 (6)	−0.08 ± 0.05 (6)	0 ± 2° (4)	−0.03 ± 0.06 (4)	0 ± (6)
Both HPC DNQX[1]	6 ± 4% (4)*	−0.53 ± 0.11 (7)*	−0.85 ± 0.16 (8)*	26 ± 16° (7)	−0.28 ± 0.2 (7)	−0.3 ± 0.2 (4)

*indicates significant difference with baseline and the vehicle group (Wilcoxon, $P < 0.05$).
[1]hippocampal EEG data on both sides were included.

(Leung, 1985). The residue spectrum was strongly suppressed by AMPA receptor antagonists administered either i.c.v. or locally into the hippocampus. Irrespective of the rat's behavior and the EEG frequency (0–100 Hz), the power of the residue spectrum was depressed after injection of DNQX/CNQX (5 μg) into the hippocampus and 10 μg DNQX i.c.v, but it was not changed after vehicle injection. During immobility, EEG power peaked at 1–2 Hz (delta frequency) and was typically devoid of theta peaks (Fig. 3A,B). After DNQX, the immobility-associated delta peak and EEG power at all frequencies were depressed as compared to after vehicle injection ($P < 0.01$, Wilcoxon; Fig. 3A,B; Table 2). The decrease of EEG power was typically of the order of 0.6–1 log units, corresponding to a two- to threefold decrease of EEG amplitude. During walking, the EEG power outside the theta range was also significantly depressed by DNQX or CNQX (Figs. 2C and 3B).

The dorsoventral coherence at the gamma frequency (hollow arrow in Fig. 2C4) was often observed to increase after local or i.c.v DNQX/CNQX. The phase changed from ~200° to near zero (hollow arrow in Fig. 2C3) after 10 μg i.c.v. DNQX. Coherence and phase changes of the same direction, but low magnitudes were found after 5 μg i.c.v. DNQX (Fig. 2B3,B4).

Despite the reduction of residue EEG power, the gamma activity was modulated by behavior before or after CNQX/DNQX. In the normal rat, an increase of gamma power was found during walking as compared to immobility (Fig. 3B). After DNQX, gamma power was still larger during walking as compared to immobility.

Hippocampal and Septal Injection of NMDA Receptor Antagonist D-APV

In a previous study, i.c.v. injection of D,L-APV (5–20 μg) was shown to decrease the power but not the frequency of the hippocampal theta rhythm (Leung and Desborough,1988). In this study, we investigated whether the hippocampus or the medial septum mediated this effect. In seven rats, D-APV (2.5 μg or 13 nmoles in 0.25 μl) injected directly into the hippocampus decreased the theta power at both the dorsal and ventral electrodes, although only the decrease of the ventral theta power was statistically significant as compared to a saline injection group (Fig. 5; Table 3). D-APV did not significantly affect the theta frequency or phase, or the peak of the commissural AEP (Table 3). Local injection of an equal volume of the saline had no significant effect on theta power or other measures (Table 3). Local injection of D-APV (2.5 μg) into the hippocampus also slightly reduced the power of the residue EEG except that at the delta (0–4 Hz) frequency (Table 2). The decrease of residue EEG power after local D-APV, as compared to after local DNQX/CNQX, showed less consistency between dorsal and ventral recording sites and less robust decrease in different frequency ranges (Table 2). The attenuated gamma EEG after hippocampal injection of D-APV still showed a difference in power between walking and immobility (data not shown), similar to the result after DNQX injection.

Medial septal injection of D,L-APV (2.24 μg or 11.7 nmoles in 0.4 μl) in 5 rats also significantly suppressed the hippocampal theta rhythm, at both the dorsal and ventral electrodes (Fig. 6; Table 2). The power decreased starting several minutes after APV injection, was maximal at 10–30 min and dissipated gradually after 30 min

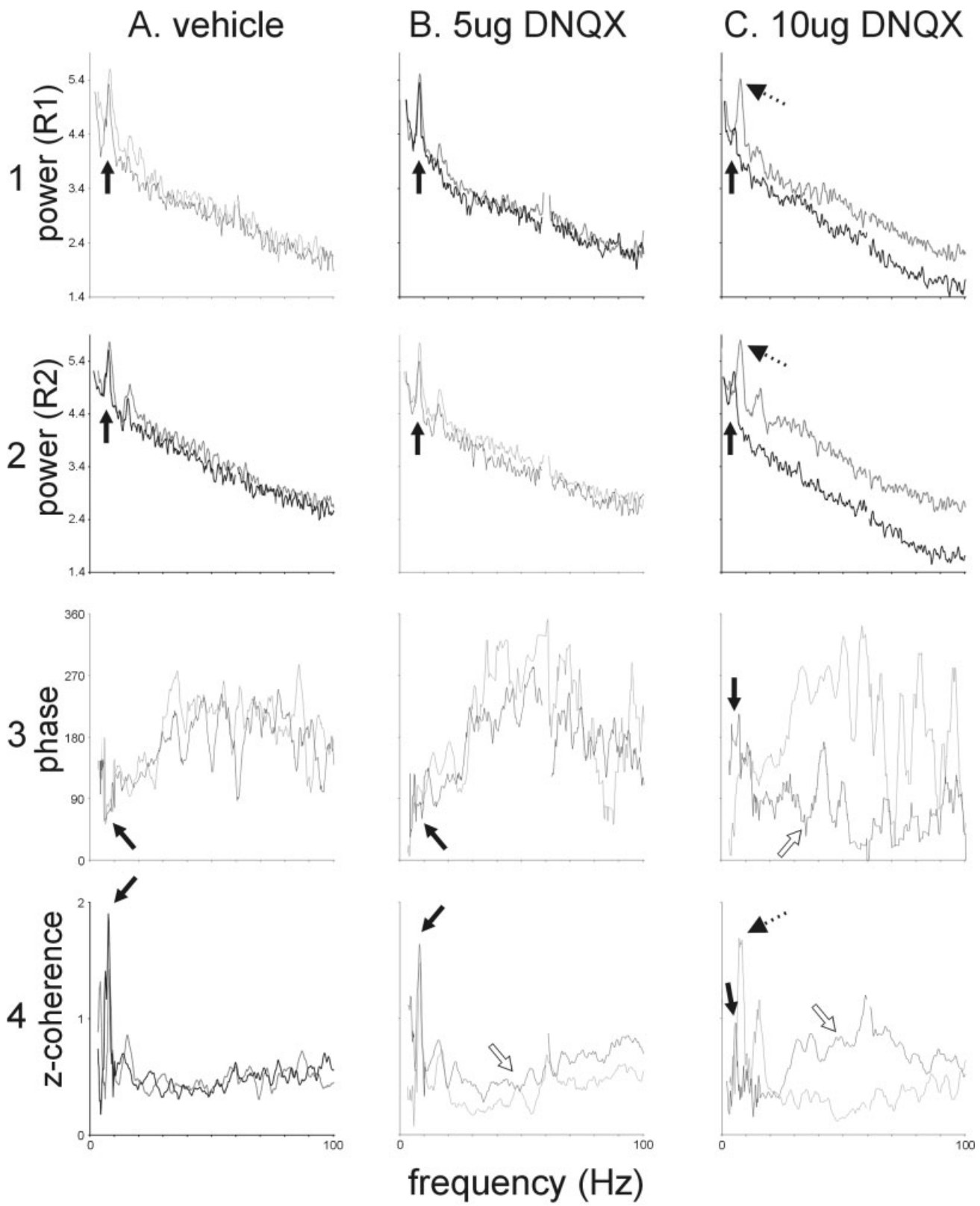
A. vehicle
B. 5ug DNQX
C. 10ug DNQX
1 power (R1)
2 power (R2)
3 phase
4 z-coherence
5.4
4.4
3.4
2.4
1.4
360
270
180
90
0
2
1
0
100
frequency (Hz)

FIGURE 2

(Fig. 6C). The effect of septal APV injection was predominantly on the theta amplitude (Fig. 6A,B) although the theta frequency may decrease by <0.5 Hz to ~6.8 Hz in some rats. As a group, theta frequency was not consistently affected by septal APV injections, but the theta power was consistently decreased (Table 3). D-APV (2.24 μg in 0.4 μl) injected into the medial septum (2 rats) gave a similar result.

DISCUSSION

The present study provides evidence that both AMPA and NMDA receptors participate in generating the currents underlying the hippocampal EEG in vivo. Intrahippocampal or i.c.v. injection of an AMPA or NMDA receptor antagonist decreased EEG power. The EEG power at all frequencies was suppressed by an AMPA receptor antagonist, and hippocampal EEG at 10–100 Hz was slightly suppressed by an NMDA receptor antagonist. The hippocampus opposite the site of unilateral hippocampal injection of an AMPA/NMDA receptor antagonist did not show a significant change in EEG power or frequency (Table 1; Fig. 4). Control injections of vehicle (saline or DMSO), locally or i.c.v., did not significantly change theta or other EEG activities.

With respect to the two hypotheses proposed in the introduction, we have found little evidence that the frequency of hippocampal theta was mediated by ionotropic glutamate receptors in the medial septum. However, postsynaptic NMDA receptors in the medial septum appear to participate in the control of the amplitude of the hippocampal theta rhythm. In addition, we found conclusive evidence that both non-NMDA and NMDA receptors in the hippocampus participate in the generation of the currents underlying the theta rhythm. These are further discussed below.

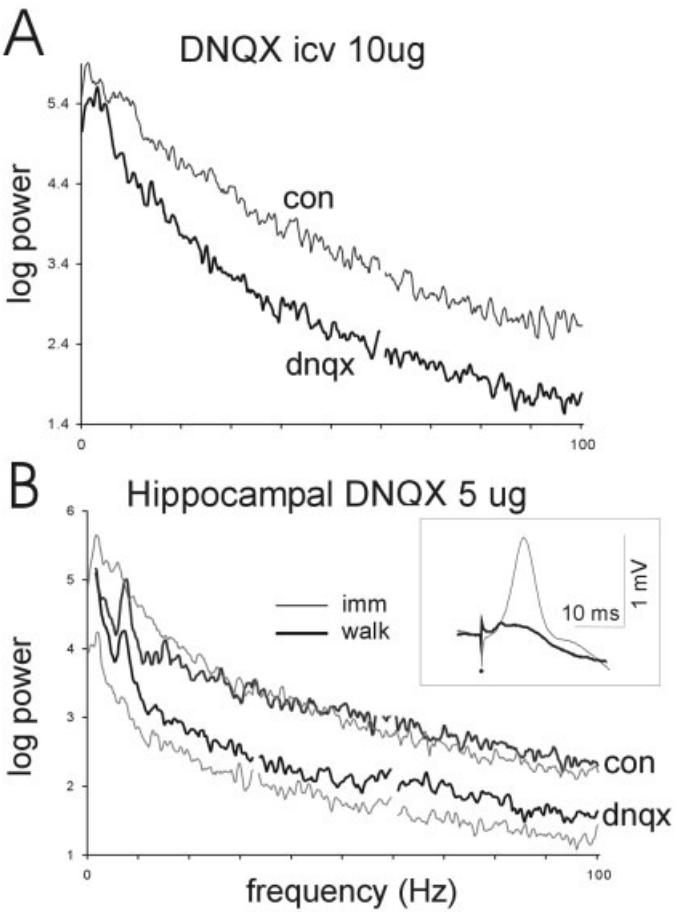

FIGURE 3. Effect of intraventricular and local 6,7-dinitroquinoxaline-2,3,dione (DNQX) on the electroencephalographic (EEG) power spectra at the stratum radiatum electrode during awake-immobility. A: Autopower EEG spectra during awake-immobility at the deep hippocampal electrodes before (light trace, con) and after (dark trace, DNQX) i.c.v 10 μg DNQX. Sample EEG traces are shown in Fig. 1B. B: Behavioral modulation of hippocampal EEG was preserved before and after bilateral local injection of DNQX (5 μg/side). EEG power spectra at the deep hippocampal electrode for walking EEG (dark trace) and immobile EEG (light trace) during baseline control (con) and after DNQX (DNQX). Inset, overlaid traces of commissural average evoked potential (AEP), stimulus at solid circle, before (light trace) and after (thick trace) DNQX. Rat 914.

FIGURE 2. Power, phase, and coherence spectra for hippocampal electroencephalography (EEG) during walking following intraventricular (i.c.v.) injection of (A) DMSO vehicle, (B) 5 μg dinitroquinoxaline (DNQX), or (C) 10 μg DNQX. Thick traces were spectra after injection, overlaid with spectra before injection (thin traces); 60-Hz artifacts in EEG were digitally removed. Rows 1 and 2, autopower of EEG at electrode R1 (row 1) and R2 (row 2), respectively. Row 3, phase shift (signals at R1 electrode with respect to reference at R2 electrode) and row 4, coherence z-transform. A high coherence indicates high correlation of the EEGs at R1 and R2 at a particular frequency. Frequency resolution was 0.195 Hz, all spectral values were smoothed across 0.95 Hz except phase and coherence of >20 Hz were smoothed across 3.12 Hz to increase reliability. DMSO and 5 μg DNQX slightly reduced the amplitude and frequency of the theta EEG (solid arrow); 10 μg DNQX resulted in a significant decrease of theta power and frequency after (solid arrow) as compared to baseline (dotted arrow). After 5 or 10 μg DNQX, open arrow indicates change in coherence and phase after DNQX. Experiments were performed on one rat (405) over the course of 4 weeks, with the chronological order 10μg DNQX, vehicle DMSO, and 5 μg DNQX. The similarity of EEG power in baseline (light) traces indicates the stability of the EEG recordings and the recovery from DNQX/vehicle injections.

Glutamatergic Involvement in Temporal (Frequency) Generation of Theta Waves

The amplitude, but not the frequency, of the hippocampal theta rhythm was suppressed significantly by medial septal injection of APV. In contrast, DNQX had no clear effects on the theta rhythm when injected directly into the medial septum. The suppression of amplitude, but not frequency, of the hippocampal theta by septal APV injection is similar to medial septal injection of muscimol, an γ-aminobutyric acid (GABA)-A receptor agonist (Bland et al., 1996). The result is also consistent with the suggestion that the frequency of the theta rhythm is already coded downstream to the medial septum, presumably in the SUM (Kirk et al., 1996; Kirk, 1998). By contrast, medial septal injection of procaine, a voltage-dependent Na^+ channel blocker (local anesthetic), reduced both

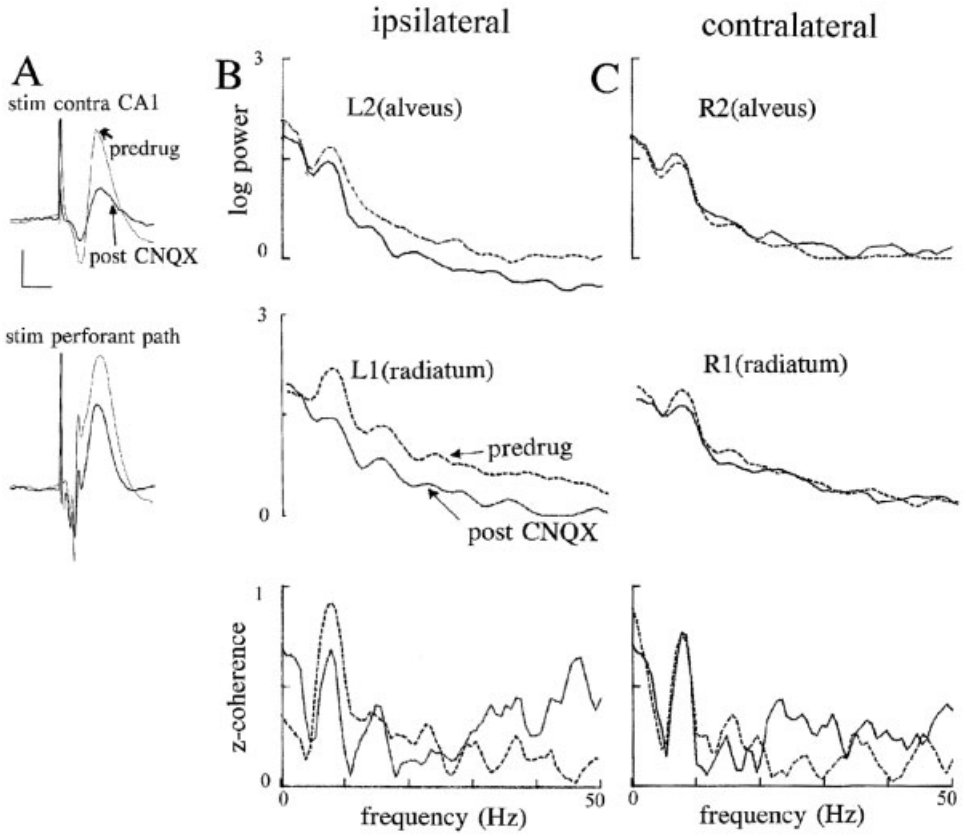

FIGURE 4. Local injection of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist CNQX into the hippocampus depressed the local electroencephalographic (EEG) power. CNQX (5 μg in 0.3 μl) was injected locally into the left hippocampus, ~0.5 mm from the CA1 recording electrode L1, which was ~1 mm ventral to L2. R1 and R2 electrodes were placed in the right hippocampus. A: At 10–15 min after CNQX, average evoked potentials recorded at L1, following stimulation (stim) of the contralateral (contra) CA1 and medial perforant path, were attenuated. Calibration, 0.5 mV and 5 ms. B,C: Spectra before and after CNQX on the left (B) and right (C) side, showing decrease in hippocampal EEG power, including theta, on the left but not the right hippocampus. B: From top down, autopower (logarithmic) spectrum at L2, autopower at L1 and L2–L1 coherence. C: From top down, autopower R2, autopower R1 and R2–R1 coherence (z-transform). EEGs were digitized simultaneously (8-bit) at 4 electrodes. Rat 610.

the frequency and amplitude of the hippocampal theta rhythm (Oddie et al., 1996).

We suggest that postsynaptic NMDA receptors on medial septal neurons were blocked by APV (cf. Armstrong and MacVicar, 2001), perhaps reducing excitation and neuronal bursting in the medial septum and the theta-frequency driving of the hippocampus by the medial septum. Injection of NMDA receptor antagonist into the medial septum was found to affect acetylcholine (ACh) turnover in the hippocampus (Giovannini et al., 1997) and disrupt behavioral functions (Izquierdo et al., 1992). APV was reported to decrease the physostigmine-induced theta in urethane-anesthetized rats (Puma et al., 1996) or decrease both hippocampal theta and movements following threshold posterior hypothalamic stimulation in behaving rats (DeClerck et al., 2002).

It is unclear why DNQX injection into the medial septum did not significantly affect hippocampal theta, since medial septal neurons are known to express AMPA receptors (Page and Everitt, 1995; Armstrong and MacVicar, 2001). It is possible that not all areas of the medial septum/DB were affected by a single injection of DNQX (39.7 nmoles in 0.4 μl). However, a lower dose and volume of DNQX injected into the hippocampus gave robust results. An AMPA receptor antagonist (12 nmoles of NBQX) injected into the medial septum was reported to decrease the physostigmine-induced theta in urethane-anesthetized rats (Puma and Bizot, 1999), but theta generation is likely different in anesthetized and behaving rats (Vanderwolf, 1988; Leung, 1998).

The results in this study emphasize the participation of NMDA receptors in the medial septum in the generation of hippocampal theta rhythm, in which cholinergic and GABAergic synapses in the medial septum have been the focus (Brazhnik and Fox, 1997; Alreja et al., 2000). Glutamatergic synapses from the hippocampus to the lateral septum were reported not to be important for hippocampal theta (Rawlins et al., 1979; Stewart and Vanderwolf, 1987). Injection of ibotenic acid, an NMDA receptor agonist, into the medial septum lesioned the dorsolateral septum and decreased hippocampal theta (Stewart and Vanderwolf, 1987; Leung et al., 1994). Whether septal NMDA receptor blockade and ibotenic lesion affect the same neurons is unknown.

A significant decrease in theta frequency, from ~7.5 to ~6 Hz during movements, was found after i.c.v. injection of 10 μg but not 5 μg of DNQX. A high dose of DNQX (i.c.v.) also reduced voluntary movements and affected postural balance of the rat,

TABLE 2.

Power of the hippocampal EEG (other than the theta rhythm) decreased, in comparison with injection of vehicle DMSO (0.4 μL), after local injection of AMPA receptor antagonist CNQX (5 μg in 0.4 μL unilaterally), DNQX (5 μg in 0.3 μL bilaterally), or NMDA receptor antagonist D-APV (2.5 μg in 0.25 μL unilaterally). EEG power was averaged over a particular frequency range for the baseline and after drug conditions (during walking or awake- immobility). Negative values indicate smaller power after as compared to before injection.

	unilateral DMSO (5)		unilateral CNQX (5)		bilateral DNQX (6 sides)		unilateral D-APV (6)	
Frequency	dorsal	ventral	dorsal	ventral	dorsal	ventral	dorsal	ventral
Walking								
10–30 Hz	0.01 ± 0.05	−0.05 ± 0.03	−0.48 ± 0.08*	−0.59 ± 0.07*	−0.74 ± 0.15*	−0.79 ± 0.18*	−0.32 ± 0.07*	−0.41 ± 0.14
30–70 Hz	−0.11 ± 0.01	−0.07 ± 0.02	−0.42 ± 0.05*	−0.46 ± 0.08*	−0.66 ± 0.19*	−0.68 ± 0.28*	−0.20 ± 0.07	−0.25 ± 0.1
70–100 Hz	−0.04 ± 0.03	−0.08 ± 0.03	−0.25 ± 0.09*	−0.37 ± 0.08*	−0.58 ± 0.2*	−0.52 ± 0.2*	−0.26 ± 0.11	−0.36 ± 0.12*
Immobile								
0.5–4 Hz	−0.04 ± 0.08	−0.11 ± 0.09	−0.48 ± 0.09*	−0.65 ± 0.07*	−0.53 ± 0.25*	−0.85 ± 0.18*	0.04 ± 0.06	−0.17 ± 0.08
5–9 Hz	0.08 ± 0.04	−0.02 ± 0.07	−0.59 ± 0.08*	−0.63 ± 0.09*	−0.64 ± 0.28*	−0.89 ± 0.2*	−0.06 ± 0.09	−0.30 ± 0.15
10–30 Hz	−0.03 ± 0.02	0.04 ± 0.06	−0.6 ± 0.1*	−0.81 ± 0.1*	−0.78 ± 0.12*	−1.18 ± 0.18*	−0.23 ± 0.08*	−0.25 ± 0.13
30–70 Hz	−0.06 ± 0.05	0.02 ± 0.04	−0.52 ± 0.1*	−0.64 ± 0.11*	−0.72 ± 0.08*	−0.98 ± 0.14*	−0.17 ± 0.05*	−0.18 ± 0.08*
70–100 Hz	0.02 ± 0.07	0.0 ± 0.03	−0.29 ± 0.09*	−0.46 ± 0.07*	−0.51 ± 0.11*	−0.84 ± 0.07*	−0.08 ± 0.06	−0.14 ± 0.08

*indicates significant depression of power ($P < 0.05$, Wilcoxon) from the baseline and from vehicle (DMSO) injection.

suggesting a relation between movement and theta frequency (Vanderwolf, 1969; Whishaw and Vanderwolf, 1973; Oddie et al., 1996). A theta rhythm of ~6 Hz was not found during movement in normal rats, but it was commonly found after some general anesthetics, such as urethane, ether and halothane (Stumpf, 1965; Leung, 1985; Vanderwolf, 1988; Ma et al., 2002). We suggest that a high dose of DNQX (i.c.v.) may act outside of the septohippocampal system, since local injections of DNQX into the medial septum or the hippocampus did not significantly affect theta frequency. Many synapses in the brainstem activation system are glutamatergic (Jones, 2003), including afferents to the SUM (Kiss et al., 2002).

Glutamatergic Involvement in the Spatial Generation of Theta EEG in the Hippocampus

Blockade of AMPA receptors after i.c.v. or intrahippocampal injection of DNQX/CNQX was confirmed by the suppression of commissural and perforant-path evoked potentials. DNQX and CNQX may also block kainate receptors, predominant on CA3 neurons and presynaptic terminals of interneurons. In addition to cholinergic and GABAergic neurons, putative glutamatergic neurons were found in the basal forebrain (Jones, 2003; Manns et al., 2003). The participation of the glutamatergic septohippocampal pathway in hippocampal theta generation is unclear.

We propose that glutamatergic synapses are involved in multiple mechanisms in different pathways that generate the hippocampal EEG (Fig. 7). We have proposed that the theta field potentials are driven by two theta-frequency inputs on CA1 pyramidal cells—a proximal inhibitory input and a distal excitatory input (Buzsaki et al., 1983; Leung, 1984c) (Fig. 7). The proximal inhibitory input mediates a rhythmic series of GABA-A receptor-mediated inhibitory postsynaptic potentials (IPSPs) impinging near the soma of pyramidal cells (Leung and Yim, 1986; Fox, 1989; Soltesz and Deschenes, 1993; Ylinen et al., 1995a). These IPSPs may be derived from pacemaker driving of the CA1 interneurons by GABAergic and cholinergic inputs from the medial septum (Freund and Buzsaki, 1996). It was suggested that theta in CA1 was partly driven by distal-dendritic excitation of the pyramidal cells by the perforant pathway from the entorhinal cortex (Vanderwolf and Leung, 1983; Leung, 1984c) (Fig. 7), a suggestion that was supported by other studies (Heynan and Bilkey, 1994; Bragin et al., 1995). A phase delay between proximal and distal driving (Leung, 1984c) accounted for a gradual phase shift of the theta rhythm in the apical dendritic layers (stratum radiatum) of CA1 in behaving rats (Winson, 1974). CA1 may also be driven by an independent pacemaker in CA3, perhaps to a smaller degree (Fig. 7). In hippocampal slices or slice-cultures in vitro, theta-frequency oscillations in CA3 were activated by cholinergic agonist like carbachol (Konopacki et al., 1987; MacVicar and Tse, 1989; Williams and Kauer, 1997; Fellous and Sejnowski, 2000; Fischer et al., 2002). In animals in vivo, ACh release (Dudar et al., 1979) or septohippocampal driving may induce theta-frequency oscillations in CA3 (Heynen and Bilkey, 1991) that, in turn, drive CA1. CA3 to CA1 driving may be particularly strong after entorhinal lesion (Kocsis et al., 1999).

Ionotropic glutamatergic antagonist may block several of the above pathways that generate theta field potentials in CA1 (Fig. 7). First, distal dendritic excitation of CA1 by the entorhinal cortex was shown to be mediated by NMDA and AMPA receptors in vitro (Colbert and Levy, 1992) and in vivo (P. Peloquin and L.S. Leung, unpublished data). Either lesion of the entorhinal cortex (Bragin et al., 1995) or inactivation of the perforant path (Heynan and Bilkey, 1994) strongly attenuated theta field potentials, and blockade of glutamatergic receptors in the distal dendritic layers of CA1 may do the same. Second, in the carbachol-induced theta rhythm in vitro, intrinsic generation of theta by CA3 and propa-

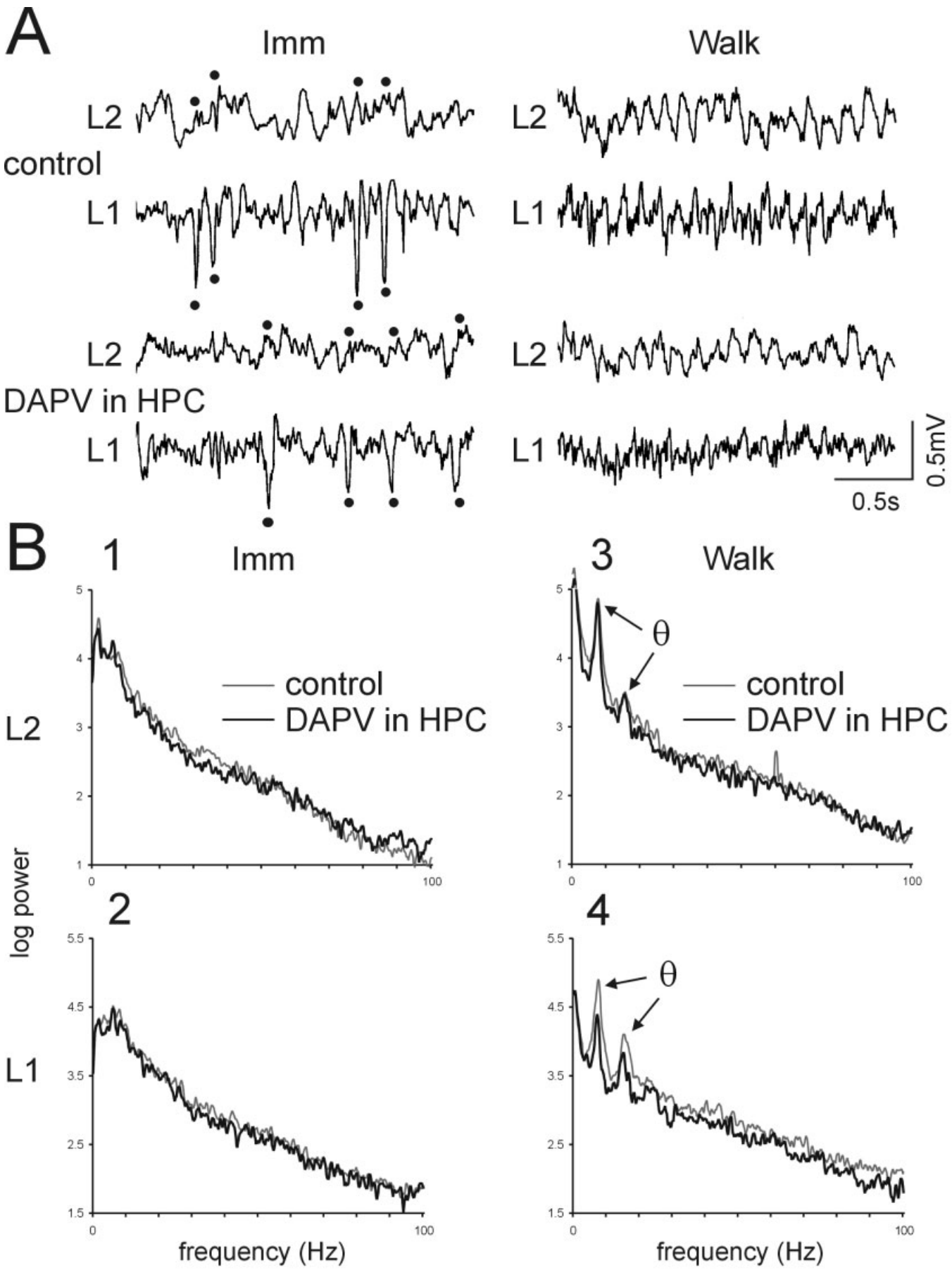
A
Imm
Walk
L2
L2
control
L1
L1
L2
L2
DAPV in HPC
L1
L1
0.5mV
0.5s
B
1
Imm
3
Walk
control
DAPV in HPC
θ
θ
L2
log power
2
4
L1
frequency (Hz)
frequency (Hz)

FIGURE 5

TABLE 3.

Summary of theta power, frequency and coherence changes after hippocampal (HPC) or septal (SEP) injections of NMDA receptor antagonist APV or saline. D-APV (2.5 μg in 0.25 μL) was used in the hippocampus, and D,L-APV (2.24 μg in 0.4 μL) in the medial septum; saline injected in each area was the same volume as the APV injected. AEP peak values are the peak of the hippocampal average evoked potential (AEP) after injection, expressed as a percent of the baseline before injection. Dorsal and ventral power indicated theta peak power at the electrode dorsal and ventral, respectively, to the CA1 cell layer. Power units are in logarithmic units, and coherence was the Fisher z-transform of the coherence. Negative values indicate smaller power, phase, coherence or frequency after, as compared to before injection. Number in bracket shows the number of rats.

	AEP peak	Dorsal power	Ventral power	Phase	Coherence	Frequency (Hz)
HPC APV	1.03 ± 0.06 (6)	−0.42 ± 0.1 (7)	−0.49 ± 0.13 (7)*	−18 ± 15° (7)	−0.24 ± 0.04 (7)*	−0.36 ± 0.15 (7)
HPC saline	0.86 ± 0.11 (6)	−0.19 ± 0.06 (7)	−0.10 ± 0.04 (7)	−3 ± 4° (7)	0.02 ± 0.07 (7)	−0.17 ± 0.17 (7)
SEP APV[1]	0.89 ± 0.09 (3)	−0.56 ± 0.09 (5)*	−0.64 ± 0.13 (7)*	−3 ± 9° (5)	−0.25 ± 0.16 (5)	0 ± 0.27 (5)
SEP saline[1]	0.94 ± 0.08 (3)	−0.08 ± 0.06 (6)	−0.09 ± 0.03 (7)	7 ± 7° (6)	0.07 ± 0.14 (6)	0.29 ± 0.31 (4)

*indicates significant difference with baseline and the saline group (Wilcoxon, $P < 0.05$).
[1]hippocampal EEG data on both sides were included, if dorsal theta peak showed > 0.2 log unit rise from the background.

gation of CA3 rhythmic outputs to CA1 were blocked by CNQX and APV, respectively (Williams and Kauer, 1997; Fellous and Sejnowski, 2000). In another in vitro theta model, D-APV blocked the theta activated by metabotropic glutamate receptor agonist in CA1 (Gillies et al., 2002). Glutamatergic blockade also removes the excitation of CA1 interneurons by CA1 and CA3 pyramidal cells (Grunze et al., 1996), although it is unclear how this may alter interneuronal oscillations at the theta frequency. While interneurons were suggested to synchronize membrane potential oscillations (MPOs) in CA1 pyramidal cells (Cobb et al., 1995), the MPOs induced by depolarizing CA1 pyramidal cells were not sensitive to CNQX or APV (Leung and Yim, 1991). In summary, both distal and proximal dendritic excitation of CA1, derived from entorhinal cortex and CA3, respectively, may carry theta-frequency signals and NMDA or AMPA receptor blockade may block either distal or proximal driving.

We did not find a consistent change in theta phase at stratum radiatum after NMDA or AMPA receptor blockade, although the change may be significant in a single rat. This suggests a variable attenuation of either the distal or the proximal theta-generating inputs (Leung and Desborough, 1988), thus giving rise to variable changes in theta phase (Leung, 1984a,b,c).

FIGURE 5. Local injection of N-methyl-D-aspartate (NMDA) receptor antagonist D-amino-5-phosphonovaleric acid (APV) into the hippocampus (HPC) depressed the power of the local theta rhythm. D-APV (2.5 μg in 0.25 μl) was injected locally into the hippocampus, ~1 mm from the L1 recording electrode at stratum radiatum CA1; L2 was at stratum oriens of CA1. A: Electroencephalographic (EEG) traces at L2 and L1, during awake-immobility (Imm) and walking, in the baseline control condition before drug and 12–15 min after local injection of D-APV. Sharp waves during Imm, pronounced as negative wave (solid circles) at L1 and reversed at L2, were found during Imm after D-APV. The theta rhythm at L1, but not that at L2, was obviously smaller after D-APV. B: Power spectra at L2 and L1, overlaid control baseline and after D-APV conditions. 1 and 2, during Imm at L2 and L1, respectively; there was a slight decline of 10–30 Hz power after D-APV, which was significant among the group of rats. 3 and 4, spectra during walking at L2 and L1, respectively; θ arrows pointing at first and second harmonic of theta, which was diminished at L1 but not L2 electrode. Rat 932.

Participation of the EPSPs in Theta Generation

Rhythmic excitatory postsynaptic potentials (EPSPs) have been proposed to contribute to the intracellular theta-frequency oscillations of anesthetized animals (Fujita and Sato, 1964; Nunez et al., 1987), but it is difficult to identify EPSPs at the dendrites definitively and to distinguish them from voltage-dependent MPOs and spikes (Leung and Yim, 1991; Kamondi et al., 1998; Bland et al., 2002). Pharmacological blockade in this study supports the participation of EPSPs in the generation of hippocampal theta of behaving rats. However, the technique was not sufficiently refined to block glutamatergic (excitatory) synapses on CA1 pyramidal cells alone, and CNQX/DNQX may suppress theta amplitudes by blocking other synapses (Fig. 7). Also, a substantial amount of behaviorally dependent theta and gamma activity remained despite near-complete suppression of the AMPA-receptor-mediated evoked potentials (Fig. 4B), suggesting that, in addition to AMPA receptors and synapses, GABAergic and other inputs may mediate hippocampal theta and gamma activities (Fig. 7).

Ionotropic Glutamate Receptors in Generation of Irregular Slow EEG and Gamma Activity

DNQX/CNQX and, less strongly, D-APV attenuated the power of hippocampal EEG other than the theta peaks (Fig. 1; Table 2). A recurrent inhibitory circuit in the hippocampus has been proposed to generate the hippocampal residue EEG, including gamma activity (Leung, 1982, 1985). Other studies suggest that hippocampal gamma activity depends on recurrent excitation and inhibition among CA3 pyramidal cells and GABAergic interneurons (Fisahn et al., 1998; Csicsvari et al., 2003), as illustrated schematically in Figure 7. Any circuit that depends on recurrent excitation or inhibition will be sensitive to AMPA receptor blockade. In addition, recurrent glutamatergic excitation (Traub and Miles, 1991) is responsible for the

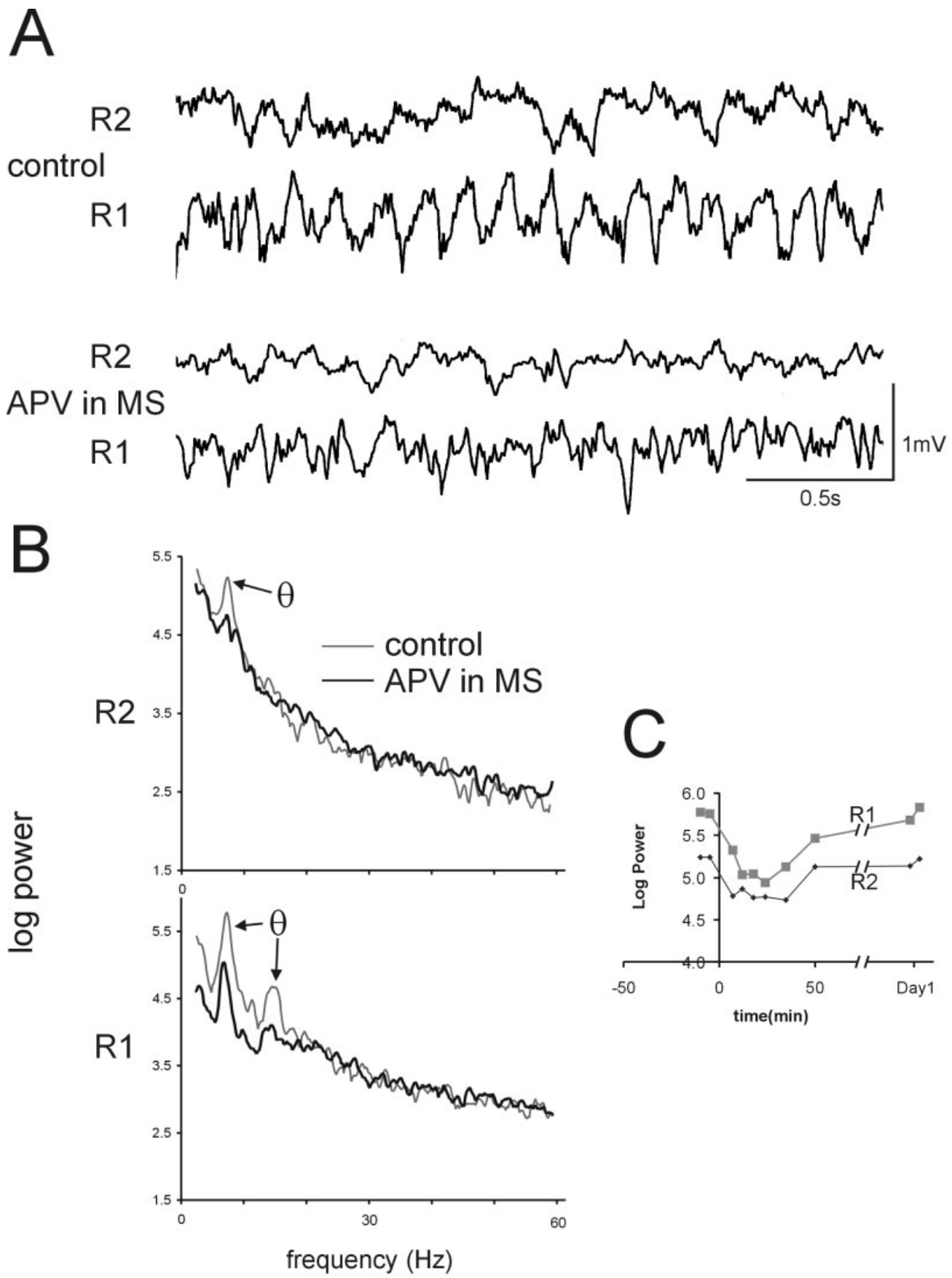
A
R2
control
R1
R2
APV in MS
R1
1mV
0.5s
B
log power
R2
R1
θ
θ
control
APV in MS
5.5
4.5
3.5
2.5
1.5
0
30
60
frequency (Hz)
C
Log Power
6.0
5.5
5.0
4.5
4.0
R1
R2
-50
0
50
Day1
time(min)

FIGURE 6

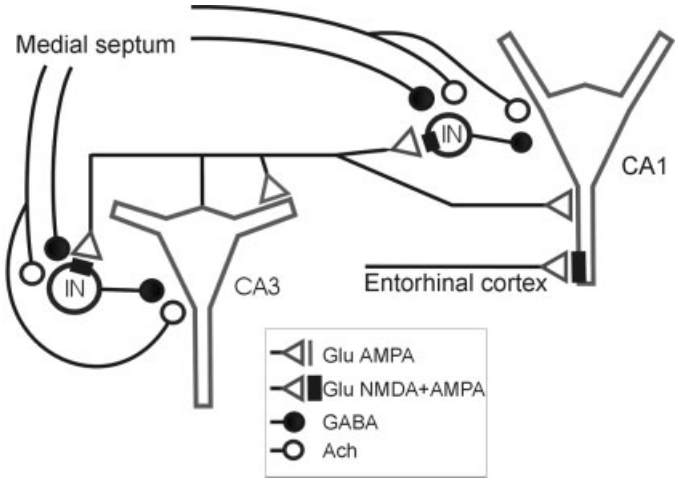

FIGURE 7. Schematic diagram illustrating possible participation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid//N-methyl-D-aspartate (AMPA/NMDA) receptors in generating hippocampal electroencephalography (EEG) in this study. All possible mechanisms for oscillations are not included, in particular, the dentate gyrus and the different types of interneurons. Medial septal afferents, containing γ-aminobutyric acid (GABA) and acetylcholine (ACh), are shown to drive hippocampal interneurons in CA3 and CA1, and ACh may also directly affect pyramidal cells and interneuronal terminals. Theta field potentials in CA1 are assumed to be generated from rhythmic driving by proximal (cell body) inhibition and distal excitation through afferents of the entorhinal cortex (EC). Minor theta generation may occur in CA3 as well. In contrast, the residue EEG (non-theta) in CA1 may be driven by activity in CA3 neurons. CA3 recurrent excitation (shown by recurrent excitation at the basal dendrite) and feedback inhibition of CA3 interneurons are presumably responsible for generation of sharp waves and gamma activity recorded in CA1. Two types of glutamate (Glu) synapses are distinguished: those that activate NMDA (and AMPA) receptors normally and those that activate only AMPA receptors. NMDA receptor blockade reduces the theta driving from the EC, and perhaps from CA3 and the interneurons as well. AMPA receptor blockade reduces the CA3 signals that generate the residue spectra in CA1 (i.e., decreases sharp waves, delta and gamma frequencies) and also suppresses the excitatory postsynaptic currents in CA1. GABAergic currents in CA1, paced by medial septal afferents, remain after combined AMPA and NMDA receptor blockade.

synchronized CA3 network bursting that drives CA1 sharp waves during awake-immobility (Buzsaki et al., 1983; Ylinen et al., 1995b). Thus, CNQX would attenuate sharp waves and the irregular slow activity during immobility (Figs. 3 and 5). Several in vitro models also manifest hippocampal delta and gamma oscillations that are attenuated by AMPA/NMDA receptor blockade. In thick hippocampal slices or isolated hippocampus in vitro, intrinsic delta activity was suppressed by CNQX (Wu et al., 2002). Gamma activity induced by carbachol in horizontal hippocampal slices in vitro was blocked by an AMPA receptor antagonist (Fisahn et al., 1998). However, in vitro gamma activity was also reported in the presence of CNQX and APV, and presumably generated by a GABAergic interneuronal network after metabotropic glutamate receptor activation (Whittington et al., 1995; Traub et al., 1996, 1997; Gillies et al., 2002). The latter narrow-band gamma activity in vitro appears different from the normal wide-band gamma activity in vivo (Leung, 1982, 1998; Ma and Leung, 2002), and the essential participation of pyramidal cells (and ionotropic glutamatergic synapses) in gamma activity in vivo (this study), but not in vitro (Traub et al., 1997, 2000), underscore this difference.

The almost synchronous gamma activity (near zero phase) concomitant with an increase in dorsoventral gamma coherence (Fig. 2C) after DNQX can be explained by the blocking of the local CA1 gamma activity. The CA1 gamma waves are expected to be ~180° phase reversed, while volume-conducted gamma waves, possibly from the dentate gyrus (Csicsvari et al., 2003), are expected to be synchronous (0° phase) at electrodes across the CA1 cell layer. Because the dentate and CA1 activities are generally not coherent (Csicsvari et al., 2003), the superposition of CA1 and dentate signals is expected to give low dorsoventral coherence, while removal of the CA1 (or dentate) signal will yield higher coherence (see appendix in Leung et al., 1982, for explanation).

In a previous study, (RS)-α-methyl-4-carboxyphenylglycine (MCPG), a broad-spectrum metabotropic glutamate receptor antagonist, when injected into the hippocampus, medial septum or i.c.v. did not significantly affect the spontaneous hippocampal EEG during normal walking and immobility in the normal rat. However, MCPG injected into the hippocampus did block the increase in gamma power after a hippocampal afterdischarge (Ma and Leung, 2002).

FIGURE 6. Medial septum injection of N-methyl-D-aspartate (NMDA) receptor antagonist amino-5-phosphonovaleric acid (APV) decreased the power of the hippocampal theta rhythm. D,L-APV (2.24 μg in 0.4 μl) was injected locally into the medial septum. R1, recording electrode at stratum radiatum CA1; R2 was at stratum oriens of CA1. A: Electroencephalographic (EEG) traces at R2 and R1, during walking, in the baseline control condition before drug and 18 min after septal injection of APV. Theta rhythm at both R1 and R2 was attenuated after APV. B: Power spectra at R2 and R1, overlaid control baseline and after APV conditions, showing a decrease of the theta (θ) peaks after APV. C. Time course of change of theta peak power at R2 and R1, showing recovery at 50 min and 1 day after injection. Rat 918.

Hippocampal EEG and Functions

NMDA and non-NMDA receptor blockade may mediate dysfunction of the hippocampus by blocking neuronal networks and oscillations in the hippocampus. While NMDA receptor antagonists have been suggested to block long-term potentiation (in the dentate gyrus) and hippocampus-mediated memory (Morris and Frey, 1997), hippocampal theta rhythm in a behaving rat was more readily attenuated by APV (Leung and Desborough, 1988; this study) than apical dendritic LTP in CA1 (Leung and Shen, 1999). Similarly, some of the behavioral effects of AMPA receptor blockade (Riedel et al., 1999) may be attributed to disruption of networks in the hippocampus, as manifested by disruption of hippocampal sharp waves and gamma waves.

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