MODULATION OF SEPTO-HIPPOCAMPAL θ ACTIVITY BY GABA_A RECEPTORS: AN EXPERIMENTAL AND COMPUTATIONAL APPROACH[1]

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Abstract—θ Frequency oscillation of the septo-hippocampal system has been considered as a prominent activity associated with cognitive function and affective processes. It is well documented that anxiolytic drugs diminish septo-hippocampal oscillatory θ activity contributing to their either therapeutic or unwanted side effects. In the present experiments we applied a combination of computational and physiological techniques to explore the functional role of GABA_A receptors in θ oscillation. In electrophysiological experiments extracellular single unit recordings were performed from medial septum/ diagonal band of Broca with simultaneous hippocampal (CA1) electroencephalogram (EEG) recordings from anesthetized rats. Neurotransmission at GABA_A receptors were modulated by means of pharmacological tools: the actions of the GABA_A receptor positive allosteric modulator diazepam and inverse agonist/negative allosteric modulator FG-7142 were evaluated on septo-hippocampal activity. Systemic administration of diazepam inhibited, whereas FG-7142 enhanced θ oscillation of septal neurons and hippocampal EEG θ activity. In parallel to these experimental observations, a computational model has been constructed by implementing a septal GABA neuron model with a CA1 hippocampal model containing three types of neurons (including oriens and basket interneurons and pyramidal cells; latter modeled by multicompartment techniques; for detailed model description with network parameters see online addendum: http://geza.kzoo.edu/theta). This connectivity made the network capable of simulating the responses of the septo-hippocampal circuitry to the modulation of GABA_A transmission, and the presently described computational model proved suitable to reveal several aspects of pharmacological modulation of GABA_A receptors. In addition, computational findings indicated different roles of distinctively located GABA_A receptors in θ generation. © 2004 IBRO. Published by Elsevier Ltd. All rights reserved.

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Experimental and clinical findings indicate a central role of the hippocampal formation in various physiological mechanisms related to cognitive and affective processes (Buzsáki, 2002; Kahana et al., 2001). Activity of the hippocampal system has been analyzed from sub-cellular to system/circuitry level in order to connect specific neuronal activity to a given behavioral state or function. Oscillatory activity of the hippocampal formation (and in fact the entire limbic system) has been recognized as a key network pattern activity (Bland and Colom, 1993; Vinogradova, 1995; Buzsáki, 2002). The best-characterized oscillatory patterns of the hippocampal formation are the θ and γ oscillations (Vertes and Kocsis, 1997; Traub et al., 1999; Buzsáki, 2002), which are functionally closely related and occur simultaneously in a variety of behavior activities (Bragin et al., 1995; Penttonen et al., 1998). Although most of our information on hippocampal oscillatory activity derived from electrophysiological recordings in anesthetized and non-anesthetized rodents, recent findings also indicate a similar oscillatory activity in the hippocampal formation of the human brain (Bódizs et al., 2001; Kahana et al., 2001; Jensen and Tesche, 2002; Cantero et al., 2003). In general, θ oscillation in the hippocampal system has been linked to mnemonic processes (Lisman and Idiart, 1995; Raghavachari et al., 2001; Jensen and Tesche, 2002; Seager et al., 2002), and power of θ oscillation seems to correlate with the anxiety level (Green and Arduini, 1954; Fontani and Carli, 1997; Yamamoto, 1998; McNaughton and Gray, 2000).

Current theories on generation of θ oscillation in the hippocampal formation attribute a central role of interconnected inhibitory GABA neurons, contributing either as oscillators or resonators to θ activity (Buzsáki, 2002). In part mediating brainstem and subcortical inputs, medial septum/diagonal band (MS/DB) projecting GABA neurons are thought to play a crucial role in regulation of θ activity (Vertes and Kocsis, 1997; Buzsáki, 2002). In addition, recent θ models incorporate several cellular (e.g. active and passive membrane properties) and network characteristics of both GABA and pyramidal neurons in order to establish a realistic functional model. Given the physiological complexity of oscillatory θ generation, computational models can offer an opportunity to analyze the impact of a given membrane property or particularity of a synaptic connectivity on oscillatory activity (White et al., 2000; Den-

ham and Borisyuk, 2000; Orbán et al., 2001; Wang, 2002). Furthermore, the precise role of various neurotransmitters and neuromodulators, or their specific receptors in oscillatory activity can be elucidated by means of highly selective drugs available lately. Conversely, it is now gradually recognized that evaluation of drug actions on brain processes must take into consideration neuronal network responses, which cannot be simply deducted from responses of individual neurons. Given the critical role of the hippocampal formation in cognitive and affective processes and the significance of its θ activity, several recent studies have investigated the actions of various drugs on the hippocampal/limbic system oscillatory activity (McNaughton and Coop, 1991; McNaughton and Gray, 2000; White et al., 2000; Whittington et al., 2000; Baker et al., 2002; Gill et al., 2002). It has been shown that anxiolytic drugs acting on $GABA_A$ receptors inhibit oscillatory activity of the limbic system, which is thought to contribute to their anxiolytic or sedative effects. More recently, combining experimental observations on drug-induced changes in $GABA_A$ receptor–ion channel kinetics and computational modeling of oscillatory network activity, it has been demonstrated that the level of drug-induced receptor desensitization critically influences network activity and its coherent oscillations (White et al., 2000; Baker et al., 2002). In the present study we performed simultaneous recordings of single units from the MS/DB and hippocampal (CA1) field potentials (electroencephalogram [EEG]) from anesthetized rats, and the actions of positive and negative allosteric modulators of $GABA_A$ receptors, diazepam and FG-7142, respectively (Mehta and Ticku, 1989) on septo-hippocampal oscillatory activity were evaluated. In parallel, a computational model of the septo-hippocampal circuit has been constructed in order to model its *in vivo* activity and its oscillatory changes induced by *in vivo* pharmacological manipulations. One of the main aims of our studies was to validate this computational model by demonstrating an analogous network model activity to our pharmacological findings. Provided with a number of adjustable parameters, our computational network model is constructed the way that, once it is validated, it could serve as a relevant network model to address more intricate pharmacological questions as receptor/channel kinetics or specific interaction of various GABA receptors.

EXPERIMENTAL PROCEDURES

Animals

All studies were conducted with male Sprague–Dawley rats (270–300 g; Harlan, Indianapolis, IN, USA). Animals were housed in groups of four, kept under conditions of controlled temperature (21 ± 1 °C) and lighting (lights on 06:00–18:00 h) and were given food pellets and water *ad libitum*. Studies were performed between 8:00 and 17:00 h. All procedures were carried out under an approved animal use protocol and were in compliance with the Animal Welfare Act Regulations (9 CFR Parts 1, 2 and 3) and with the Guide for the Care and Use of Laboratory Animals (ILAR 1996). All efforts were made to minimize the number of animals and their suffering.

Electrophysiological experiments

Surgical procedures and drug solutions. Rats were anesthetized with chloral hydrate anesthesia (400 mg/kg, i.p.; Hajós et al., 2003a,b), and the femoral artery and vein were cannulated for monitoring arterial blood pressure and administration of drugs or additional doses of anesthetic, respectively. The anesthetized rat was placed in a stereotaxic frame, and craniotomy was performed above the regions of the medial septum and unilateral CA1 hippocampus. Body temperature of the rat was maintained at 36° to 37 °C by means of an isothermal (37 °C) heating pad (Braintree Scientific, Braintree, MA, USA). Solutions of diazepam, a positive allosteric modulator of the $GABA_A$ receptors and FG-7142, a negative allosteric modulator of $GABA_A$ receptors were made up based upon their salt weights in H_2O and concentrations adjusted so that injection volumes equaled 1 ml/kg body weight. After conclusion of experiments animals were killed with an i.v. bolus of chloral hydrate. Brains were removed, blocked and frozen on a cryostat stage for histological verification of electrode placements.

Single unit recordings. Single units were recorded from the medial septum and vertical limb of the diagonal band of Broca (co-ordinates: 0.2–0.6 mm anterior to bregma, lateral 0 mm and 5–7 mm below the dura; Paxinos and Watson, 1986) using glass microelectrodes filled with 2 M NaCl and saturated with Pontamine Sky Blue (impedance 4–10 MOhms). Extracellularly recorded potentials were amplified, filtered, displayed, discriminated and recorded for off-line analysis using conventional electrophysiological methods (Hajós et al., 2003a,b). Neuronal activity was followed by constructing firing rate, frequency and interspike interval histograms using Spike3 program (Cambridge Electronic Design, Cambridge, UK). Oscillation of neuronal activity was analyzed by auto-correlation; power of oscillation was calculated by fast Fourier transformation analysis of autocorrelation (Hajós et al., 2003b). Location of the recording electrode was marked with iontophoretic ejection of Pontamine Sky Blue and revealed by routine histological procedure. Only neurons located within the MS/DB are included in the study.

EEG recording. Unilateral hippocampal field potential (EEG) was recorded by a metal monopolar macroelectrode (Rhodes Medical Instruments Co) placed into the CA1 region (co-ordinates: 3.0 mm posterior from the bregma, 2.0 mm lateral and 3.8 mm ventral; Paxinos and Watson, 1986). Field potentials were amplified, filtered (0.1–100 Hz), displayed and recorded for on-line and off-line analysis (Spike3 program; Cambridge Electronic Design). Rhythmic synchronized (θ) and large amplitude irregular hippocampal activities were distinguished in the EEG; quantitative EEG analysis was performed by means of fast Fourier transformation (Hajós et al., 2003a,b). Power spectrum density of EEG for θ activity was calculated at peak frequency between 3 and 6 Hz. Location of the recording electrode was verified histologically.

Data analysis and statistics

Mean firing rates were determined in periods of 300 s before and after drug treatment. Interspike interval histograms, autocorrelograms and hippocampal EEG power spectra were determined in periods of 300 s, for an identical duration preceding and following drug treatment. Differences between baseline and drug treatment were assessed by paired Student's *t*-test.

Septo-hippocampal model

Hypotheses deduced from *in vivo* experiments were tested in network models of septum and the hippocampus. Computer simulations were conducted with biophysically realistic models of networks of neurons described by the Hodgkin-Huxley formalism. Detailed description of model cells and additional parameters of

the network can be found in the online supplementary materials (see: http://geza.kzoo.edu/theta and http://geza.kzoo.edu/theta/theta.html for links to script files).

We used three different types of cells in the hippocampal CA1 region and one neuron population was explicitly taken into account in the medial septum, while cholinergic innervation was accounted by a constant inducing current only. In the hippocampus, only those cell types were included, which were previously demonstrated to participate in θ activity during *in vivo* conditions (Csicsvári et al., 1999; Ylinen et al., 1995; Klausberger et al., 2003). We used a multicompartmental realization of pyramidal neurons (consisting of 256 compartments) modified from the previous model of Warman et al. (1994) by including hyperpolarization activated by currents (Magee 1998). The model showed firing properties characteristic of pyramidal neurons of the CA1 region, including action potential firing, burst firing, multiple forms of depolarizing afterpotentials, rebound spikes and calcium spikes. Based on location, two types of interneurons were taken into account, representing stratum oriens interneurons and basket neurons. Stratum oriens interneurons are known to project to the lacunosum moleculare (O-LM interneurons), innervating the most distal dendrites of pyramidal neurons (Lacaille and Williams, 1990). Stratum oriens neurons showed adaptation and low-frequency autonomous firing in the θ band, and contained hyperpolarization-activated current (Maccaferri and McBain, 1996; Lupica et al., 2001). Basket neurons were non-adaptive fast spiking cells, previously described by Wang and Buzsáki (1996), and Orbán et al. (2001). Single compartmental model was applied for both stratum oriens (Wang, 2002) and basket neurons.

Hippocampal circuitry

In the CA1 region of the hippocampus the two main neural populations, pyramidal neurons and interneurons, establish intricate synaptic connections (Freund and Buzsáki, 1996). However, functional significance of several of these connections during different behavioral states remains to be established. We confined our study to those afferents and efferents, which seemed to be necessary for the generation of θ oscillation (Fig. 1). Recurrent collaterals of pyramidal neurons are virtually absent in the CA1 region (Knowles et al., 1982). The main excitatory afferents to oriens interneurons are projections from pyramidal neurons, involving AMPA receptors (Lacaille et al., 1987; Blasco-Ibanez and Freund, 1995). Interneurons with cell bodies in the stratum oriens either project to the septum (Tóth and Freund, 1992; Jinno and Kosaka, 2002) or innervate distal apical dendritic regions of the pyramidal neurons (Lacaille and Williams, 1990). Although the physiological homogeneity of these two interneuron populations has not been demonstrated, we used the same single-compartment model (detailed description: http://geza.kzoo.edu/theta). Since, oriens interneurons establish synaptic contacts with parvalbumin containing putative basket neurons (Katona et al., 1999) a small number of oriens interneurons innervating basket cells were also incorporated in the model circuitry. Oriens interneurons established synaptic contacts with postsynaptic neurons through GABA$_A$ receptor mediated synapses. Basket interneurons show sprouting of their axons in the stratum pyramidale and radiatum (Freund and Buzsáki, 1996), therefore, in our model, basket neurons established GABAergic connections both with pyramidal neurons and other basket neurons. Under conditions where the perforant path mediated excitation is attenuated, hippocampal θ activity is observable (Bragin et al., 1995; Ylinen et al., 1995). Furthermore, in specific conditions θ activity *in vitro* can be induced in a CA1 slice, which oscillation reflects action potential timings characteristic of *in vivo* experiments (Gillies et al., 2002). Therefore, at this level of study perforant path and Schaffer-collateral input were also neglected.
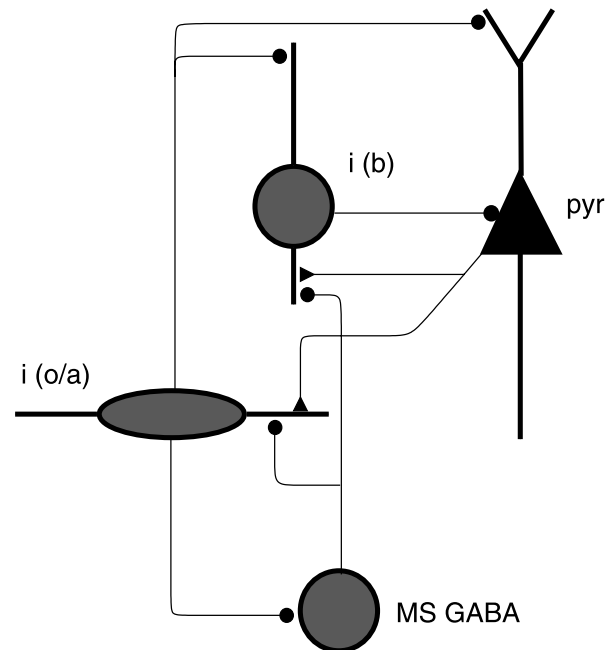

Fig. 1. Structure of the medial septum-hippocampal CA1 model. Three hippocampal neuron populations include pyramidal neurons (pyr), basket interenurons (i(b)), horizontal oriens interneurons (i(o/a)). In the septum only hippocampally projecting GABAergic neurons (MS-GABA) were explicitly taken into account. Hippocampo-septally projecting neurons and those interneurons projecting to the lacunosum moleculare were non-overlapping subpopulations of neurons with same electrophysiological characteristics.

The model of the medial septum consisted of a single GABAergic neuron population. Periodic θ-frequency oscillations have been shown to exist in septal non-cholinergic neurons (Serafin et al., 1996) and ionic basis for these discharges has also been established (Wang, 2002; Alonso et al., 1996). Septal GABAergic neurons (MS-GABA cells) projected both to other MS-GABA cells and to hippocampal interneurons (Freund and Antal, 1988; Varga et al., 2002a).

Modeling the effects of allosteric modulators

FG-7142 and diazepam are known to bind to the benzodiazepine binding site of the GABA$_A$ receptors (Little, 1991). Furthermore, the FG-7142 was shown to reduce the chloride current at the synaptic channel (Mehta and Ticku, 1989). In our model the synaptic current represented the chloride current assigned to the GABA receptor complex. Therefore, we modeled the effect of negative/positive allosteric modulators by decreasing/increasing the maximal conductance of GABA$_A$ synapses.

Synthetic EEG

In order to get a measure of the population activity, an extracellular field potential was calculated using data obtained from pyramidal cells. The overwhelmingly larger number of pyramidal cells in the real neural tissue legitimated the method of using only pyramidal cell activity for this calculation. Simulated pyramidal cells were placed on the circumference of a 400 μm radius circle and total transmembrane current divided by the inverse of the distance was summed for each compartment. Filtering properties of the extracellular medium was taken into account by low pass filtering the signal (0–10 Hz), thus reducing the contribution of fast events to the EEG. θ Power was determined as the peak of the power spectrum of the EEG trace in the range of 3–8 Hz.

Data analysis

Autocorrelograms. We calculated the autocorrelograms of membrane potential traces of individual neurons. As our aim was to use these autocorrelograms to characterize the periodicity of neuronal firing we defined a measure of periodicity based on the rate of attenuation of the autocorrelograms. To achieve this, we filtered simulated EEGs with a low-pass filter (the first 2 s transient of a simulation was ignored, upper bound of low-pass filter was at 9 Hz), selected maxima of EEG, and finally an exponential was fitted on the maxima in the form of $A\exp(-\lambda)$, where A was the height of the central peak in the autocorrelogram and INSERT GRAPHIC was the fitted variable. If the exponent was smaller than a given threshold, which was set to be 1.2, the firing of the neuron was considered to be periodic. Ratio of periodic cells was calculated as the number of periodic cells divided by the total number of cells. Although the transition from classifying a neuron as periodic to classifying it as non-periodic is arbitrary, the rate of autocorrelogram attenuation proved to be a reliable measure.

Synchrony measure. A measure to quantify synchronization in networks of model neurons was introduced and was denoted by κ (Wang et al., 1996; Orbán et al., 2001). We used the following definition to calculate the value of κ:

$$\kappa = \frac{1}{N(N-1)} \sum_{i=2}^{N} \sum_{j=1}^{i-1} \frac{\sum_{l=0}^{K-1} F_i(l)\,F_j(l)}{\sqrt{\sum_{l=0}^{K-1} F_i^2(l) \sum_{l=0}^{K-1} F_j^2(l)}} \tag{1}$$

Membrane potential vs. time function of each of the N neuron was binned into K bins and the $F_x(l)$ function was constructed such that:

$$F_x(m) = \begin{cases} 1, & \text{if cell } x \text{ fired in bin number } m \\ 0, & \text{if cell } x \text{ was silent in bin number } m \end{cases} \tag{2}$$

In our analysis bin width, $\tau = \dfrac{T}{K} = \dfrac{\text{Total simuation time}}{\text{Number of bins}}$, was chosen to be 10 ms. Note that the maximum of κ is one which corresponds to a totally synchronized state, its minimum is 0 representing the absolutely desynchronized state.

RESULTS

In vivo electrophysiology of the septo-hippocampal system

Activity of MS/DB neurons recorded from anesthetized rats showed a considerable variability in their activity and firing pattern. The mean firing rate of MS/DB neurons calculated during the control period was 19.4 ± 3.7 spikes/s ($n=17$), ranging from 0.4 to 50.3 spikes/s. As it has been previously established (Bland et al., 1999; Ford et al., 1989; Vertes and Kocsis, 1997), firing pattern of MS/DB neurons was directly related to hippocampal EEG activity, showing oscillation during hippocampal θ wave activity and irregular, non-oscillatory activity during hippocampal large amplitude irregular activity (Figs. 2 and 3).

Effects of GABA$_A$ receptor allosteric modulators on the septo-hippocampal oscillatory activity

Systemic (i.v.) administration of the positive allosteric GABA$_A$ receptor modulator diazepam (0.03–0.1 mg/kg,

$n=5$) instantaneously attenuated or abolished θ oscillation of MS/DB neurons and significantly reduced their firing rate in all tested rats (Fig. 2 and Table 1). Power of oscillation of MS/DB neurons was significantly reduced by diazepam (32% of baseline, $P<0.01$), as revealed by fast Fourier transformation analysis of their autocorrelation. Parallel to changes of MS/DB neuronal activity, diazepam significantly reduced hippocampal EEG power at θ frequency range (between 3 and 6 Hz, corresponding to θ frequency in anesthetized rats; Fig. 2 and Table 1). Subsequent administration of the negative allosteric GABA$_A$ receptor modulator beta-carboline FG-7142 (1 mg/kg, i.v., $n=5$) reversed diazepam-induced inhibition of θ activity of both MS/DB neurons and hippocampal EEG (Fig. 2). Furthermore, when FG-7142 (0.1–1 mg/kg, i.v., $n=12$) was administered to control rats, it significantly increased firing rate of MS/DB neurons (Fig. 3, Table 1) and induced or enhanced their θ oscillation indicated by an increase in power of oscillation at θ frequency (181% of baseline, $P<0.05$). In addition to increased firing rate and θ oscillation of MS/DB neurons, FG-7142 significantly enhanced θ wave activity of hippocampal EEG (Fig. 3, Table 1).

Validation of the computational model

The computational neural network model outlined in the Experimental Procedures (and detailed in the online supplementary material) was used to explore the effects exerted by GABA$_A$ modulators on the septo-hippocampal system. Synthetic hippocampal EEG, together with firing rate and firing patterns of septal neurons, characterized by interspike interval histograms and auto-correlograms, provided reference points in our simulations to establish the range of computational parameters that resulted in patterns and activities corresponding to *in vivo* observations. Once these computational parameters were fixed at default values (as detailed in the supplementary material), actions of pharmacological manipulations were determined on septo-hippocampal activity (including synthetic EEG, network rhythm generation as well as septal and hippocampal cell firing and pattern activity) by the modulation of maximal conductance of GABA$_A$ synapses. As we describe it in detail below, an increase in maximal synaptic conductance, simulating the effect of the positive allosteric modulator diazepam, inhibited θ activity both in synthetic EEG and in septal or hippocampal cell activity model, resembling our *in vivo* pharmacological observations. Furthermore, a similar correlation was found between pharmacological and computational findings when the negative allosteric modulator FG-7142 was modeled by a decrease in maximal synaptic conductance at GABA$_A$ receptors.

Modeling of septal GABA neurotransmission and its modulation

Using the computational model of the isolated septal neuron network, with interconnections between septal cells, the modeled septal GABAergic neurons showed rhythmic discharge properties, characterized by clusters of one to five spikes generated within the θ frequency band (Fig.
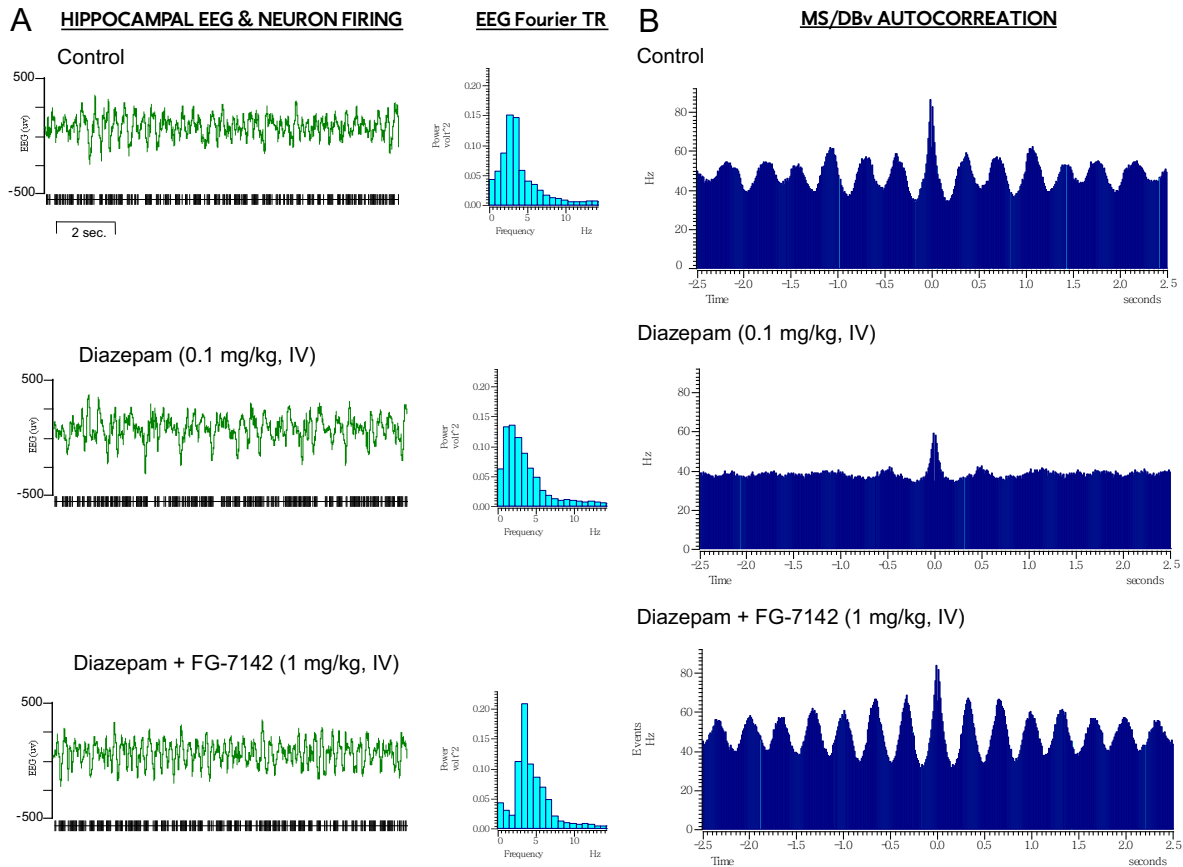
Fig. 2. (A) Typical recording showing θ activity of the septo-hippocampal system inhibited by systemic administration of diazepam and reversal by the subsequent administration of FG-7142. Left columns show periods of simultaneously recorded hippocampal EEG (upper trace) and firing activity of MS/DB neuron (lower trace) during control baseline period (Control), after i.v. administration of Diazepam (0.1 mg/kg) and subsequent administration of FG-7142 (1 mg/kg). Voltage calibration corresponds to hippocampal EEG. Right columns show power spectra (V^2) of the hippocampal EEG using fast Fourier transformation. (B) Columns demonstrate amount of oscillation (autocorrelation) of same MS/DB neuron shown in Fig. 2A.

4Aa, Ba). This periodicity of action potential generation (as revealed by autocorrelation of membrane potentials), was observed over a broad range of maximal synaptic conductance, representing various levels of synaptic transmission. However, autocorrelograms of membrane potentials of several septal neurons did not show periodicity despite of the fact that they discharged spikes-clusters within 100–180 ms time interval, i.e. within the θ frequency range (Fig. 4Ab, Bb). Indeed, in a network of septal GABA neurons with given conditions (i.e. with a given maximal synaptic conductance) both θ-periodic and θ-non-periodic neurons were present.

Periodicity of individual septal neurons was influenced by network characteristics, such as connectivity, including both convergent and divergent connections. In a more densely connected network there was a lower number of neurons showing periodicity (data not shown). In order to model pharmacological manipulation of GABA$_A$ receptors of septal neurons, the maximal synaptic conductance was altered (see Experimental Procedures). Simulations showed that increased synaptic conductance resulted in a decrease of average firing rate of septal neurons (10.4±1.5 Hz and 8.5±2.1 Hz at

low and high synaptic conductance, respectively) and, in addition, a reduction in number of neurons firing periodically (Fig. 4A). In order to quantify this observation, the periodicity of a neuron was evaluated by the decay constant and was qualified as periodic (i.e. $\lambda < 1.2$) or non-periodic (i.e. $\lambda \geq 1.2$) cell (see Experimental Procedures) and a relationship was established between maximal synaptic conductance and the ratio of periodic cells (Fig. 5). As it can be seen on Fig. 5, throughout the examined conductance interval the ratio of periodic cells is a monotonously decreasing function of the maximal synaptic conductance. Data were best fit with an exponential of the form $r = a \exp(-g/g_o)$, where g_o was 2.23±0.11 nS and a was set to 1 reflecting that at 0 nS maximal synaptic conductance the network is uncoupled hence the ratio of periodically firing neurons is 1. This finding shows that halving the maximal synaptic conductance results in an increase of the number of periodic cells to the square root of original one. In line with previous observations (Wang, 2002), no synchronization between septal neurons was found (data not shown).
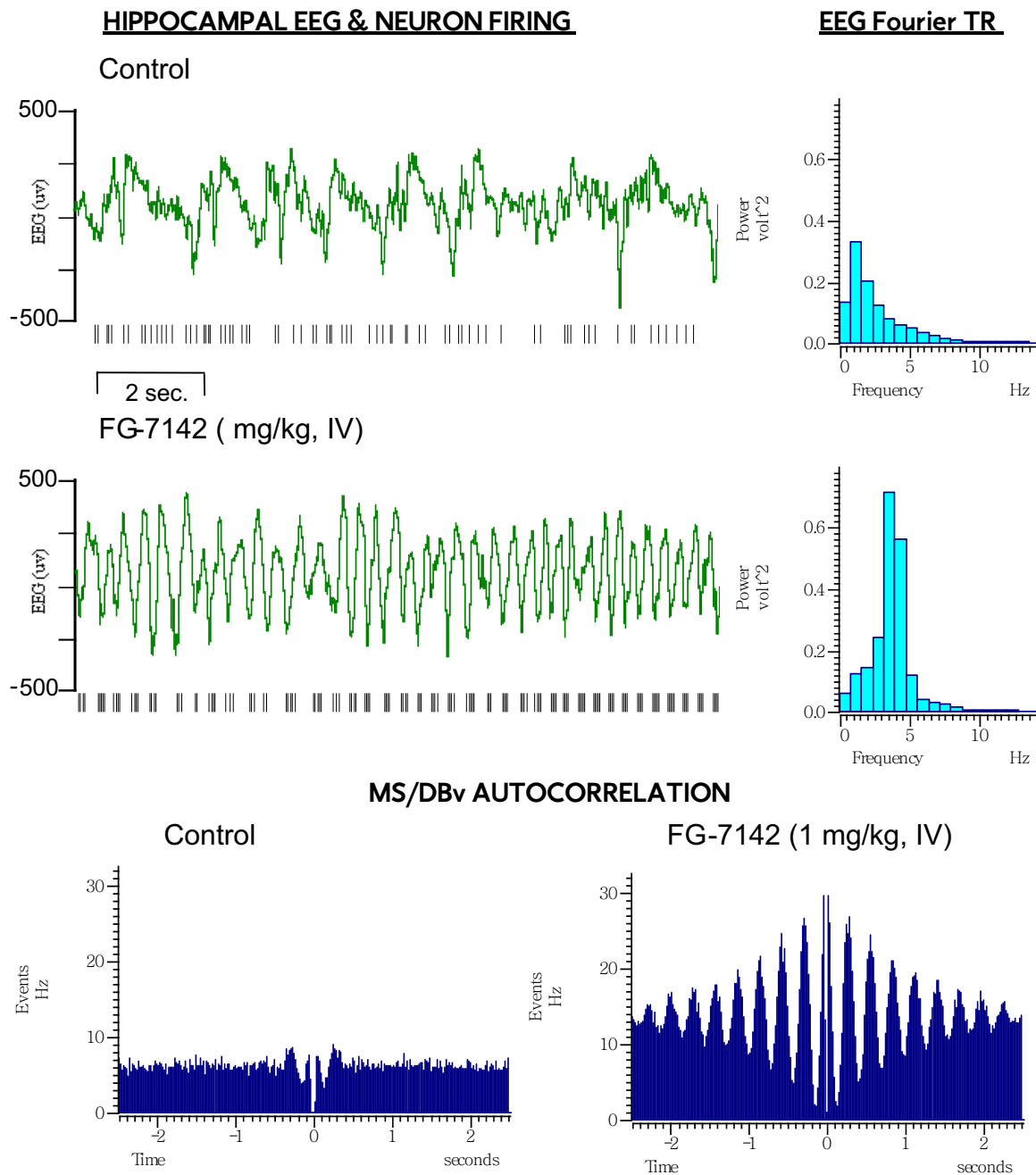
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EEG Fourier TR

Fig. 3. Typical recording showing θ activity of the septo-hippocampal system induced by systemic administration of FG-7142. Upper left columns show periods of simultaneously recorded hippocampal EEG (upper trace) and firing activity of MS/DB neuron (lower trace) during control baseline period (Control), and after i.v. administration of FG-7142 (1 mg/kg). Upper right columns show power spectra (V^2) of the hippocampal EEG (using fast Fourier transformation). Voltage calibration corresponds to hippocampal EEG. Simultaneously induced oscillation of the MS/DB neuron (auto-correlation) is shown in the lower row.

Modeling of modulation of GABA neurotransmission in the septo-hippocampal system

Computational results show that θ activity in the hippocampus might arise from the interplay of three neuron populations: pyramidal cells, basket, and oriens interneurons. In our model tonic depolarization of pyramidal and basket neurons was required for θ generation, but in accordance with recent *in vitro* observations (Gillies et al., 2002) no phasic input was needed. This excitatory input could represent cholinergic input in physiological conditions. In the following we studied the modulation of θ activity emerging in the hippocampus in the framework of our four-population septo-hippocampal model.

Pharmacological modification of GABA$_A$ receptors in the septo-hippocampal system was modeled as described in the previous section. Replicating the action of positive and negative allosteric modulators, maximal synaptic con-

Table 1. Effects of IV administration of FG-7142 and diazepam (doses indicated in parenthesis) on neuronal firing rate in the MS/DB and power of hippocampal EEG at θ frequency (3–6 Hz; as revealed by fast Fourier transformation)[a]

Treatment	MS/DB firing rate (% of baseline)	EEG power (θ) (% of baseline)
FG-7142 (0.1 mg/kg, $n=6$)	160±12*	155±33
FG-7142 (1 mg/kg, $n=6$)	218±67**	290±83*
Diazepam (0.1 mg/kg, $n=5$)	36±16*	70±8.7*

[a] Values (mean±S.E.M.) are expressed as % change from baseline.
* $P<0.05$, ** $P<0.01$, paired t-test.

ductance at synapses between basket and basket neurons (b→b), basket and pyramidal neurons (b→p), oriens and pyramidal neurons (o→p), oriens and basket neurons (o→b), oriens and septal GABA neurons (o→m), septal GABA neuron and oriens neurons (m→o), septal GABA and basket neurons (m→b), and septal GABA and septal GABA neurons (m→m) were increased and decreased, respectively. Simulations showed that at high maximal GABA synaptic conductance level no spike-clustering could be observed on the timescale characteristic of θ activity (Fig. 6A). In contrast, a decreased GABA$_A$ transmission (i.e. lower maximal GABA$_A$ synaptic conductance values) induced spike-clustering in all modeled neurons, including pyramidal neurons (Fig. 6B). Firing histogram maxima showed a well-defined timing of action potentials of neuron populations and a phase difference between neuron populations (basket cell maximal firing approximately 130 ms before that of pyramidal and oriens neurons). Besides showing periodicity in firing pattern, basket neurons displayed an increase in firing rate (8.6±7.2 Hz and 14.7±6.4 Hz for high and low synaptic conductance, respectively). Reflecting spike clustering, a significant increase in hippocampal field θ (4.75 Hz) power was observed (Fig. 6C).

Since it was unknown whether θ oscillation induced by lowering maximal synaptic conductance was due to changes of all septo-hippocampal GABA synapses or a subset of them, we modulated GABA synapses at pathways of the septo-hippocampal circuitry selectively. Averaged ($n=15$) EEG θ power confirmed our observation shown by individual simulations that simultaneous reduction of maximal synaptic conductance at all pathways leads to a significant increase in θ activity (Fig. 6E). The most pronounced increase in θ power of EEG (Fig. 6D) and synchrony of pyramidal neurons (Fig. 6E) was apparent when maximal synaptic conductance was decreased between both septal and septal neurons and basket and basket neurons simultaneously. Interestingly enough, selective reduction in maximal synaptic conductance between septal and septal neurons did not enhance θ activity (Fig. 6D and 6E).

Dependence of θ EEG power on overall synaptic conductance was established by systematically changing maximal synaptic conductance at all pathways. We found a clear increasing tendency of EEG power with lower synaptic conductance (Fig. 7), showing that a limited (less than 50%) reduction in synaptic conductance results in observable fall of θ amplitude. Further decreasing synaptic conductance at GABA$_A$ synapses (below 40%) naturally resulted in an abrupt breakdown in θ power.

DISCUSSION

The present findings demonstrate that systemic administration of the anxiolytic diazepam, a positive allosteric modulator of GABA$_A$ receptors inhibits, whereas the anxiogenic FG-7142, a negative allosteric modulator of GABA$_A$ receptors enhances θ activity of the septo-hippocampal system. Responses of the septo-hippocampal system observed in pharmacological experiments are mirrored by our computational septo-hippocampal model, at both neuronal and circuitry levels, when the actions of allosteric modulators have been represented by corresponding changes in maximal synaptic currents. In addition, the computational model provided further insight into the underlying θ rhythm generating mechanisms.

It is well documented that interactions between septal and hippocampal GABA neurons are key contributing mechanisms to θ activity, a rhythmic, synchronized activity of the septo-hippocampal system (Petsche et al., 1962; Tóth et al., 1997; Buzsáki, 2002). Consequently, pharmacological manipulation of GABA receptors has profound effects on θ activity, along with behavioral or physiological processes associated with this septo-hippocampal activity. It has been shown that the GABA$_A$ receptor positive allosteric modulators like diazepam inhibit (Hirose et al., 1990; McNaughton and Coop, 1991; Yamadera et al., 1993), while the negative allosteric modulator FG-7142 induces hippocampal θ activity (Ongini et al., 1983; Marrosu et al., 1988). In the present studies we demonstrated that activation of GABA$_A$ receptors desynchronized septo-hippocampal activity: diazepam simultaneously inhibited θ oscillation of MS/DB neurons and induced large amplitude irregular hippocampal EEG activity. Diazepam-induced changes of both septal neuron activity and hippocampal EEG were readily reversed by the negative allosteric modulator FG-7142, indicating that converse modulation of GABA$_A$ receptor-mediated current evokes opposite responses of the septo-hippocampal system. In addition, FG-7142 induced or significantly enhanced both hippocampal θ wave activity and θ oscillation of MS/DB neurons in control animals. These findings demonstrate that uniform and simultaneous modulation of all GABA$_A$ receptors by systemic administration of allosteric modulators evokes a coherent response, regardless of the elaborate synaptic connectivity utilizing GABA$_A$ receptors within the septo-hippocampal system.

Our computational model of the septo-hippocampal system, based on four physiologically different cell populations contributing to θ rhythm generation, showed similar responses to those measured in *in vivo* experiments. A
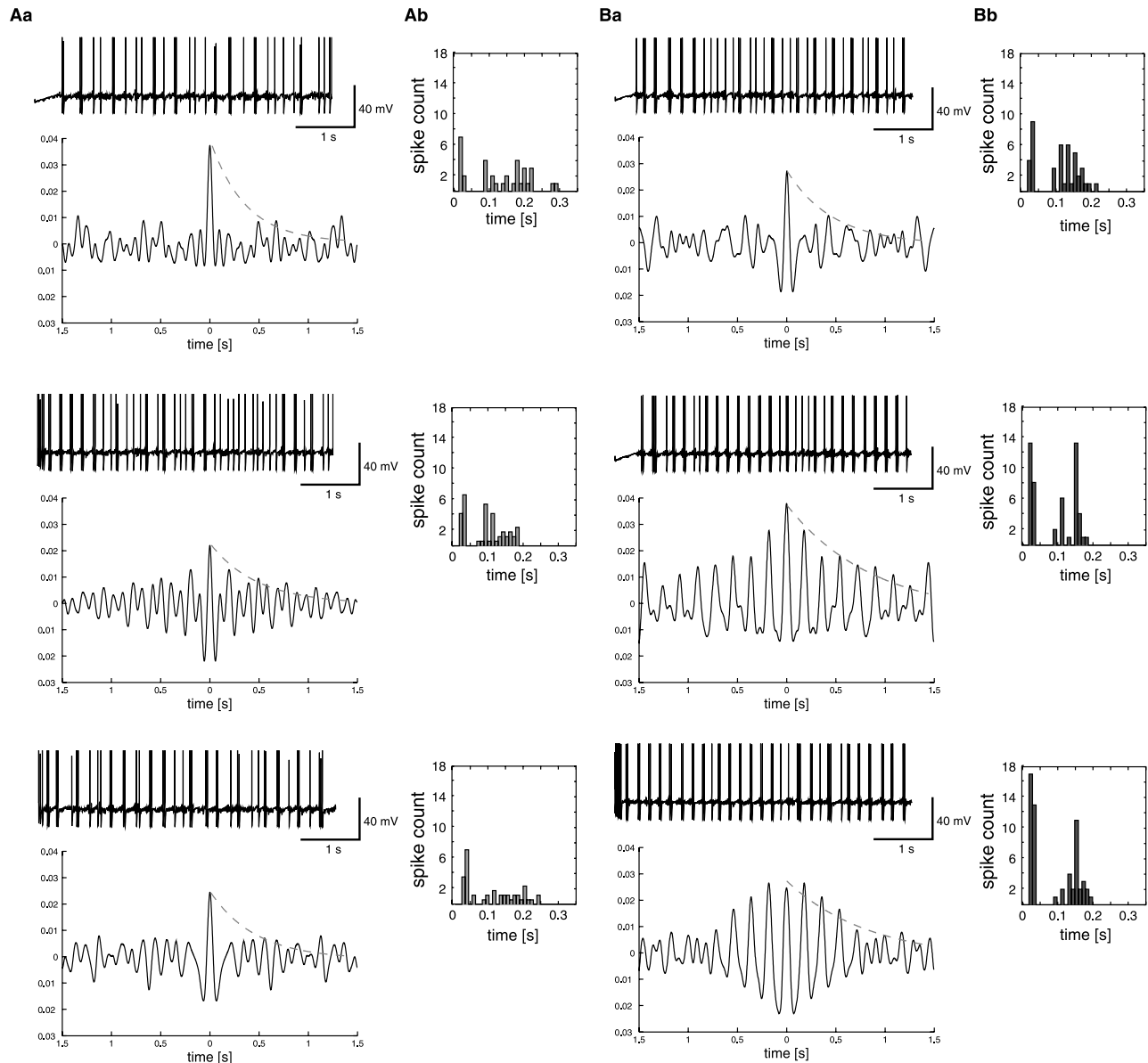
Fig. 4. Characterization of MS-GABA neuron behavior. An isolated MS-GABA cell population with random connectivity was simulated (number of neurons 50, connection probability was 30%) and maximal synaptic conductance was varied. Three arbitrarily chosen neuron at high (1.25 nS) and low (0.63 nS) synaptic conductance values are shown (A and B, respectively). Membrane potential traces, their autocorrelograms (a, black, solid lines) and corresponding ISI histograms (b) are shown. While at high synaptic conductance periodicity of membrane potential traces in θ band was almost absent (Aa, with λ=2.60, 1.69, 2.04, for the top, middle and bottom panel, respectively), at lower values autocorrelograms revealed θ-periodic behavior with different levels of attenuation (Ba, with λ=2.01, 1.09, 1.16, for the top, middle and bottom panel, respectively). Note, that with a given synaptic conductance not all neurons are classified as either periodic or non-periodic. Also, ISI histograms reflect this duality. ISI histograms of neurons connected with stronger synapses resemble more to that of a Poisson distribution (Ab), while with weak synapses bimodal distributions are prevalent (Bb). Depolarizing current given to each neuron was set to be 2 μA/cm². Dashed gray lines on (a) represent the exponentials fitted with the given values of λ.

fraction of septal GABA neurons, simulated in an interconnected network, showed θ related rhythmic discharge properties, although both periodic and non-periodic neurons were present simultaneously. This finding is in line with physiological observations showing septal neurons with highly different levels of θ oscillation in anesthetized animals (Vertes and Kocsis, 1997; Varga et al., 2002b; Hajós et al., 2003a,b). However, increasing maximal synaptic conductance (simulating the effect of positive alloste-

ric modulation of GABA_A receptors) resulted in a reduced proportion of septal neurons showing firing periodicity, and a reduction in both firing rate and periodicity (oscillation) of individual septal neurons. The latter observations, i.e. significant reduction in θ oscillation and firing rate of individual septal neurons have been demonstrated in our parallel *in vivo* experiments.

Regarding network characteristics of the septo-hippocampal model, a tonic depolarizing input to pyramidal
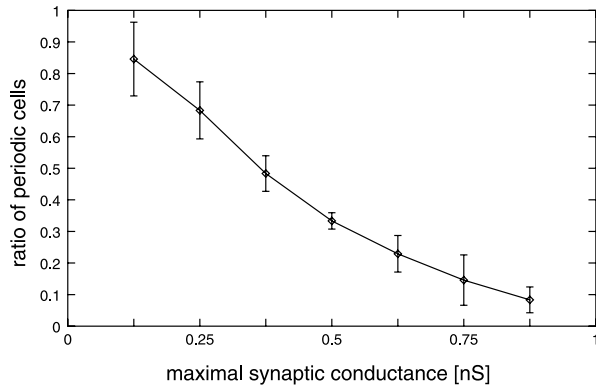
Fig. 5. Drop in the ratio of periodically firing MS-GABA neurons in a randomly connected network as a result of increasing synaptic conductance. Average number of periodically firing neurons as a function of maximal synaptic conductance of synapses connecting MS-GABA neurons. At low synaptic conductance elements of the network act independently, generating action potentials autonomously, thus spike-trains of neurons reflect the inherent periodicity of cells. With rising synaptic conductance the effect of other neurons becomes larger and this perturbation reduces the coherence of the periodic oscillation causing the autocorrelograms to attenuate faster. During the simulations we used 50 neurons, connection probability was held at 20%, the level of depolarizing current was 2 μA/cm^2. Error bars denote standard deviation.

and basket neurons was necessary for its θ generation. This tonic depolarizing current could correspond to the presumed excitatory cholinergic input (Cole and Nicoll, 1983; Madison et al., 1987; Hasselmo and Fehlau, 2001) necessary for θ activity of the septo-hippocampal system under general anesthesia (Lee et al., 1994). However, synchrony of pyramidal neurons, correlating with θ power in the extracellular field potential, was determined by maximal synaptic conductance representing GABA$_A$ receptor activation. Thus, at high maximal synaptic conductance level no spike-clustering could be observed on the time-scale characteristic of θ activity (200 ms), but at decreased synaptic conductance values (i.e. attenuated GABA$_A$ neurotransmission) spike-clustering was observed in all modeled neurons, including pyramidal neurons.

In order to determine how GABA$_A$ receptor-mediated connections between individual populations of neurons of septo-hippocampal system impact θ generation, selective modulation of these pathways have been modeled. As we discussed above, increasing synaptic conductance induced a shift from θ-periodic to non θ-periodic behavior in a number of neurons in the septal GABA network. This observation shows that the level of perturbation arising form inhibitory postsynaptic potentials of afferent GABA neurons is a crucial factor contributing to θ-periodic characteristics of neuronal activity. On the other hand, it was found that in the hippocampus different GABA connections have different roles in synchronizing neuron populations in θ frequency. Thus, we showed that strength of interconnections between basket neurons had a great influence on septo-hippocampal θ activity. Similarly, simultaneous modulation of connections between septal and septal neurons together with the modulation of synaptic transmission between basket

and basket neurons also induced strong septo-hippocampal θ activity. In contrast, selective modulation of GABA$_A$ connection between septal and septal neurons failed to alter θ activity of the septo-hippocampal system, although it had a clear effect on activity of individual septal neurons. This finding is in line with *in vivo* electrophysiological observations, demonstrating that medial septal neurons activity phase-locked to various hippocampal EEG phases, and there is no phase correspondence between individual medial septal neurons (King et al., 1998; Varga et al., 2002a). These computational observations clearly indicate that θ activity of the septo-hippocampal system is regulated differently by separate connections within the circuitry.

Alterations in strength of interconnections between basket neurons influenced firing rate of basket neurons, as reflected by their lower firing rate at higher synaptic conductance, parallel to changes in septo-hippocampal θ activity. Since it has been shown that activity of basket neurons is the main contributor to hippocampal γ activity (Whittington et al., 1995; Wang and Buzsáki, 1996; Csicsvári et al., 1999; Orbán et al., 2001), a reduction of firing activity would strongly impact both the frequency of γ oscillation, and the synchronization of target pyramidal neurons. Basket cells are in a unique position in the hippocampal circuitry given that they innervate the perisomatic region of pyramidal cells and, consequently have a great impact on the pattern and timing of their output (Freund and Buzsáki, 1996; Klausberger et al., 2003). In a heterogeneous network of basket neurons (i.e. random connection pattern between cells) lower average firing rate causes a less effective gating of pyramidal cell firing and, accordingly lower synchrony of pyramidal neurons at θ frequency.

As we demonstrated, systematic attenuation of GABAergic transmission (up to 50%) in the septo-hippocampal system increased the power of θ oscillation (36% on average at 50% attenuation). However, standard deviation from mean reflected a considerable heterogeneity of individual simulations, indicating that with a given network structure (connection patterns and synaptic transmission levels) both non-synchronized and synchronized states could be observed. This is in accordance with the notion that although with stronger synaptic conductance the probability of θ emergence increases, synaptic conductance itself is not sufficient to fully determine the state of the septo-hippocampal system; additional modulators and inputs can affect it as well.

In summary, the present pharmacological observations support the presumed connection between anxiety and septo-hippocampal θ activity, since the θ blocking benzodiazepines are clinically proven anxiolytics, and FG-7142 is known to be anxiogenic both in animal models (Little, 1991; Adamec, 1997) and in humans (Horowski and Dorrow, 2002). However, given the multiplicity of behaviors associated with θ activity (Buzsáki, 2002), relationship between θ oscillation of the septo-hippocampal system (or any sub-regions of the limbic system) and anxiolytic drug action has to be further
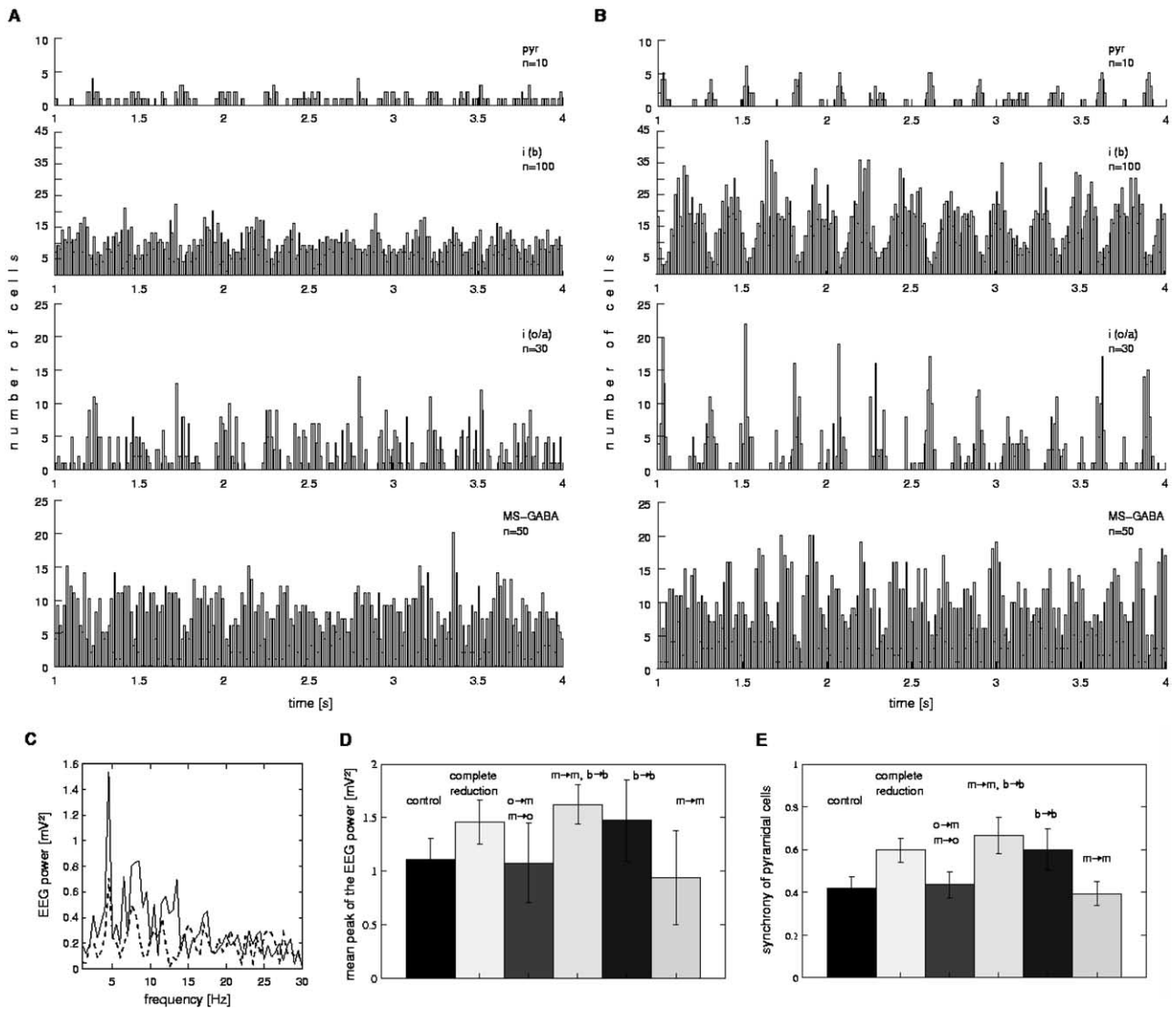
Fig. 6. Controlling the behavior of septal and hippocampal neuron populations by modulation of GABA$_A$ synapses. (A, B) Firing histograms of different neuron populations for high and low GABA$_A$ synaptic conductances, respectively (pyr: pyramidal neurons, I(b): basket neurons, I(a/o): alveus/oriens interneurons, MS-GABA: medial septal GABAergic neurons). GABAergic synaptic transmission was attenuated at all pathways (ratio of high and low conductances was 0.5, high conductance was the double of default values; see Supplementary Material: http://geza.kzoo.edu/theta). All hippocampal neuron populations show a clear θ-frequency firing rate modulation at lower GABA$_A$ conductance levels and a moderate modulation of firing rate of medial septal GABAergic cell population is observable. (C) Power spectra of the extracellularly measured EEGs calculated for simulations shown in A (dashed line) and B (solid line). (D, E) Mean amplitude of the θ band oscillation (4–5 Hz) deducted from the power spectra of EEG traces and average level of synchrony between pyramidal cells. Bars correspond to the modulation of GABAergic transmission at different pathways (labels of bars denote the specific pathways where synaptic transmission was attenuated; see text; reduction was 50%, control: simulation with default values, complete reduction: all pathways). The two measures showed similar characteristics. Significant increase in θ power relative to the control state was found when synaptic conductances at all pathways were reduced (complete reduction), or partly reduced among MS-GABA cells and basket cells (m→m, b→b), or reduced in the basket neuron network (M=1.5, S.D.=0.2), Z=3.6, P<0.01; (M=1.6, S.D.=0.1), Z=5.7, P<0.01; (M=1.5, S.D.=0.4), Z=2.5, P<0.01 relative to the control situation (M=1.1, S.D.=0.2), respectively. In the other two cases no significant change was observed. Investigating the pyramidal cell synchrony, significant increase was observed in the same cases: when synaptic conductances at all pathways were reduced (complete reduction), or partly reduced among MS-GABA cells and basket cells (m→ m, b→ b), or reduced in the basket neuron network (M=0.60, S.D.=0.06), Z=6.8, P<0.01; (M=0.67, S.D.=0.08), Z=4.7, P<0.01; (M=0.60, S.D.=0.1), Z=4.8, P<0.01 relative to the control situation (M=0.42, S.D.=0.05), respectively. In the other two cases no significant change was observed.

substantiated. In addition, it is still unclear whether inhibition of hippocampal θ activity is a contributing factor to the well-demonstrated negative effects of anxiolytic benzodiazepines on cognitive functions (Coull et al., 1999). The present computational model proved suitable to reveal several aspects of pharmacological ac-

tions of GABA$_A$ receptor modulators. Moreover, our computational findings indicate different roles of distinctively located GABA$_A$ receptors in θ generation. The currently described compartmental model of the septo-hippocampal system allows us to investigate interactions with the circuitry at a highly specific way, currently
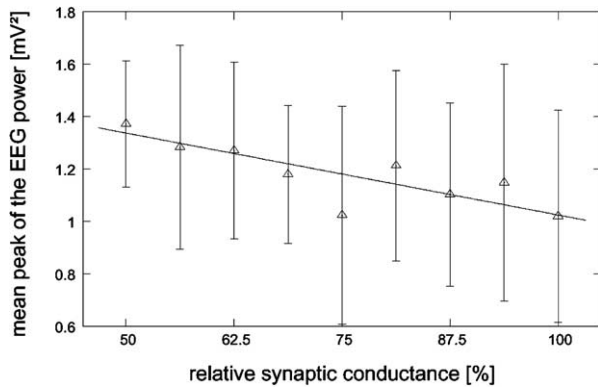
Fig. 7. θ Activity decreases monotonously with increasing GABA$_A$ synaptic conductance. Mean amplitude of θ band oscillation (4–5 Hz, number of simulations run=24) at different levels of relative strength of GABAergic transmission (diamonds), 100% corresponding to the control and 50% corresponding to the complete reduction state in Fig. 3. Amplitude is normalized with level calculated at non-synchronized network measured at blocked GABA transmission. Error bars denote S.D. Data were fit with a linear function (solid line) with slope −0.77±0.23 and offset 235±17. Note, that linear relation cannot hold for very low synaptic conductance levels because in this case pyramidal cells become practically uncoupled (as reflected by the low baseline level).

not accessible experimentally. Combined pharmacological and computational experiments would provide us with further opportunities to investigate mechanism of septo-hippocampal θ generation and modulation, and its role in therapeutic action of psychotherapeutic agents.

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