Pharmacological and computational analysis of alpha-subunit preferential GABA$_A$ positive allosteric modulators on the rat septo-hippocampal activity

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Abstract

Clinically most active anxiolytic drugs are positive allosteric modulators (PAMs) of GABA$_A$ receptors, represented by benzodiazepine compounds. Due to their non-selective profile, however, they potently modulate several sup-type specific GABA$_A$ receptors, contributing to their broad-range side effects. Based on observations in genetically altered mice, however, it has been proposed that anxiolytic action of benzodiazepines is predominantly mediated by GABA$_A$ $\alpha$2/3 subunit-containing receptors. In the present study we analyzed the actions of the preferential GABA$_A$ $\alpha_1$ and $\alpha_2/3$ PAMs, zolpidem and L-838417, respectively on hippocampal EEG and medial septum neuronal activity in anesthetized rats. In parallel, a computational model was constructed to model pharmacological actions of these compounds on the septo-hippocampal circuitry. The present results demonstrated that zolpidem inhibited theta oscillation both in the hippocampus and septum, and profoundly inhibited firing activity of septal neurons. L-838417 also inhibited hippocampal and septal theta oscillation, however, it did not significantly alter firing rate activity of septal neurons. Our computational model showed that cessation of periodic firing of hippocampo-septal neurons, representing absence of hippocampal theta activity, disrupted oscillation of septal units, without altering their overall firing activity, similar to changes observed in our in vivo experiments following administration of L-838417. Understanding the correlation between changes in septo-hippocampal activity and actions of selective modulators of GABA$_A$ subtype receptor modulators would further advance design of anxiolytic drugs.

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1. Introduction

Benzodiazepines, the positive allosteric modulators (PAMs) of GABA$_A$ receptors are clinically proven anxiolytic drugs. These compounds potently modulate several sup-type specific GABA$_A$ receptors, including $\alpha_1$, $\alpha_2$, $\alpha_3$ or $\alpha_5$ subunit-containing receptors with comparable efficacy. This non-selective action is presumed to be responsible for their broad-range side effects, including muscle relaxant, sedative, ethanol-potentiating and amnesic effects (Mohler et al., 2002). Based on observations in genetically altered mice, it has been proposed that the anxiolytic...
action of benzodiazepines is predominantly mediated by α2 and/or α3 subunits containing GABA A receptors, whereas α1 subunit-containing GABA A receptors mediate their sedative effects (McKernan et al., 2000; Rudolph et al., 2001). Since effective anxiolytic drugs are clearly in high clinical demand, there is an intensive research on subunit selective GABA A PAM compounds, which are devoid of the most common side effects, like sedation or abuse liability. Recently, several GABA A receptor PAMs have been developed, which display preferential modulation towards α2/α3 and α5, but not α1 subunit-containing receptors (Atack, 2005). One of these modulators, L-838417, has a similar efficacy (as a partial agonist at the benzodiazepine binding site) at α2/α3 and α5 subunit-containing receptors, but it lacks efficacy at α1 subtype receptors (McKernan et al., 2000; Atack, 2005). Anxiolytic effects of L-838417 have been demonstrated in rat and mice anxiety models (McKernan et al., 2000; Mathiasen and Mirza, 2005) and in primates (Rowlett et al., 2005).

It has been shown that anxiolytic drugs inhibit oscillatory neuronal network activity of the limbic system, an effect that is thought to contribute to their anxiolytic or sedative effects (Hirose et al., 1990; McNaughton and Gray, 2000). Diazepam, a non-selective PAM of GABA A receptors inhibits hippocampal field potential (electroencephalography, EEG) theta band activity (Caudarella et al., 1987; McNaughton and Gray, 2000; van Lier et al., 2004). We have recently reported that systemic administration of diazepam simultaneously inhibits theta oscillation of medial septum/diagonal band of Broca (MS/DB) neurons and theta wave activity in the hippocampus in chloral hydrate anesthetized rats (Hajós et al., 2004). In contrast, FG-7142, a negative allosteric modulator of GABA A receptors inhibits hippocampal field potential (electroencephalography, EEG) theta band activity (Hajós et al., 2003, 2004). Since interconnected GABAergic interneurons are critical to neuronal network oscillations, including hippocampal theta rhythm (Buzsáki, 2002; Freund, 2003), either positive or negative modulation of GABA A receptors (i.e. enhancing or reducing efficacy of the neurotransmitter GABA within the circuitry) is expected to have a profound effect on network activity, like theta oscillation. However, morphologically and neurochemically distinct hippocampal GABAergic interneurons show highly specialized synaptic connectivity, indicating different functional roles in generating or modulating oscillatory activity (Freund and Buzsáki, 1996; Freund, 2003; Henderson et al., 2004; Klausberger et al., 2003). In addition, different subunit specific GABA A receptors are localized in synapses involved between distinct neurons in the septo-hippocampal formation (Freund, 2003). It has been demonstrated, for example, that α2 subunit-containing GABA A receptors are predominantly located on the axon hillock/initial segments of pyramidal neurons, which are predominantly innervated by axo-axonal neurons in the hippocampus (Nusser et al., 1996). Therefore, it is a possibility that selective modulation of different subsets of GABA A receptors will have different impacts on hippocampal oscillatory activity (Howard et al., 2005). In fact, our recent computational work indicated that selective modulation of distinct GABA A mediated synapses could modify oscillatory activity differently (Hajós et al., 2004). Consequently, the aims of the present experiments were to analyze the effects of L-838417, a preferential modulator of α2, α3 and α5 GABA A receptors, and zolpidem, a preferential modulator of α1 GABA A receptors on activity of the septo-hippocampal system by using simultaneous recordings of single units from the MS/DB and hippocampal (CA1) field potentials (EEG) from anesthetized rats. Furthermore, a computational model of the septo-hippocampal circuit has been utilized in order to model in vivo activities of the GABA A receptor PAMs on hippocampal and septal neuronal and network activities.

2. Materials and methods

2.1. Electrophysiological experiments

2.1.1. Animals and surgical procedures

Experiments were performed on male Sprague–Dawley rats (weighing 250–300 g) in chloral hydrate anesthesia (400 mg/kg i.p.), under an approved animal 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, National Institutes of Health guidelines. The femoral vein was cannulated for administration of test compounds or additional doses of anesthetic. The anesthetized rat was placed in a Kopf 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 36–37°C by means of an isothermal (37°C) heating pad (Branticef Scientific, Braintree, MA).

2.1.2. Single unit recordings

Single units were recorded from the medial septal 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 MΩms). Extracellularly recorded potentials were amplified, filtered, displayed, discriminated and recorded for off-line analysis using conventional electrophysiological methods (Hajós et al., 2003, 2004). Neuronal activity was followed by constructing firing rate, frequency and interspike interval histograms using the 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., 2003). Effects of GABA A receptor PAMs were tested on septal neurons showing comparable baseline activities during the control period; average firing rates of neurons were 25.7 ± 5.1 Hz and 22.4 ± 5.2 Hz in the zolpidem and L-838,417 groups, respectively.

The tip 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.

2.1.3. 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, Cambridge, UK). Rhythmic synchronized (theta) 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., 2003, 2004). Power spectrum density of EEG for theta activity was calculated at peak frequency between 3 and 6 Hz. Location of the recording electrode was verified histologically.
2.1.4. Data analysis and statistics

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

2.1.5. Materials

Solutions of zolpidem (Tocris), L-838417 (7-tert-butyl-3-(2,5-difluorophenyl)-6-(2-methyl-2H-[1,2,4]triazol-3-ylmethyl)-[1,2,4]triazol(4,3-b)pyridazine) and Ro 15-1788 (flumazenil) were made up based upon their salt weights in H2O and concentrations adjusted so that injection volumes equaled 1 mL/kg body weight. L-838417 and Ro 15-1788 were synthesized at Pharamcia Corporation, Kalazamoo, MI.

2.2. Computational methods

2.2.1. Single cell models

2.2.1.1. Septal GABAergic cell. Results of in vivo experiments were reproduced by using a mathematical model of the MS/DB neurons. The model was based on the Hodgkin–Huxley equation (Hodgkin and Huxley, 1952) using a single compartmental model developed by Wang (2002) representing intrinsic properties of the putative GABAergic MS/DB neurons. Membrane potential change is given by the following current balance equation (the membrane noise term, originally introduced by Wang was omitted and heterogeneity is included in the app term):

\[
c_m \frac{dV}{dt} = -I_{Na} - I_K - I_L - I_{syn} + I_{app}
\]

where \(V\) is the cell’s membrane voltage, \(C_m = 1 \text{F/cm}^2\) is the membrane capacitance, \(I_{Na}, I_K, I_{KCa}, I_L, I_{syn}\), are the sodium, delayed rectifier potassium, slow potassium, leakage and synaptic currents, respectively. The detailed form of these currents together with the parameters used in the simulations can be found in Appendix A. \(I_{app}\) is the applied current, is a depolarizing current representing background current mostly due to cholinergic innervation. Its heterogeneity is introduced into the cell model via \(I_{app}\) by means of its value every 5 ms according to a Gaussian distribution of standard deviation \(\sigma(I_{app}) = \mu(I_{app})/6\). The noise modeled this way is a good approximation of a 100 Hz upper limit frequency noise (Dayan and Abbott, 2001). For numerical integration of these equations the initial membrane potential \(V_{init}\) is the cell’s membrane voltage, \(C_m = 1 \text{F/cm}^2\) is the membrane capacitance chosen from a Gaussian distribution of mean \(\mu(C_m) = 1 \text{F/cm}^2\) and standard deviation \(\sigma(C_m) = 0.3 \text{F/cm}^2\). \(I_{Na}, I_K, I_{KCa}, I_L, I_{syn}\) are the sodium, delayed rectifier potassium, hyperpolarization activated non-specific cation, high threshold calcium, calcium activated potassium, leakage and synaptic currents, respectively. Details of these currents and appropriate parameters are given in Appendix A. \(I_{app}\) again represents the background depolarizing current, the effect of the tonic septal cholinergic innervation, while \(I_{hid}\) is a phasic current representing the summation of all phasic synaptic currents. When modeling the hippocampal theta state this current either takes the form of a 4–5 Hz frequency, 36 pA amplitude sine wave or is taken from experimental measurements. In latter case \(I_{hid}\) is the signal measured by the hippocampal CA1 field electrode and transformed to a similar shape (amplitude \(\sim 36\) pA and \(I_{hid} \sim 3\) pA). This method is justified by the fact that the majority (>80%) of the innervation of hippocampo-septal cells originates from axon collaterals of local pyramidal neurons (Blasco-Ibanez and Freund, 1995).

When modeling a non-theta case evoked in the physiological experiments by the IV application of L-838417 or zolpidem, \(I_{hid}\) again is the CA1 field signal scaled to similar amplitude. This signal is analogous to a white noise; peaks are not observable in its Fourier spectrum. In control conditions hippocampo-septal cells fire periodically, 1–3 spikes/theta cycle, while under the effect of L-838417 they fire aperiodically with a similar firing rate (6–14 Hz). In all cases \(I_{hid}\) comes from the measurements described by the present paper (see Fig. 1A, B for representative examples).

2.2.2. Synapse and network models

2.2.2.1. GABA A synapse model. Our simulations focus on the generation and modification of theta frequency population rhythm generated in a network of mutually inhibitory cells of the medial septum. The interconnections between our simulated network units were based on the modeled GABA A synapses developed by Wang and Buzsaki, (1996)

\[
I_{syn} = g_{syn}^{a}(V - E_{syn})
\]

where \(g_{syn}^{a}\) is the maximal synaptic conductance (see below), and the parameters between two septal cells \(E_{syn} = -75 \text{mV}, \alpha = 14/\text{ms}, \beta = 0.07/\text{ms}\); for the hippocampo-septal connections \(E_{syn} = -80 \text{mV}, \alpha = 1/\text{ms}, \beta = 0.05/\text{ms}\). \(K_{pre} = -2 \text{mV}, \Theta_{syn} = 0 \text{mV}\) for both synapse types.

2.2.2.2. Network structure. Recent anatomical and physiological findings suggest that a delicate synaptic connection pattern might account for the pace-maker capability of septal inhibitory cells. First, it was shown that the distribution of preferred firing phases of medial septal parvalbumin-positive (PV+) GABAergic neurons is a bimodal distribution: a subpopulation of these cells preferentially fire at the peak and others at the trough of the hippocampal field theta oscillation (Borhegyi et al., 2004). Second, anatomical studies (Henderson et al., 2004) revealed two PV+ cell populations in the medial septum: one medially and another more laterally located. These two populations differ in their GABAergic innervation: PV+ basket-like terminals are on medially located cells, while there are fewer PV+ synapses on the laterally located neurons. It is possible that these parvalbumin-positive populations correspond to the two, antiphasically oscillating cell-populations described by Borhegyi et al. (2004).

These experimental findings led us to develop a septal network model in which two cell populations (A and B) are distinguished by their preferential firing phase relative to the hippocampal theta rhythm. Neurons of both populations are described by the same equation (Eq. (1)) but cells of population A send axon collaterals to several cells of population B but only to a few other cells in population A, and vice versa (Fig. 3E). To create this network connection probabilities \(p_{AB}-p_{BA}\) and \(p_{BA}\) are defined to give the probability of connecting a given cell of population A to an other cell of A, etc. The strength of individual synapses \(g_{syn}^{a}(A,B)\) i.e. the strength of a single synapse between any two neurons was \(g_{syn}^{a} = 0.0125 - 1 \text{ mS/cm}^2\), while the total synaptic strength \(g_{syn} = \sum_{A,B}(A,B)\) of a cell using networks of 40 or 80 cells typically varied between \(2 \text{mS/cm}^2\). Respectively. To create septal networks of the type described above \(p_{AB} > p_{BA}\) were used, i.e. the connection probability within a subpopulation is less than between cells from different subpopulations.

The effect of the hippocampus was taken into account via the hippocampo-septal cells. In the model these cells do not form connections between each other but each hippocampo-septal cell innervates each septal GABAergic...
cell of population A by the synapses described above. The strength of these synapses ($g_{ij}$) varied in the 0.0−0.6 mS/cm$^2$ interval.

2.2.3. Modeling drug effects

Zolpidem and the L-838417 compound (Atack, 2005) are known to bind to the benzodiazepine binding site of the GABAA receptors. Modeling the effects of these drugs was based on the following observations. First, benzodiazepines, such as zolpidem are known to increase the affinity of the GABA-A receptor to its intrinsic agonist GABA (Mohler et al., 2002), increasing the frequency of opening of the associated chloride ion channel in the presence of the GABA. This causes an increase in the decay time constant and also the amplitude of the IPSCs when the receptor occupancy is incomplete. In the case of hippocampal interneurons (and pyramidal cells) 10$^{-5}$ M zolpidem increased the conductance of IPSCs to 140% (135%) and the decay time constant to 184% (173%) of the control value (Hájos et al., 2000), i.e. more than a two fold increase in the net synaptic current. Second, zolpidem is a preferential $\alpha_1$ GABA-A receptor positive allosteric modulator, while the L-838417 is a preferential $\alpha_2/\alpha_3/\alpha_5$ subunit selective positive allosteric modulator of the GABAA receptors. Third, $\alpha_1$ GABAA receptors are expressed by septal GABAergic neurons (Gao et al., 1995), but they present at the perisomatic region of hippocampal pyramidal cells (Nusser et al., 1996).

Thus, in our mathematical model application of zolpidem was hypothesized to increase the maximal synaptic conductance of every GABAA synapses ($g_{syn}$ in Eq. (3)) to account for the direct effect of zolpidem and decreased the mean of the tonic background depolarizing current of all cells ($I_{app}$ in Eqs. (1) and (2)) to describe decreased excitatory innervation. Typically, in the control situation $g_{syn}$ was in the 0.12−0.48 mS/cm$^2$ and $I_{app}$ was in the 35−45 pA interval; when modeling the effect of zolpidem $g_{syn}$ was increased to 0.6−1 mS/cm$^2$ and $I_{app}$ was decreased to 15−25 pA. In the simulations we altered either the time constant or the amplitude of the IPSPs. Both of these modifications

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**Fig. 1.** Effects of systemic administration of GABAA receptor PAMs on activity of the septo-hippocampal system in anesthetized rats. Upper panel: typical recording showing the effects of zolpidem (1 mg/kg, IV, A) and L-838417 (1 mg/kg, IV, B) on hippocampal EEG and MS/DB single neuron activities. Scale of y-axis indicates microV. Lower panels: bar diagrams showing percent changes in hippocampal EEG theta activity, power of theta oscillation (periodicity) and firing rate of MS/DB neurons after administration of zolpidem (Z, 1 mg/kg, IV, $n=6$) and L-838417 (L, 1 mg/kg, IV, $n=9$), and following subsequent administration of the benzodiazepine binding site antagonist flumazenil (R, Ro-15 1788, 1 mg/kg, IV). Error bars show the standard error of mean; asterisks indicate significant changes (ANOVA and paired t-test).

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**Fig. 2.** Autocorrelation functions of septal unit activities and power spectra of hippocampal EEG. Both zolpidem (dotted line) and L-838,417 (solid, light gray line) abolish temporal correlation of septal unit activity (A) and reduce the theta power of hippocampal EEG (B).
resulted in an approximately two fold increase of the net synaptic current, and the simulations gave very similar results (data not shown).

Contrary to this, the L-838417 exerts its direct effect in the septo-hippocampal system solely on hippocampal pyramidal cells. \( g_{\text{syn}} \) and \( I_{\text{app}} \) were not changed at any modeled neuron types. To model the effects of L-838417 the phasic component of the input of hippocampo-septally projecting cells was varied. In the control situation this current was a 4–5 Hz frequency, 36 pA amplitude sine wave or the signal recorded from the CA1 field electrode during the control situation scaled to 36 pA amplitude. In the case when administration of the L-838417 was modeled the \( I_{\text{calc}} \) was the signal of the CA1 field electrode recorded during IV administration of the L-838417 scaled to similar amplitude.

2.3. Data analysis

2.3.1. Population activity

In numerical simulations of the mathematical model networks of 40–80 septal GABAergic and 20–40 hippocampo-septal cells were used. Half of the septal cells made up subpopulation \( A \), the other half subpopulation \( B \). The activity of the subpopulations was approximated by the sum of its cells instantaneous firing rate. Instantaneous firing rates were calculated by convolving the series of firings by a Gaussian of 1 ms standard deviation.

2.3.2. Periodicity of septal cell firing

We define a quantitative measure to characterize periodicity of spike trains. First, the autocorrelation function is calculated from a series of firings. Second, maximal (\( Q_{\text{max}} \)) and minimal (\( Q_{\text{min}} \)) values of the autocorrelation function are identified in the 50–300 ms interval. Finally, periodicity is defined as the difference of these two values, \( P = Q_{\text{max}} - Q_{\text{min}} \).

3. Results

3.1. Effects of GABA\(_A\) receptor positive allosteric modulators on the septo-hippocampal oscillatory activity in vivo

Systemic administration of zolpidem (0.1–1 mg/kg, IV, \( n = 6 \)), a preferential PAM of \( z1 \) GABA\(_A\) receptors, instantaneously attenuated or abolished theta oscillation of the septo-hippocampal system. Since lower zolpidem doses (0.1–0.3 mg/kg, IV) elicited only a transient inhibition of theta activity, quantitative analysis on hippocampal EEG and septal neuronal activity were performed after administration of the 1 mg/kg (IV) dose. Thus, the power of oscillation (or periodicity) of MS/DB neurons was significantly reduced by zolpidem (32% of baseline, \( p < 0.01 \)), as revealed by fast Fourier transformation analysis of their autocorrelation (Fig. 2). In addition, zolpidem significantly inhibited the firing activity of...
Section 2 is capable of robust theta periodic population activity of both MS/DB neurons and hippocampal EEG (Fig. 1A). Induced inhibition of theta activity of both MS/DB neurons was mediated by the preferential PAM of α2/3/5 GABA_A receptors, reversed, at least partially, zolpidem-induced inhibition of theta activity of both MS/DB neurons and hippocampal EEG (Fig. 1A).

Administration of L-838417 (0.1–1 mg/kg, IV, n = 9), the preferential PAM of α2/3/5 GABA_A receptors, significantly inhibited theta oscillation of the septo-hippocampal system; quantitative analyses on hippocampal EEG and septal neuronal activity were performed after administration of the 1 mg/kg (IV) dose. L-838417 significantly reduced the power of theta oscillation in the hippocampus (p < 0.05) to a similar level to what was observed after zolpidem (1 mg/kg, IV) administration (Figs. 1B and 2). In parallel, oscillation of MS/DB neurons was also significantly abolished (p < 0.05). However, the firing activity of MS/DB neurons was not significantly altered by L-838417, in contrast to zolpidem. In fact, the small decrease in firing rate activity could reflect the decrease of the firing rate of the “theta on” subpopulation of MS/DB neurons, which shows a reduction in firing rate when theta activity of septo-hippocampal system ceases (Ford et al., 1989). Inhibition of theta oscillation of the septo-hippocampal system was reversed, at least in part, by the subsequent administration of flumazenil (Ro 15-1788, 1 mg/kg, IV). Flumazenil, given to control rats, did not change hippocampal EEG, or activity and oscillation of MS/DB neurons (n = 3, data not shown).

3.2. Computational modeling of rhythm generation in the septo-hippocampal system

The computational neural network model outlined in Section 2 was used to explore the effects exerted by GABA_A allosteric modulators on the septo-hippocampal system. Periodicity of firing, firing rate and firing pattern of single septal neurons characterized by autocorrelation functions, together with septal population activity and autocorrelation of the population activity, provided reference points in our simulations to establish the range of computational parameters that resulted in patterns and activities corresponding to in vivo observations. First, an extensive parameter-space search was conducted by varying the connection probabilities \( p_{ij} \), \( g_{ij} \), \( I_{app} \) and building several randomly connected networks to determine the properties of the septal part of the model alone. Our numerical results show that the septal model consisting of subpopulations A and B as described in Section 2 is capable of robust theta periodic population activity generation (Fig. 3A) in a wide parameter regime. Based on the characteristic frequency and periodicity of single cell firing, on the population activity (Fourier spectrum of the population activity), anti-correlation of the population activity of subpopulation A and B (determined by cross-correlation functions) a parameter-space region was identified where stable theta rhythm generation was achieved. According to our numerical simulations this region is given by the following parameter intervals: \( g_{ij} \in [0.075, 0.3] \) nS; \( I_{app} \in [20, 50] \) pA.

Investigations also show that synchronization properties within the septal part of the computer model are mostly determined by the total innervation of the septal cell, which was found to be in the \( g_{ij} \in [0.12, 0.48] \) mS/cm² interval for robust theta rhythm generation. Note that in accordance with recent experimental findings (Botteghi et al., 2004) the preferred firing phase distribution of septal cells in our numerical simulations is bimodal as shown in Fig. 3A.

Second, further simulations show that the basic properties of septal temporal pattern generation remain unchanged when the innervation of the septal two-subpopulation system by the hippocampo-septally projecting interneurons is taken into account (Fig. 3B).

The parameter regime identified above where stable, anti-phase theta periodic rhythmic activity is observed in the septo-hippocampal model is considered to be the equivalent of the control situation of electrophysiological experiments. As we describe in detail below, an increase in the maximal synaptic conductance of every GABA_A synapse and a decrease in the background current of every modeled cell, simulating the effect of the positive allosteric modulator zolpidem, inhibited theta activity. Inhibition of theta activity was reflected by a decrease of periodicity, the disappearance of the characteristic theta peak in the Fourier spectrum of the septal population activity and a decrease in the septal single unit firing rate, resembling our in vivo pharmacological observations. Furthermore, a similar correlation was found between pharmacological and computational findings when the L-838417 compound was modeled by changing the phasic excitation of hippocampo-septally projecting cells from periodic to aperiodic. In this case only the disappearance of the theta peak and a decrease of periodicity was observed without a significant drop in the firing rate.

3.3. Computational modeling of the effects of the positive allosteric modulator zolpidem

In the control situation of our computational model describing the electrical activity of the rat septo-hippocampal system during chloral hydrate anesthesia, a 4–6 Hz frequency, large amplitude oscillation was the dominant hippocampal pattern, while septal GABAergic cells fired in clusters of 3–9 above Hz spikes per cluster phase locked to the hippocampal field theta activity. The calculated frequency of the large amplitude population activity of the septal cells was also in the 4–6 above Hz frequency range in the control situation (Fig. 3A, B). Cells of the septal subpopulations A and B fired in antiphase, subpopulation A (the one being innervated by hippocampo-septal cells) at the trough, B at the peak of the hippocampal theta. Autocorrelation functions of single cell events show long-range temporal correlations (Fig. 4, solid line). Modeling of the effects of the GABA_A α1 subunit selective positive allosteric modulator zolpidem required taking two phenomena into consideration. First, the direct effect of zolpidem is an
increase of the maximal synaptic conductance, \( g^{ij}_{\text{syn}} \), of all GABAergic synapses from \( g^{ij}_{\text{syn}} = 0.125 \text{ nS} \) to \( g^{ij}_{\text{syn}} = 0.25 \text{ nS} \) resulting only in a decrease of the frequency of spike clusters of septal cells. Modeling of the increase of synaptic transmission at GABA\(_A\) synapses among the septal GABAergic pacemaker cells, however, was not enough to describe the complete action of zolpidem. Indeed, to explain the decrease of the firing rate together with the desynchronization of septal GABAergic cells a decrease of excitation has to be taken into account as an indirect effect of zolpidem. Thus, as a second step, the background depolarizing current, \( I_{\text{app}} \), both at the septal GABAergic neurons and at the hippocampo-septally projecting horizontal interneurons were decreased from \( I_{\text{app}} = 44 \text{ pA} \) to \( I_{\text{app}} = 22 \text{ pA} \) and from \( I_{\text{app}} = 3 \text{ pA} \) to \( I_{\text{app}} = -8 \text{ pA} \), respectively. A decrease of \( I_{\text{app}} \) at the hippocampal horizontal cells means that cells of this cell type also decrease their firing rate. Simultaneous modification of both \( g^{ij}_{\text{syn}} \) and \( I_{\text{app}} \) resulted in a septal firing pattern comparable to our electrophysiological results (Figs. 3C and 4, dotted line).

3.4. Computational modeling of the effects of the positive allosteric modulator L-838417

The L-838417 compound was identified as a GABA\(_A\) \( \alpha 2 \) subunit selective positive allosteric modulator and given that no \( \alpha 2 \) immunoreactivity was found in the medial septum, we hypothesize that the effect of the L-838417 on the medial septal GABAergic cells emerges from the hippocampus via the hippocampo-septally projecting interneurons. Thus, in our computer simulations of the administration of L-838417 the septal part of the network model was not altered at all. Instead, the phasic input of the hippocampo-septal cells, \( I_{\text{field}} \), which is a periodic signal in control situations representing recurrent innervation of horizontal interneurons by local pyramidal cells is modified. In cases when application of L-838417 is simulated, \( I_{\text{field}} \) takes the form of an aperiodic signal, which makes the firing of horizontal interneurons aperiodic. Depending on the synaptic strength between horizontal neurons and septal cells, \( g_{\text{HS}} \), aperiodic hippocampal innervation can make the septal activity aperiodic as reflected by the population activity, the firing pattern of the single cells (Fig. 3D), and the autocorrelation function of septal cell firing (Fig. 4, gray line). Specifically, we found that when the ratio of \( g^{ij}_{\text{syn}} \) and \( I_{\text{app}} \) is greater than or equal to approximately 0.4, septal cell firing becomes aperiodic. Comparing the results of the simulations with the experimental findings, we see that the effects of the two drugs in the model and on the experiments are similar: while zolpidem significantly decreases hippocampal theta power, medial septal theta power (Fig. 4) and septal firing periodicity together with the firing rate of septal GABAergic neurons, the L-838417 compound expresses its effect only on the hippocampal and septal theta power (Fig. 4) without significantly modifying the firing rate of septal cells (compare Figs. 1 and 3F). Our modeling results suggest that besides modulating the GABA\(_A\) synapses among the septal GABAergic cells, zolpidem also had an effect on GABA\(_A\) synapses among other cells. This indirect effect also had a critical influence on the septal theta generation. Furthermore, we showed that the effect of the L-838417 compound on the septal theta generation can be explained through the hippocampo-septal projection alone only if the application of the drug turns the firing profile of the hippocampo-septal cells from periodic to aperiodic.

4. Discussion

The present findings demonstrate that preferential modulation of \( \alpha 1 \) subunit-containing GABA\(_A\) receptors by zolpidem as well as preferential modulation of \( \alpha 2, \alpha 3 \) and \( \alpha 5 \) subunit-containing GABA\(_A\) receptors by L-838417 inhibit theta oscillation of the septo-hippocampal circuitry in anesthetized rats with a similar potency. Although both zolpidem and L-838417 abolished theta-related oscillatory activity of MS/DB neurons, only zolpidem, but not L-838417 inhibited their firing rate. Our computational model, utilizing a conductance-based description of neurons and an interconnected septal inhibitory neural network, revealed similar responses when drug actions were represented by modification of maximal synaptic conductances of respective GABA\(_A\) synapses, supporting our pharmacological observation on differential modulation of MS/DB neurons by subunit selective GABA\(_A\) modulators.

Recent studies on genetic deletion of a discrete subunit of GABA\(_A\) receptors have not only revealed their potential physiological role in CNS functions, but also provided novel...
opportunities to analyze the involvement of different GABA_A receptors in the diverse pharmacological effects of GABA_A receptor allosteric modulators. Moreover, in order to overcome compensatory developmental changes in knockout mice, GABA_A subtype selective receptor point-mutated knockin mice have also been generated (Rudolph and Mohler, 2004). Thus, studies on genetically modified mice with the α1 subunit-containing GABA_A receptors knocked out, or in α1(H101R) knockin mice, have revealed that the sedative effects of benzodiazepines are mediated via α1 GABA_A receptors (Atack et al., 2005). Interestingly, the diazepam-induced anterograde amnesia was also absent in these α1 receptor mutant mice (Rudolph et al., 2000). Based on observations in these genetically altered mice, as well as the anatomical distribution and function of GABA_A receptors, it has been proposed that anxiolytic action of benzodiazepines is predominantly mediated by α2 and/or α3 subunit-containing GABA_A receptors (Atack et al., 2005). Thus, a considerable effort has been made to synthesize α2 subunit selective GABA_A receptor PAMs (Atack, 2005), highlighted by the discovery of L-838417, a preferential α2, α3 and α5 GABA_A receptor PAM (McKernan et al., 2000). L-838417, which shows selective efficacy for these receptors has anxiolytic activity in various preclinical animal models without inducing sedation (Atack, 2005).

It has been shown that anxiolytic GABA_A receptor PAMs inhibit spontaneous theta oscillation, shift frequency of theta oscillation to lower frequencies, reduce theta frequency and responsiveness to brainstem or medial septum stimulation (McNaughton and Gray, 2000; Kopp et al., 2004; Hajós et al., 2004). In line with these observations, our current findings show that the septo-hippocampal theta oscillation is effectively disrupted by either zolpidem or L-838417 in chloral hydrate anesthetized rats, similar to the non-selective GABA_A PAM diazepam (Hajós et al., 2004). In fact, the two compounds differ only in their effects on the firing rate of MS/DB neurons. While L-838417 did not inhibit the firing rate of MS/DB neurons, zolpidem reduced the firing rate of these neurons by approximately 90%. These findings most likely reflect differences in distribution of α1 and α2/3/5 GABA_A receptors. Thus, it has been shown that α1 GABA_A receptors are expressed by both hippocampal and MS/DB GABAergic neurons (Gao and Fritschy, 1994; Gao et al., 1995); consequently their activation would lead to reduction in firing rate activity, as observed after application of zolpidem. However, MS/DB GABAergic neurons do not express α2 GABA_A receptors (Gao et al., 1995), and in our recordings these presumed GABAergic neurons were not significantly inhibited by L-838417. Furthermore, L-838417- or zolpidem-induced changes in septo-hippocampal activity were sensitive to the GABA_A receptor benzodiazepine binding site antagonist flumazenil, indicating selective involvement of GABA_A receptors in the observed pharmacological effects.

The present computational modeling provided further theoretical support to our hypothesis on the mode of action of GABA_A receptor positive allosteric modulators. Our computational model of the septo-hippocampal system, based on a detailed description of the septal inhibitory network and an abstract model of the hippocampal CA1 area, showed similar responses to those measured in our electrophysiological experiments. The computational model incorporated a novel process for generating a synchronized theta rhythm in the septum, as well as relevant and appropriate neuronal attributes for modeling the effects of GABA_A receptor PAMs on the septo-hippocampal activity. Since in vivo recordings of identified septal GABAergic neurons demonstrate cluster-firing behavior (Brazhnik and Fox, 1997; Borhegyi et al., 2004), these neurons were represented in our mathematical model as cluster-firing type units (Wang, 2002). The modeled septal GABAergic neurons were interconnected into two subpopulations in order to generate emergent population activities similar to those observed in a recent in vivo electrophysiological experiment (Borhegyi et al., 2004). Typically, this model system generated robust synchronized activity where clusters of spikes emitted by septal units were synchronized after a very short initial transient. Units of one of the two subpopulations fired at the peak, others at the trough of the simulated hippocampal field theta. In the present computational approach hippocampal rhythm generation was not explicitly modeled; detailed modeling of the hippocampal theta was considered in an earlier work (Hajós et al., 2004). However, particular emphasis was put on the hippocampo-septal projection, which derives from GABAergic interneurons of the hippocampus CA1 and CA3 regions, and terminates predominantly on GABAergic septal neurons (Toth et al., 1993). In our mathematical model this hippocampo-septal projecting neuronal population was taken into account in order to model the interactions between the hippocampus and septum. This mathematical scheme enabled us to numerically study how different modes (periodic and aperiodic) of the hippocampal innervation modify the autonomous septal pacemaker function, serving as a primary tool to understand subunit specific drug action.

Utilizing our computational model, mathematical representation of the effects of zolpidem on septal neuronal activity was described by direct and indirect effects. Although strengthening of all GABA_A synapses in the model via increasing maximal synaptic conductance by a pharmacologically relevant extent (direct effect) impacted the oscillatory behavior of septal units, it failed to reproduce the experimentally observed decrease in their firing rate. This fact warranted decreasing the tonic depolarizing input impinging septal units (indirect effect), to simulate our in vivo electrophysiological observations. These findings might indicate that zolpidem alters the activity of various neuron populations projecting to the MS/DB, which could contribute to the dramatic decrease in their firing rate. On the other hand, this could reflect the fact that benzodiazepines increase the conductance of extrasympathetic GABA_A channels (Eghball et al., 1997) activated by low concentration of extracellular GABA. In contrast, the effects of the L-838417 were modeled only by changing the activity of the hippocampo-septal projecting inhibitory neurons from periodic to aperiodic fashion, but parameters of septal units (e.g. representing direct modulation of GABA_A receptors) and the intra-septal circuitry were unchanged. It was presumed that this
modification alone could only account for the observed change in the septal behavior if (1) during hippocampal theta the firing pattern of the hippocampo-septal cells is theta periodic; (2) the \(\alpha_2\) agonist L-838417 changes the firing pattern of these cells from periodic to aperiodic; (3) the hippocampo-septal coupling is strong enough to modulate the firing activity of MS/DB neurons. Our experimental findings demonstrated that L-838417 disrupted theta activity in the hippocampus with similar efficacy to zolpidem. This is not unexpected, however, given the fact that \(\alpha_2\) GABA\(_A\) receptors are expressed at the initial segments of pyramidal neurons, a functionally excellent position to regulate generation and propagation of action potentials (Howard et al., 2005). The hippocampo-septal neurons receive local axon collaterals from pyramidal neurons (Blasco-Ibanez and Freund, 1995), enabling them to effectively transfer the pyramidal cell synchrony to the septum (Freund and Buzsáki, 1996). On the other hand, Dragoi et al. (1999) found that hippocampal GABAergic inhibition of MSDB neurons could be strong enough to suppress MSDB neurons firing, e.g. during hippocampal sharp waves or theta oscillation. Thus, the combination of our experimental and computational findings indicate that changes in hippocampal oscillatory activity can shift oscillatory activity of MS/DB neurons to the same direction without impacting significantly on their firing rate (Nerad and McNaughton, 2006).

The present results demonstrating inhibition of theta oscillation by both zolpidem and L-838417 in a similar fashion to the non-selective GABA\(_A\) PAM diazepam (Hajós et al., 2004) provide further support for the hypothesis that anxiolytic drugs effectively interact with the oscillatory activity of the septo-hippocampal system (McNaughton and Gray, 2000). In a recent study, recordings of hippocampal EEG from non-anesthetized rats showed similar significant reductions in theta activity after administration of diazepam and zolpidem (van Lier et al., 2004). Although studies using genetically altered mice suggest that \(\alpha_2\) and/or \(\alpha_3\) GABA\(_A\) receptors mediate anxiolysis of benzodiazepines, anxiolytic effects of zolpidem have also been established in certain anxiety models (Nazar et al., 1997; Rowlett et al., 2001). Interestingly, a recent publication disclosed an azaisostere analogue of zolpidem, showing exceptional selectivity towards \(\alpha_1\) GABA\(_A\) receptors, with very potent anxiolytic action (Selleri et al., 2005). In summary, our electrophysiological findings, together with the theoretical support of computational modeling, suggest that responses of the septo-hippocampal system can discriminate between \(\alpha_1\) and \(\alpha_2/3/5\) preferential GABA\(_A\) PAMs. Our current results further support a connection between septo-hippocampal theta oscillation and anxiety. However, additional studies are warranted to reveal the functional relevance of the dissimilar firing rate activities of MS/DB neurons in response to GABA\(_A\) receptor PAMs having distinct \(\alpha\) subunit preferential activity.

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**Appendix. Supplementary data**


**Appendix A**

**A.1. Septal GABAergic cell model**

The version of the Wang (2002) model neuron used in the simulations consists of a single compartment where the membrane potential is described by the following current balance equation (the membrane noise term, originally introduced by Wang was omitted)

\[
C_m \frac{dV}{dt} = -I_Na - I_K - I_{KS} - I_L - I_{syn} + I_{app} \quad (A1)
\]

The membrane currents are given by

\[
I_{Na} = g_{Na}m^3h(V - E_{Na}) \quad (A2a)
\]
\[
I_K = g_Kn^4(V - E_K) \quad (A2b)
\]
\[
I_{KS} = g_{KS}p_q(V - E_K) \quad (A2c)
\]
\[
I_L = g_L(V - E_L) \quad (A2d)
\]

A gating variable \(x (x \in \{h, n, p, q\})\) satisfied first order kinetics

\[
\frac{dx}{dt} = \varphi_x[\alpha_x(V)(1 - x) - \beta_x(V)x] \equiv \left(\frac{x_{\infty}(V) - x}{r_x(V)}\right) \quad (A3)
\]

The rate constants are:

\[
\alpha_m = \frac{-0.1(V + 33)}{\exp[-0.1(V + 33)] - 1} \quad (A4a)
\]
\[
\beta_m = 4 \exp\left[\frac{V + 58}{18}\right] \quad (A4b)
\]
\[
\alpha_h = 0.07 \exp\left[-\frac{V + 51}{10}\right] \quad (A4c)
\]
\[
\beta_h = \frac{1}{\exp[-0.1(V + 21)] + 1} \quad (A4d)
\]
\[
\alpha_n = \frac{-0.01(V + 38)}{\exp[-0.1(V + 38)] - 1} \quad (A4e)
\]
\[
\beta_n = 0.125 \exp\left[-\frac{V + 48}{80}\right] \quad (A4f)
\]
\[
p_a = \frac{1}{\exp\left[-\frac{V + 34}{6.5}\right] + 1} \quad (A4g)
\]
\[ \tau_p = 6 \text{ ms} \]
\[ q_s = \frac{1}{\exp\left[\frac{V + 65}{6.6}\right] + 1} \]
\[ \tau_q = 100 \left\{ \frac{1}{1 + \exp\left[\frac{V + 50}{6.8}\right]} \right\} \]

The kinetic variable \( m \) was approximated by its asymptotic value \( m = (V(t)) = \alpha_m/(\alpha_m + \beta_m) \) instantaneously.

Parameter values were set according to Wang (2002): \( C_m = 1 \mu \text{F/cm}^2, g_L = 0.1 \text{ mS/cm}^2, g_{Na} = 50 \text{ mS/cm}^2, g_K = 8 \text{ mS/cm}^2, g_{Kss} = 12 \text{ mS/cm}^2, E_L = -50 \text{ mV}, E_{Na} = 55 \text{ mV, } E_K = -85 \text{ mV}; \) the temperature factors \( \varphi_h = \varphi_n = 5. \)

### A.2. Hippocampo-septal cell model

The horizontal cell model used to convey the hippocampal effect to the septal network is a single compartmental model. Evolution of the membrane potential is governed by the following current balance equation:

\[ C_m \frac{dV}{dt} = -I_{Na} - I_K - I_H - I_{Ca} - I_{KCa} - I_L - I_{syn} + I_{app} + I_{field} \]  

The membrane currents in this model are given by

\[ I_{Na} = g_{Na} m_h^3 h (V - E_{Na}) \]
\[ I_K = g_K n^4 (V - E_K) \]
\[ I_H = g_H (V - E_H) \]
\[ I_{Ca} = g_{Ca} n^2 (V - E_{Ca}) \]
\[ I_{KCa} = g_{KCa} [Ca^{2+}] / ([Ca^{2+}] + K_D) (V - E_K) \]
\[ I_L = g_L (V - E_L) \]

The intracellular calcium concentration is described by

\[ \frac{d[Ca^{2+}]}{dt} = -\alpha m_{Ca^{2+}} - \frac{[Ca^{2+}]}{\tau_{Ca^{2+}}} \]

The rate constants are:

\[ \alpha_m = \frac{-0.1(V + 35)}{\exp[-0.1(V + 35)] - 1} \]
\[ \beta_m = 4 \exp\left[\frac{-V + 60}{18}\right] \]

\[ \alpha_h = 0.07 \exp\left[\frac{V + 58}{20}\right] \]
\[ \beta_h = \frac{1}{\exp[-0.1(V + 28)] + 1} \]
\[ \alpha_n = \frac{-0.01(V + 34)}{\exp[-0.1(V + 34)] - 1} \]
\[ \beta_n = 0.125 \exp\left[\frac{-V + 44}{80}\right] \]
\[ H_m(V) = \frac{1}{\exp\left[\frac{V + 80}{10}\right] + 1} \]
\[ \tau_m(V) = \frac{200}{\exp\left[\frac{V + 70}{20}\right] + \exp\left[\frac{V + 70}{20}\right] + 5} \]

The kinetic variable \( c \) was substituted by its steady state value

\[ c_a(V) = \frac{1}{\exp\left[\frac{V + 20}{9}\right] + 1} \]

The parameter values of this model according to Wang (2002) are: \( \varphi_h = \varphi_n = 5 \) for the temperature factors, \( K_D = 30 \mu \text{M}, \alpha = 0.002, \tau_{Ca} = 80 \text{ ms}, \) and for the maximal conductances \( g_L = 0.1 \text{ mS/cm}^2, g_{Na} = 55 \text{ mS/cm}^2, g_K = 9 \text{ mS/cm}^2, g_{H} = 0.15 \text{ mS/cm}^2, g_{Ca} = 1 \text{ mS/cm}^2 \) and \( g_{KCa} = 10 \text{ mS/cm}^2). \) Reversal potentials were set to \( E_L = -65 \text{ mV, } E_{Na} = +55 \text{ mV, } E_K = -90 \text{ mV and } E_{Ca} = +120 \text{ mV.} \) The membrane capacitance was \( C_m = 1 \mu \text{F/cm}^2. \)

### References


