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Short communication

# Effects of nicotine, pilocarpine, and tetrahydroaminoacridine on hippocampal theta waves in freely moving rabbits

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**Abstract**

The effects of three cholinergic agents on hippocampal theta waves were investigated by analyzing electroencephalographic power spectra in freely moving rabbits. In the hippocampal spectra, nicotine (a nicotinic receptor agonist, 0.03 mg/kg) increased the theta wave frequency, but caused no change in its power. Pilocarpine (a muscarinic receptor agonist, 0.3 and 1.0 mg/kg) and tetrahydroaminoacridine (a cholinesterase inhibitor, 3.0 mg/kg) increased the power and deceased the frequency. These results suggest that the activating effect of nicotinic receptor agonists on the hippocampus may be different from that of muscarinic receptor agonists or cholinesterase inhibitors. 

*Keywords:* Nicotine; Pilocarpine; Tetrahydroaminoacridine; Hippocampal theta wave; EEG power spectrum; Rabbit, freely moving

## 1. Introduction

With a view to activating cholinergic neurons in the cerebral cortex and hippocampus in Alzheimer's disease patients, esterase inhibitors, muscarinic receptor agonists, and nicotinic receptor agonists have been developed as cholinergic agents (Fisher et al., 1994; Giacobini, 1997; Newhouse et al., 1997). In recent years, a new therapeutic strategy consisting of combining esterase inhibitors with muscarinic or nicotinic receptor agonists has been tested for its ability to enhance the efficacy of the former (Giacobini, 1997). Esterase inhibitors have been reported to cause cognitive enhancement and to have central inhibitory effects on behavior, such as drowsiness or decline (Honma and Miyamoto, 1994; Kumar, 1994). Combination therapy is expected to moderate the central inhibitory effects of esterase inhibitors on behavior. Nicotine has been reported to improve attention deficit and depression (Gilbert, 1996; Levin et al., 1996), but there is little evidence that muscarinic receptor agonists improve mental disorders. It seems to be necessary to elucidate the effects of cholinergic agents on both learning impairment and mental symptoms.

In the present study, the electroencephalographic (EEG) power spectra of hippocampal theta waves were analyzed in freely moving rabbits after administration of the three kinds of cholinergic agents, nicotine (a nicotinic receptor agonist), pilocarpine (a muscarinic receptor agonist), and tetrahydro-aminoacridine (an esterase inhibitor), and the central activating effects of these drugs were compared.

## 2. Materials and methods

### 2.1. Animals and surgical procedure

Eighteen male Japanese White rabbits weighing 2.6–3.5 kg were used. The animals were anesthetized with pentobarbital sodium (30 mg/kg i.v.) and bipolar stainless steel wire electrodes (0.25 mm in diameter, insulated except for 0.5 mm at the tip; polar distance 0.5–1.0 mm) were implanted in the hippocampus (A: − 4, L:4, H:5) according to the brain atlas of Sawyer et al. (1954). Two silver-plated stainless screw electrodes (1.0 mm in diameter) were placed subdurally 2–3 mm apart on the surface of the parietal cortex (A:2, L:2). Each electrode was fixed to a hole in the skull with dental cement and was soldered to a connector socket. The animals were allowed at least 1 week to recover before the experiments were started. Groups of three rabbits were used for each dose of test drug.

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### 2.2. EEG recording

Each animal was accustomed to being placed inside a transparent plastic box (26 × 42 × 34 cm, same size as the home cage) in a soundproof, shielded room for 4 h, 1 day before recording EEG. Bipolar cortical and hippocampal EEGs were recorded on a polygraph (NEC San'ei, Model 361) at a time constant of 0.1 s with a low-pass filter setting of 25 Hz. During recording, power spectra analysis of the EEG data was performed for 15 min with a signal processor (Model 1000, NEC San'ei), followed by fast Fourier transformation at frequencies from 0 Hz to 25 Hz. The percentage of the total power was calculated for theta (T1: 4.0–5.9, T2: 6.0–7.9 Hz) waves, and the spectra were printed as histograms at 0.1-Hz intervals. Prior to the start of the experiments, the animals were sufficiently acclimatized to the observation box because stable EEGs without novelty stress were obtained. With the spectra obtained

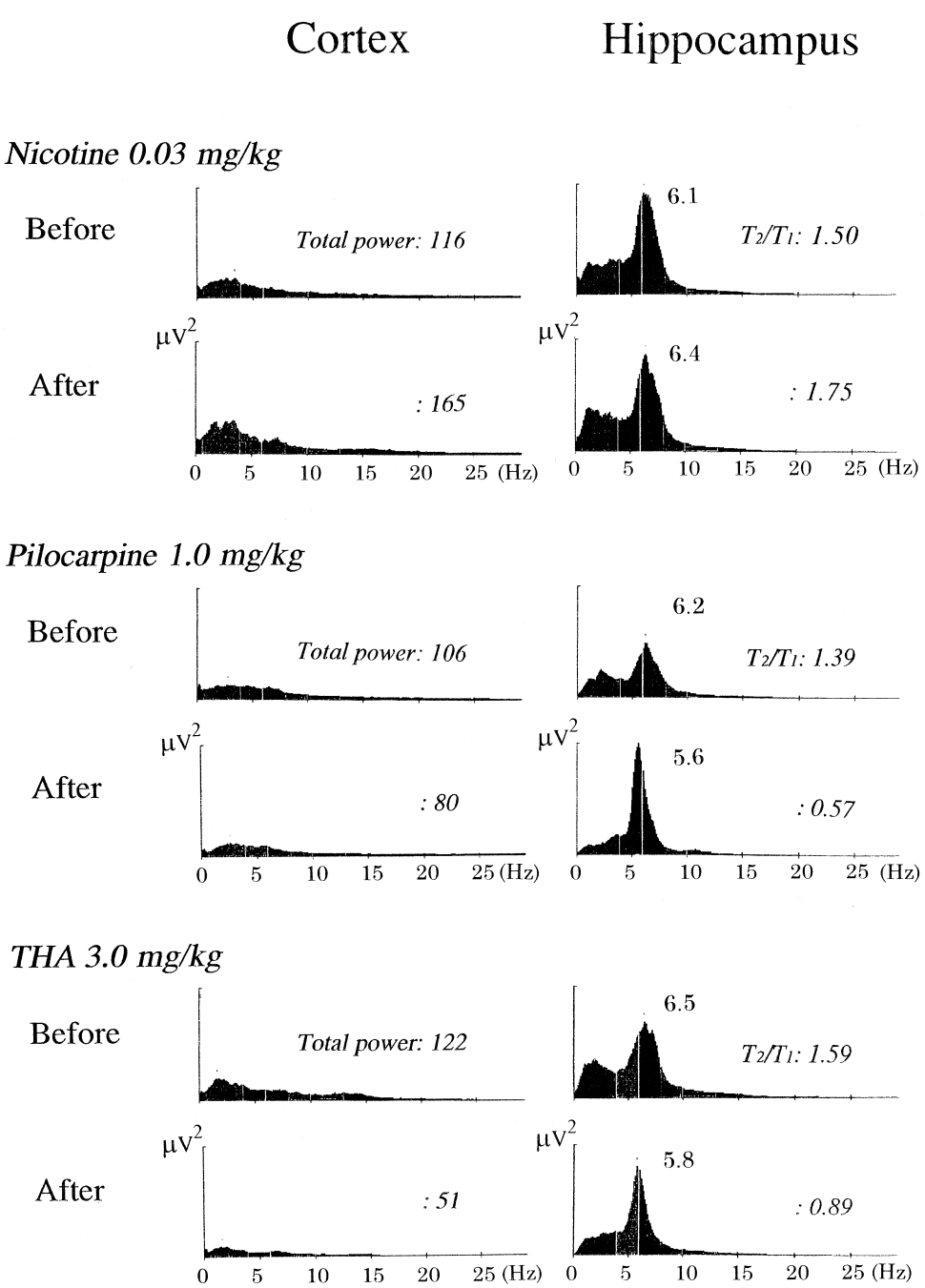


Fig. 1. Cortical and hippocampal EEG power spectra of rabbits after i.v. administration of nicotine, pilocarpine and tetrahydroaminoacridine (THA). Power spectra were analyzed by fast Fourier transformation for 15- to 30-min periods, and the spectra were printed as histograms at 0.1-Hz intervals. The percentage of the total power was calculated for theta waves (T1: 4.0–5.9 Hz, T2: 6.0–7.9 Hz), and small numbers in the hippocampal spectra represent the peak frequency of theta waves.

during wakefulness before drug administration as the baseline, the hippocampal T2/T1 ratio and theta wave power were compared.

### 2.3. Drugs

The drugs used in this study were nicotine (Wako), pilocarpine (Sigma), tetrahydroaminoacridine (Aldrich) and pentobarbital sodium (Nembutal, Abbott Pharm.). For intravenous administration, the drugs were dissolved in saline (0.9% NaCl, Otsuka Pharm.) and injected into an ear vein. First, twelve rabbits were used for testing the effects of saline and three doses of nicotine. After a washout period of 1 week or longer, these twelve rabbits and another six rabbits were used to test the three doses of pilocarpine and tetrahydroaminoacridine, at random.

### 2.4. Statistics

The significance of differences was assessed by using the *t*-test (Statistical Analysis System Institute).

## 3. Results

### 3.1. Cortical and hippocampal EEG power spectra

The cortical and hippocampal power spectra obtained from 15 to 30 min are shown in Fig. 1. When nicotine was given at a dose of 0.03 mg/kg, i.v., the power spectra of

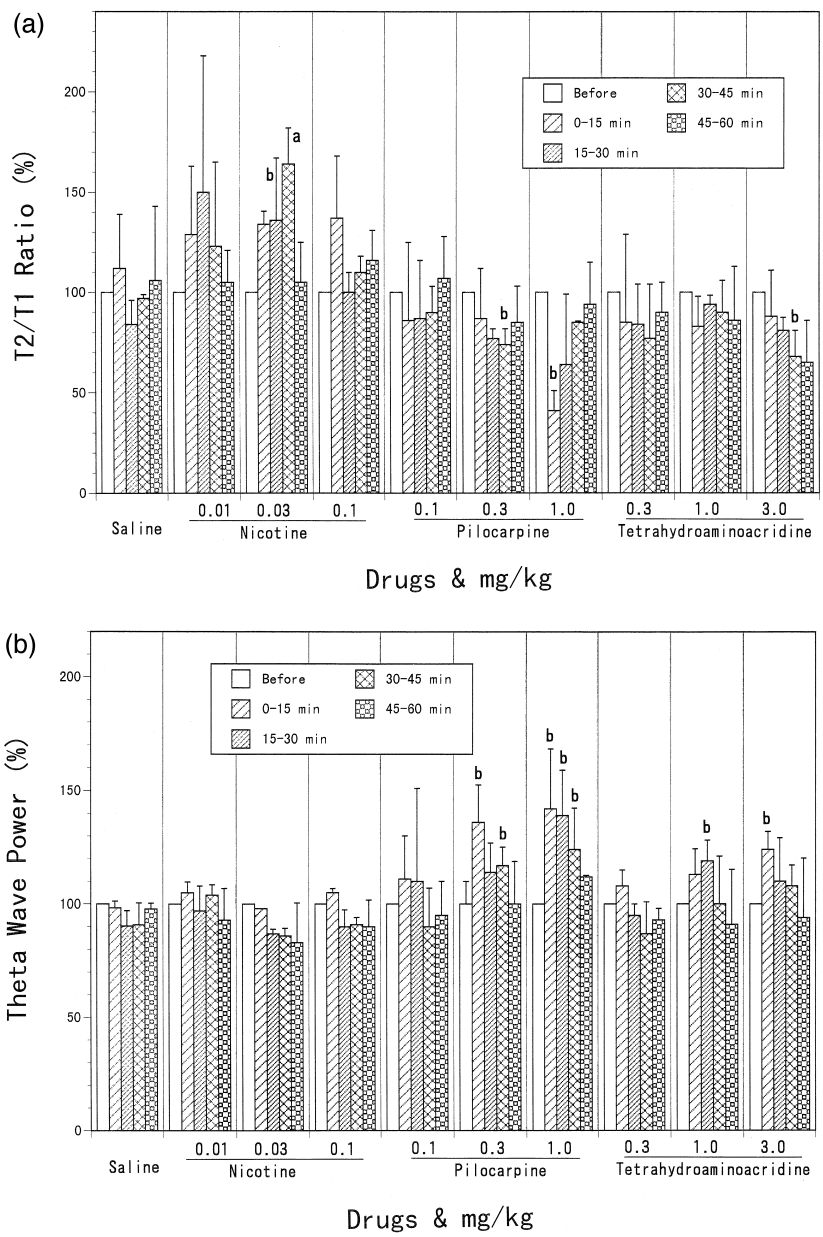


Fig. 2. Changes of T2/T1 ratio and theta wave power (T1 + T2) of hippocampal EEG in rabbits for four 15-min periods after i.v. administration of nicotine, pilocarpine and tetrahydroaminoacridine. The values of T2/T1 and theta power are expressed as a percentage of that before, and each value represents the mean ± S.D. for three rabbits. [a]$P < 0.01$, [b]$P < 0.05$ vs. saline control.

the cortical and hippocampal EEGs were similar to those during wakefulness before treatment, but the hippocampal theta wave peak was slightly shifted toward a higher frequency band and the T2/T1 ratio tended to increase. High-amplitude hippocampal theta waves and sustained cortical EEG desynchronization were produced by i.v. administration of pilocarpine (1.0 mg/kg) or tetrahydoaminoacridine (3.0 mg/kg). The hippocampal theta wave peak increased and became symmetrical with a decrease in the T2/T1 ratio. The total power of the cortical EEG spectrum was decreased compared to that for pretreatment wakefulness.

### 3.2. Hippocampal T2 / T1 ratio

Nicotine produced no significant change in the T2/T1 ratio at a dose of 0.01 mg/kg, but induced a sustained increase over 45 min at a dose of 0.03 mg/kg (the ratio increased by 34%, 36% ($P < 0.05$) and 64% ($P < 0.01$) at 0–15, 15–30 and 30–45 min, respectively). At a dose of 0.1 mg/kg, there was no dose-dependent increase in action, except for a 37% increase in the T2/T1 ratio at 0–15 min. Pilocarpine tended to decrease the T2/T1 ratio for 45 min after administration at a dose of 0.1 mg/kg, and produced a dose-related decrease of the T2/T1 ratio when given at doses of 0.3 mg/kg (15–30 min: 23%, 30–45 min: 26% ($P < 0.05$)) and 1.0 mg/kg (0–15 min: 59% ($P < 0.05$), 15–30 min: 36%). Tetrahydroaminoacridine tended to decrease the T2/T1 ratio after administration at doses of 0.3 and 1.0 mg/kg, and produced a significant decrease at a dose of 3.0 mg/kg (30–45 min: 32%) (Fig. 2).

### 3.3. Hippocampal theta wave power (T1 + T2)

The power of the hippocampal theta waves was 90–98% of the pre-treatment value within 60 min of saline administration, showing a slight decrease. Nicotine did not produce a significant change for 60 min after administration at doses of 0.01, 0.03 and 0.1 mg/kg (83–105% of the pretreatment value). Hippocampal theta power increased immediately after pilocarpine administration and showed a dose-related increase thereafter. At doses of 0.3 and 1.0 mg/kg, power was increased by 36% ($P < 0.05$) and 42% ($P < 0.05$) respectively at 0–15 min, by 14% and 39% ($P < 0.05$) at 15–30 min and by 17% ($P < 0.05$) and 24% ($P < 0.05$) at 30–45 min. There was also a dose-related increase in hippocampal theta power after tetrahydroaminoacridine administration. At doses of 1.0 and 3.0 mg/kg, power was increased by 13% and 24% ($P < 0.05$) respectively at 0–15 min and by 19% ($P < 0.05$) and 10% at 15–30 min (Fig. 2).

## 4. Discussion

Pharmaco-EEG analysis in unrestrained conscious animals is considered useful for evaluation of the effects of drugs on behavior or psychological function. In the present study, the hippocampal EEG was selected for analysis of the pharmacological action of three kinds of cholinergic agents, and the T2 (6.0–7.9 Hz)/T1 (4.0–5.9 Hz) ratio was calculated to analyze changes in theta wave frequency and power. The overall features of the hippocampal and cortical spectra were characterized to assess the portion that could not be expressed numerically.

### 4.1. Hippocampal and cortical EEG spectra

An arousal pattern (an increase of theta wave power in the hippocampal spectra and a decrease of total power in the cortical spectra) was produced after treatment with the three cholinergic agents. The hippocampal theta wave peak was shifted toward the higher frequency range after nicotine (0.03 mg/kg) administration, while it was high and symmetrical with pilocarpine (1.0 mg/kg) and tetrahydroaminoacridine (3.0 mg/kg). The power of cortical waves in all frequency bands was lower with pilocarpine and tetrahydroaminoacridine than with nicotine.

### 4.2. T2 / T1 ratio and theta power of hippocampal EEG

Administration of nicotine at a dose of 0.03 mg/kg (a moderate dose level) increased the T2/T1 ratio (i.e., increased the theta wave frequency), but produced no change in theta power. Pilocarpine produced a dose-related increase in theta power (at doses of 0.3 and 1.0 mg/kg), and decreased the T2/T1 ratio, unlike nicotine. These results suggest that the increase in theta wave frequency was mediated by nicotinic receptors, and that muscarinic receptors specifically play a role in the increase of theta power. Therefore, it seems that the mechanism of cholinergic activation of hippocampal theta waves differs between the nicotinic system and the muscarinic system. Arecoline, a muscarinic receptor agonist, has been reported to induce low-frequency rhythmic slow activity in freely moving rats (Krug et al., 1981). It is also suggested that muscarinic receptors are not associated with the increase in theta wave frequency. Like pilocarpine, tetrahydroaminoacridine produced a dose-related increase in theta power (at 1.0 and 3.0 mg/kg) and decreased the T2/T1 ratio at higher doses. The esterase inhibitor-induced increase in acetylcholine concentration in the synaptic cleft was comparable to the cholinergic activation induced by the muscarinic receptor agonist.

As to the relationship of the cholinergic system with the production of hippocampal theta waves, it has been reported that this is mediated by muscarinic receptors, but the involvement of nicotine receptors has not been reported. Ott et al. (1983) reported that oxotremorine (a muscarinic receptor agonist) caused a long-lasting increase of rhythmic slow activity (5.0–7.5 Hz) in freely moving rats. Similarly, Bland and Colom (1988) found that carbachol (a muscarinic receptor agonist) caused long trains of

slow-wave theta (type 2, 2–8 Hz) in urethane-anesthetized rats. With regard to the present study, the involvement of muscarinic receptors in the production of hippocampal theta waves (4.0–7.9 Hz) is compatible with the increase in power, while the increase in frequency could be involved with the production of theta waves by nicotine. The nicotine-induced increase in theta wave frequency was not so great (within 1 Hz), and so the results of evaluation may vary depending on the method of dividing the theta wave frequency range (T1: 4.0–5.9 Hz, T2: 6.0–7.9 Hz in this study).

In a previous study, it was found that the power of hippocampal theta waves increased according to the level of consciousness, and that the frequency of hippocampal theta waves increased or decreased after administration of psychostimulants or antipsychotics, respectively (Yamamoto, 1989, 1997, 1998). Nicotine increased the frequency of hippocampal theta waves, while the effect of nicotine on the T2/T1 ratio was moderate (maximum; 0.03 mg/kg, $1.76 \pm 0.64$, $n = 3$) compared to that of psychostimulants such as methamphetamine (1 mg/kg, $3.4 \pm 1.0$, $n = 3$) and apomorphine (1 mg/kg, $3.1 \pm 0.9$, $n = 3$). Pilocarpine and tetrahydoaminoacridine clearly decreased the total cortical power and increased the power of hippocampal theta waves; however, higher doses of these agents decreased the frequency of hippocampal waves (i.e., decreased the T2/T1 ratio). In conclusion, these results suggest that the activating effect of nicotinic receptor agonists on the hippocampus is different from that of muscarinic receptor agonists and cholinesterase inhibitors, and that these agents may produce emotional activation by different mechanism.

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