ELSEVIER

European Journal of Pharmacology 282 (1995) 39–55

ejp

Modulation of rat neocortical high-voltage spindle activity by 5-HT_1/5-HT_2 receptor subtype specific drugs

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Received 1 August 1994; revised 18 April 1995; accepted 2 May 1995

Abstract

To investigate the role of serotonin (5-hydroxytryptamine; 5-HT) receptors in the modulation of rat thalamocortical oscillations, we studied the effects of 5-HT_1/5-HT_2 receptor subtype specific drugs on neocortical high-voltage spindle activity in adult male rats. A 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (0.03, 0.1, 0.3 and 1.0 mg/kg s.c.), had no effect on neocortical high-voltage spindle activity. Furthermore, a mixed 5-HT_1/5-HT_2 receptor antagonist, methysergide (1.0, 5.0 and 15.0 mg/kg i.p.), had no effect, whereas a non-specific mixed 5-HT_1/5-HT_2 receptor antagonist, methiothepin (0.2, 1.0 and 5.0 mg/kg i.p.), significantly increased neocortical high-voltage spindles. Of the 5-HT_2 receptor antagonists, ritanserin (0.1, 1.0 and 5.0 mg/kg s.c.) had no effect, whereas ketanserin (1.0, 5.0 and 20.0 mg/kg s.c.) increased neocortical high-voltage spindles, but only at the highest dose used. A 5-HT_2 receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (0.5, 1.0 and 2.0 mg/kg s.c.), at the two highest doses significantly decreased neocortical high-voltage spindle activity, and this effect was blocked by the 5-HT_2 receptor antagonists, ketanserin (1.0, 5.0 and 20.0 mg/kg s.c.) and ritanserin (1.0 and 5.0 mg/kg s.c.), as well as by methiothepin (0.2, 1.0 and 5.0 mg/kg i.p.) and methysergide (1.0, 5.0 and 15.0 mg/kg i.p.). Furthermore, unilateral intrathalamic infusions, but not intrahippocampal control infusions, of DOI (10 and 50 μg/1.0 μl/rat) decreased neocortical high-voltage spindle activity and systemic administration of ketanserin (20.0 mg/kg s.c.) completely blocked this effect. The present results suggest that (1) the serotonergic system modulates rat thalamocortical oscillations as measured by neocortical high-voltage spindle activity, (2) activation of 5-HT_2 receptors, possibly located in the thalamus, with a specific 5-HT_2 receptor agonist, DOI, causes a reduction in rat neocortical high-voltage spindle activity.

Keywords: 5-HT_1 receptor; 5-HT_2 receptor; Neocortical high-voltage spindle, rat; Thalamocortical oscillation

1. Introduction

The neocortical electroencephalographic (EEG) activity of waking alert rats is typically composed of desynchronized low-voltage fast activity (Vanderwolf and Baker, 1986; Buzsáki et al., 1988; Riekkinen, Jr. et al., 1991a). During periods of relaxed waking-immobility, however, brief bursts of highly synchronized oscillatory high-voltage spindle activity may occur in the neocortical EEG in some of the rats (Micheletti et al., 1987; Buzsáki et al., 1988; Sirviö et al., 1989; Buzsáki et al., 1990a,b,c; Riekkinen, Jr. et al., 1991a). The appearance of neocortical high-voltage spindles seems to be genetically determined (Buzsáki et al., 1990b; Vergnes et al., 1990), and the number and duration of high-voltage spindles increases with age in rats (Buzsáki et al., 1988, 1990a; Buzsáki et al., 1990b,c; Sirviö et al., 1989; Riekkinen, Jr. et al., 1991a). Typically, neocortical high-voltage spindles occur during low arousal and low vigilance states, being virtually absent during high vigilance states (Buzsáki et al., 1990a).

It is thought that rhythmically active γ-aminobutyric acid (GABA)-containing nucleus reticularis thalamus neurons, via their divergent projections, can phasically hyperpolarize their thalamocortical target neurons. In the absence of other depolarizing inputs, voltage-and time-dependent rebound Ca^{2+} spikes occur in a phase

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SSDI 0014-2999(95)00272-3

locked manner in thalamocortical relay neurons, as a result of the deinactivation of low-threshold Ca^{2+} channels located on the thalamocortical relay neurons (Steriade and Deschênes, 1984; Steriade and Llinás, 1988; Buzsáki et al., 1990a,b,c; McCormick, 1990, 1992; Steriade et al., 1993). In turn, these periodic bursts of thalamocortical neurons converge by means of their axon collaterals onto reticular neurons and facilitate their rhythmic oscillation. The bursts of thalamocortical neurons are also transferred to the cortex, where they induce excitatory post-synaptic potentials in cortical pyramidal neurons, thereby generating EEG high-voltage spindles (Steriade et al., 1993). Finally, cortical neurons of layer 6 innervate topographically both the dorsal thalamus and the nucleus reticularis thalamus, thereby completing the oscillatory network (Steriade and Llinás, 1988; Steriade et al., 1993). It has been suggested that the transfer of information through the thalamus to the cortex and other structures may be disrupted during thalamic oscillatory activity, since during thalamocortical oscillations the output of a thalamocortical relay neuron may be highly non-linear in relation to the input that triggers it (McCormick, 1992).

Factors that suppress rhythmic bursts in the thalamic pacemaker zone, the nucleus reticularis thalamus, and factors that produce tonic depolarization or block hyperpolarization in thalamocortical relay neurons may be regarded as anti-oscillatory factors (Buzsáki et al., 1990a,b,c), whereas factors that produce hyperpolarization of the thalamocortical relay neurons and release low-threshold Ca^{2+} spikes in these neurons are regarded as oscillation-promoting factors (Buzsáki et al., 1990a,b,c). Previous data suggest that the ascending subcortical neurotransmitter systems, e.g. cholinergic (Levey et al., 1987; Buzsáki et al., 1988; McCormick, 1989, 1990, 1992; Riekkinen, Jr. et al., 1990a,b, 1991b, 1993a,c; Steriade and Buzsáki, 1990; Danober et al., 1993, 1994), noradrenergic (Micheletti et al., 1987; McCormick, 1989, 1992; Buzsáki et al., 1990a,b; Riekkinen, Jr. et al., 1990b, 1991b, 1993b,d; Jäkälä et al., 1992), dopaminergic (Warter et al., 1988; Buzsáki et al., 1990b,c), histaminergic (McCormick, 1992), and serotonergic (Riekkinen, Jr. et al., 1990a; Marescaux et al., 1992; McCormick, 1992) systems, which innervate the nucleus reticularis and other thalamic nuclei, modulate the generation of these thalamocortical oscillations and their related neocortical high-voltage spindles as well as spike-and-wave discharges, another phenomenon reflecting thalamocortical oscillations (Micheletti et al., 1987; Warter et al., 1988; Marescaux et al., 1992; Danober et al., 1993, 1994). The contributions of the noradrenergic, cholinergic and dopaminergic systems, as well as the roles of α_1- and α_2-adrenoceptor and muscarinic and nicotinic acetylcholine receptor subtypes, in the regulation of thalamocortical oscillations are known quite well. For example, the noradrenergic locus coeruleus system may have a dual role in the regulation of thalamic oscillations: promoting high-voltage spindles by acting on thalamic α_2-adrenoceptor and suppressing them via α_1-adrenoceptors (Buzsáki et al., 1990a,b; Riekkinen, Jr. et al., 1993b,d). Furthermore, α_1-adrenoceptor antagonists and α_2-adrenoceptor agonists increase, and α_1-adrenoceptor agonists and α_2-adrenoceptor antagonists reduce, spike-and-wave discharges in rats, whereas drugs which interact with β-adrenoceptors have no effect (Micheletti et al., 1987). Acetylcholine may also reduce the ability of thalamic neurons to generate rhythmic burst firing since partial lesions of the cholinergic nucleus basalis (Riekkinen, Jr. et al., 1990a, 1991b) or systemic administration of a muscarinic or nicotinic receptor antagonist both increase the incidence of rat high-voltage spindles (Riekkinen, Jr. et al., 1990b, 1991a, 1993a) and spike-and-wave discharges (Danober et al., 1993), and systemic injections of drugs which potentiate cholinergic transmission suppress rat high-voltage spindles and spike-and-wave discharges in a dose-dependent manner and induce arousal-like cortical EEG activity (Riekkinen, Jr. et al., 1990b, 1991b, 1993a,c; Danober et al., 1993). High doses of acetylcholine receptor antagonists or almost total lesions of the cholinergic nucleus basalis decrease spike-and-wave discharges (Danober et al., 1993, 1994). Finally, the decreased release of dopamine in the striatum and consequent release of the GABAergic nigro-thalamic and entopeduncule-thalamic systems from striatal GABAergic inhibition may promote burst firing and oscillation in the thalamus (Buzsáki et al., 1990b,c). Intrastriatal injections of dopamine receptor blockers increase the incidence of neocortical high-voltage spindles (Buzsáki et al., 1990c), whereas systemic administration of dopamine receptor antagonists increases spike-and-wave discharges (Warter et al., 1988).

However, much less is known about the role of the serotonergic system and serotonin (5-hydroxytryptamine; 5-HT) receptor subtypes in the regulation of thalamocortical oscillations. In vitro serotonin controls the excitability and firing pattern of thalamocortical neurons by enhancing a mixed Na^+/K^+ current that is activated by hyperpolarization (Pape and McCormick, 1989; Nicoll et al., 1990; McCormick, 1992). This action reduces the ability of thalamic neurons to generate rhythmic burst firing and promotes a state of excitability that may increase the efficacy of transfer of information through the thalamus to the cortex during periods of increased arousal and attentiveness (Pape and McCormick, 1989; McCormick, 1992). In addition, in slice preparations from guinea-pig nucleus reticularis neurons and cat perigeniculate nucleus, application of serotonin results in pronounced and prolonged excitation associated with the occurrence of single spike activity, and this excitatory response to serotonin appli-

cation is specifically mimicked by 5-HT_2 receptor agonists and blocked by 5-HT_2 receptor antagonists (McCormick and Wang, 1991). Previously, 5,7-dihydroxytryptamine-induced partial lesions of the nucleus raphe dorsalis, the source of the serotonergic innervation of the rat forebrain (Steinbusch, 1981; Jacobs and Azmitia, 1992), alone did not affect neocortical high-voltage spindle activity of adult rats, but significantly aggravated the increase in high-voltage spindle activity induced by partial lesions of the cholinergic nucleus basalis (Riekkinen, Jr. et al., 1990a), suggesting that the serotonergic system might play some role in the modulation of rat thalamocortical oscillations. However, previous studies investigating the effects of serotonin receptor subtype specific drugs on rat neocortical high-voltage spindle activity (Riekkinen, Jr. et al., 1991a) or spike-and-wave discharge activity (Marescaux et al., 1992) have not revealed any significant role for serotonin or different serotonin receptor subtypes in the modulation of this kind of activity. Importantly, those thalamic nuclei associated with sensory transmission may receive a relatively dense serotonergic innervation (Steinbusch, 1981; Jacobs and Azmitia, 1992), and 5-HT_{2C} receptors (previously called 5-HT_{1C} receptors) (Humphrey et al., 1993; Boess and Martin, 1994; Martin and Humphrey, 1994) have been found to be expressed in the neurons of the rat thalamic sensory relay nuclei (Molineaux et al., 1989). Therefore, the aim of the present experiment was to study further whether the 5-HT_1/5-HT_2 receptor subtypes play a role in the modulation of rat thalamocortical oscillations as measured by neocortical high-voltage spindle activity. To address this question, we studied (1) the effects of systemic single or combined administrations of different 5-HT_1/5-HT_2 receptor subtype specific drugs on neocortical high-voltage spindle activity in adult rats, and (2) in an attempt to directly modulate the generation of thalamocortical oscillations at the level of thalamocortical nuclei, we investigated the effects of direct intrathalamic infusions with a systemically effective drug, namely 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a 5-HT_2 receptor agonist.

2. Materials and methods

2.1. Animals

72 male Han:Wistar rats (aged 6–10 months) were used in the present study (group I: $n = 10$; group II: $n = 11$; group III: $n = 14$; group IV: $n = 13$; group V: $n = 9$; group VI: $n = 10$; group VII: $n = 5$). The rats were singly housed in a controlled environment (National Animal Center, Kuopio, Finland) (temperature 20°C, lights on 07:00–19:00 h) with water and food available ad libitum. The study design was approved by the Local Ethical Committee.

2.2. Drugs

The selection of drug doses was based on previous electrophysiological and behavioral tests (Bendotti and Samanin, 1986; Dedeoglu and Fisher, 1991; Garratt et al., 1991; Carli et al., 1992; Riekkinen, M. et al., 1992; Watson et al., 1992; Jäkälä et al., 1993; Martin and Humphrey, 1994). Methysergide (Sandoz, a mixed 5-HT_1/5-HT_2 receptor antagonist) (1.0, 5.0 and 15.0 mg/kg, i.p. 4.0 ml/kg), methiothepin mesylate (Research Biochemical, USA, a mixed 5-HT_1/5-HT_2 receptor antagonist) (0.2, 1.0 and 5.0 mg/kg, i.p., 4.0 ml/kg), ketanserin tartrate (Research Biochemicals, USA, a 5-HT_2 receptor antagonist) (1.0, 5.0 and 20.0 mg/kg, s.c. 4.0 ml/kg), ritanserin (Research Biochemicals, USA, a 5-HT_2 receptor antagonist) (0.1, 1.0 and 5.0 mg/kg, s.c. 4.0 ml/kg) and (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) hydrochloride (Research Biochemicals, USA, a 5-HT_2 receptor agonist) (0.5, 1.0 and 2.0 mg/kg, s.c. 2.0 ml/kg) were dissolved in saline and injected 30 min before recordings of neocortical high-voltage spindles. Before they were diluted in saline, ketanserin and ritanserin had to be dissolved with a few drops of glacial acetic acid (final pH 5–6). (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) hydrobromide (Research Biochemicals, USA, a 5-HT_{1A} receptor agonist) (0.03, 0.1, 0.3 and 1.0 mg/kg, s.c. 2.0 ml/kg) was dissolved in distilled water and injected 30 min before recordings. DOI was also administered intrathalamically (10.0 and 50.0 μg/1.0 μl/rat; pH 6–7) and for control purposes intrahippocampally (10.0 and 50.0 μg/1.0 μl/rat; pH 6–7) 10 min before recordings of the high-voltage spindles.

2.3. Surgery

The animals were anesthetized with Equithesin (3.0 ml/kg, i.p.) and placed in a stereotaxic frame with the incisor bar set at −3.3 mm and the bregma and lambda in the horizontal plane. Active recording electrodes (stainless steel screws 0.5 mm in diameter) were located symmetrically on both sides above the frontal cortex (A = 1.0 mm and L = ±2.0 mm relative to the bregma). Ground and indifferent electrodes were located in the midline above the cerebellum and nasal bone, respectively. The screw electrodes and connecting female pins were embedded in dental acrylic. For intrathalamic delivery of DOI, 22-gauge guide cannulas were unilaterally implanted in ten 6-month-old rats at the following coordinates: AP = −3.8 mm, L = 2.6 mm, V = 6.0 mm, aiming at the right nucleus ventralis posteromedialis thalami. In addition, for control purposes,

22-gauge guide cannulas were unilaterally implanted in five 6-month-old rats, aiming at the right CA1 area of the hippocampus at the following coordinates: AP = −3.8 mm, L = 2.0 mm and V = 2.6 mm. The guide cannula accepted a 28-gauge inner cannula through which DOI in 1.0 μl volume was delivered. DOI was freshly dissolved in saline (pH 6–7) and delivered by a 5.0 μl Hamilton syringe connected to the inner cannula with flexible tubing, at a speed of 0.2 μl/min with an automatic pump (Harvard Apparatus, pump 22, USA). During drug (DOI) or vehicle (saline) delivery the rat was gently restrained. The inner cannula was removed 1 min after the delivery of a drug or a vehicle, and the EEG recordings were started 10 min after this. At least 48 h elapsed between successive drug or vehicle infusions and no rat was used for more than 4 microinjections. A 2-week recovery period after implantation of the high-voltage spindle recording electrodes and cannulas was allowed before any recordings were started.

2.4. High-voltage spindle recordings

Before recordings, the rats were twice placed into the recording cages for 10 min to habituate them to the recording environment. To ascertain that the high-voltage spindle levels would be as constant as possible three 20-min cumulative waking-immobility (eyes open, head held up) baseline recordings without any drug injections and one baseline recording with saline injections were made before the drug experiments. No differences in the total duration of high-voltage spindle activity were observed between the second, the third or the fourth baseline recording sessions (data not shown). Recordings after the test drug injections were made at the same time at the various recording days for individual animals. Recordings were made between 09:00 and 15:00 h. During the recordings, the rats were allowed to move freely in the recording cages. High-voltage spindle activity of four rats was recorded simultaneously and analyzed automatically. The IBM-compatible software separated high-voltage spindles from background EEG and counted the number (incidence), mean duration and total duration (incidence × mean duration) of high-voltage spindles from the right active recording electrode during a 20-min cumulative waking-immobility period. The EEG epoch was considered as a high-voltage spindle if it met the following criteria: (1) the amplitude of the EEG was more than twice that of the background EEG (threshold), (2) the duration of each epoch which exceeded the threshold was more than 0.5 s, (3) the frequency of the EEG exceeding the threshold was 6–12 Hz (in previous studies in our laboratory high-voltage spindle activity has been observed within these frequency limits in male Han:Wistar rats: Riekkinen, Jr. et al., 1993a,c,d), (4) the time

Fig. 1. Example of a typical neocortical high-voltage spindle episode recorded from the right active recording electrode above the frontal cortex (A = 1.0 mm and L = 2.0 mm relative to the bregma) during a period of relaxed behavioral waking-immobility (eyes open, head held up). The amplitude scale (vertical bar) indicates 300 μV. The time scale (horizontal bar) indicates 1.0 s.

between two separate spindles had to be at least 0.5 s (if the time between two spindles was less than 0.5 s, it was calculated as one high-voltage spindle), (5) no movement registered by the magnetic coil binding of the EEG cable on the head except vibrissal or head tremor was allowed 1 s before or during each high-voltage spindle epoch. A typical neocortical high-voltage spindle is shown in Fig. 1. After intrathalamic drug or vehicle infusions, the EEG of the rats was also recorded on paper polygraph charts to verify by a ruler whether infusions to the right ventroposteromedial thalamic area would have similar or differential effects on neocortical high-voltage spindle activity as recorded from above the right or left frontal cortex.

Following the baseline recordings, the effects of 8-OH-DPAT on high-voltage spindle activity were tested in group I ($n = 10$) rats. The effects of saline and different doses of 8-OH-DPAT (0.03, 0.1, 0.3 and 1.0 mg/kg s.c.) were tested in a counterbalanced order every third day.

The effects of methysergide on high-voltage spindle activity were tested in group II rats ($n = 11$). Saline and the different doses of methysergide (1.0, 5.0 and 15.0 mg/kg i.p.) were tested in a counterbalanced order every third day. Thereafter, a one-week wash-out period was allowed. After that, the effects of saline and different doses of methiothepin (0.2, 1.0 and 5.0 mg/kg i.p.) were tested in a counterbalanced order every third day.

In group III rats ($n = 14$) the effects of saline and different doses of ketanserin (1.0, 5.0 and 20.0 mg/kg s.c.) were tested in a counterbalanced order every third day, and thereafter a one-week wash-out period was allowed for the rats. After this, the effects of saline and different doses of ritanserin (0.1, 1.0 and 5.0 mg/kg s.c.) were tested in a counterbalanced order every third day.

Group IV ($n = 13$) rats were used to study the effects of saline and different doses of DOI (0.5, 1.0 and 2.0 mg/kg s.c.) on high-voltage spindle activity. The recordings were made in a counterbalanced order every third day, and thereafter a one-week recovery period was allowed. Then, the effects of combined injections of DOI (1.0 mg/kg s.c.) and saline or differ-

ent doses of ketanserin (1.0, 5.0 and 20.0 mg/kg s.c.) were tested in a counterbalanced order every third day. Thereafter, a one-month recovery and wash-out period was allowed. During this period, five rats lost their EEG electrode dental acrylic connections and they were excluded from further recordings. The effects of combined injections of DOI (1.0 mg/kg s.c.) and saline or different doses of ritanserin (0.1, 1.0 and 5.0 mg/kg s.c.) were subsequently tested in a counterbalanced order every third day.

Group V rats ($n = 9$) were used to study the effects of combined administrations of DOI (1.0 mg/kg s.c.) and saline or different doses of methiothepin (0.2, 1.0 and 5.0 mg/kg i.p.) on high-voltage spindle activity. The recordings were made in a counterbalanced order every third day, and thereafter a one-week wash-out period was allowed. During the wash-out period, two rats lost their EEG electrode dental acrylic connections and they were excluded from further recordings. Then, the effects of combined administration of DOI (1.0 mg/kg s.c.) and saline or different doses of methysergide (1.0, 5.0 and 15.0 mg/kg i.p.) were tested in a counterbalanced order every third day.

The EEGs of rats with intrathalamically implanted cannulas (group VI, $n = 10$) were recorded every third day in the following order: (1) saline s.c. + saline intrathalamically, (2) DOI 10 μg intrathalamically, (3) DOI 50 μg intrathalamically, (4) ketanserin 20 mg/kg s.c., (5) ketanserin 20 mg/kg s.c. + DOI 50 μg intrathalamically, (6) saline s.c. + saline intrathalamically.

The EEGs of rats with intrahippocampal cannulas (group VII, $n = 5$) were recorded every third day in the following order: (1) saline intrahippocampally, (2) DOI 10 μg intrahippocampally, (3) DOI 50 μg intrahippocampally, and (4) saline intrahippocampally.

2.5. Histology

The rats with intrathalamically or intrahippocampally implanted cannulas (groups VI and VII) were decapitated on the day after the last recordings. The brains were rapidly removed, dissected on ice and placed in 4% formalin until they were sectioned in the coronal plane at 40 μm on a freezing vibratome. Then serial sections were stained with cresyl violet to verify the accurate placement of the cannulas.

2.6. Statistics

The multivariable analysis of variance (MANOVA) was used to analyse the overall drug treatment effects on neocortical high-voltage spindles. Post-hoc Wilcoxon signed rank test was used to analyse the differences between various drug doses. High-voltage spindle incidences, mean durations and total durations (= incidence × mean duration) as well as the total recording times (i.e. the total recording time needed to achieve a 20-min period of behavioral waking-immobility related EEG; movement periods automatically excluded by the magnet coil movement-sensor binding on the rats head) were analyzed separately for each of the drugs. The changes seen in rat neocortical high-voltage spindle activity in the present experiments were mainly due to changes in high-voltage spindle incidence and total duration, so that when high-voltage spindle incidence was either increased or decreased by the drug treatment, the high-voltage spindle total duration was correspondingly increased or decreased. When a neocortical high-voltage spindle episode appeared, its mean duration was rather constant. Therefore, as an index of neocortical high-voltage spindle activity only the results of the drug treatment effects on high-voltage spindle total duration are shown. Furthermore, total recording times, which provide indirect information about the behavioral/motor activity of the rats, are reported.

3. Results

3.1. EEG measurements

Group I

8-OH-DPAT (s.c.) (Table 1: total recording time). 8-OH-DPAT had no significant effect on high-voltage spindle total duration ($F(4,36) = 1.35$, $P < 0.1$; % group means ± S.E.M of control (saline treated) values (100%): 0.03 mg/kg: 64.1 ± 98.5; 0.1 mg/kg: 58.5 ± 104.7; 0.3 mg/kg: 55.4 ± 81.9; and 1.0 mg/kg: 49.2 ± 49.6), but significantly affected total recording time ($F(4,36) = 33.42$, $P < 0.001$). 0.03 and 0.1 mg/kg had no effect ($Z(5,3) = 0.56$, $P > 0.1$ and $Z(3,7) = -1.32$, $P > 0.1$ vs. saline, respectively), whereas 0.3 and 1.0 mg/kg significantly increased total recording times ($Z(0,10) = -2.80$, $P < 0.01$ vs. saline for both). Furthermore, 0.1 mg/kg increased total recording time vs. 0.03 mg/kg dose ($Z(2,8) = -2.14$, $P < 0.05$), the 0.3 mg/kg dose increased total recording time vs. the 0.03 mg/kg ($Z(0,10) = -2.80$, $P < 0.01$) and 0.1 mg/kg ($Z(0,10) = -2.80$, $P < 0.01$) doses, and the 1.0 mg/kg dose increased total recording time vs. the 0.03 mg/kg ($Z(0,10) = -2.80$, $P < 0.01$), 0.1 mg/kg ($Z(0,10) = -2.80$, $P < 0.01$) and 0.3 mg/kg ($Z(0,10) = -2.80$, $P < 0.01$) doses.

Group II

Methysergide (i.p.) (Fig. 2, part a: high-voltage spindle total duration; Table 1: total recording time). There was no significant drug treatment effect of methysergide on high-voltage spindle total duration ($F(3,30) = 2.94$, $P < 0.05$) or total recording time ($F(3,27) = 2.68$, $P > 0.05$).

Table 1
The effects of 5-HT_1/5-HT_2 receptor subtype specific drugs on total recording times (the total time needed to achieve a cumulative 20-min period of relaxed quiet behavioral waking-immobility = immobility + movement periods)

Treatment	Total recording time (min) ± S.E.M.
Group I (n = 10)	
Saline (s.c.)	22.9 ± 0.8
8-OH-DPAT (s.c.)	
0.03 mg/kg	22.7 ± 0.8
0.1 mg/kg	24.7 ± 1.2
0.3 mg/kg	32.7 ± 1.7 [a]
1.0 mg/kg	45.4 ± 3.9 [a]
Group II (n = 11)	
Saline (i.p.)	26.8 ± 2.1
Methysergide (i.p.)	
1.0 mg/kg	29.2 ± 2.7
5.0 mg/kg	23.9 ± 3.2
15.0 mg/kg	22.9 ± 1.5
Saline (i.p.)	25.3 ± 0.9
Methiothepine (i.p.)	
0.2 mg/kg	24.4 ± 1.0
1.0 mg/kg	22.0 ± 0.6 [b]
5.0 mg/kg	20.8 ± 0.5 [a]
Group III (n = 14)	
Saline (s.c.)	23.8 ± 0.6
Ketanserin (s.c.)	
1.0 mg/kg	23.3 ± 0.6
5.0 mg/kg	23.6 ± 0.8
20.0 mg/kg	21.8 ± 0.4 [a]
Saline (s.c.)	24.9 ± 0.7
Ritanserin (s.c.)	
0.1 mg/kg	24.4 ± 1.0
1.0 mg/kg	23.6 ± 0.6
5.0 mg/kg	23.5 ± 0.6
Group IV (n = 13)	
Saline (s.c.)	26.0 ± 1.2
DOI (s.c)	
0.5 mg/kg	31.9 ± 2.9
1.0 mg/kg	38.0 ± 2.5 [a]
2.0 mg/kg	39.6 ± 2.6 [a]
Saline + saline (s.c. + s.c.)	32.3 ± 2.1
DOI 1.0 mg/kg + saline	36.9 ± 2.8
DOI 1.0 mg/kg + ketanserin 1.0 mg/kg	24.9 ± 1.3 [b]
DOI 1.0 mg/kg + ketanserin 5.0 mg/kg	23.9 ± 1.8 [b]
DOI 1.0 mg/kg + ketanserin 20.0 mg/kg (n = 8)	23.6 ± 0.8 [a]
Saline + saline (s.c. + s.c.)	20.6 ± 0.7
DOI 1.0 mg/kg + saline	21.8 ± 1.2
DOI 1.0 mg/kg + ritanserin 0.1 mg/kg	20.6 ± 0.7
DOI 1.0 mg/kg + ritanserin 1.0 mg/kg	21.8 ± 1.2
DOI 1.0 mg/kg + ritanserin 5.0 mg/kg	21.1 ± 0.7
Group V (n = 9)	
Saline + saline (s.c. + i.p.)	25.1 ± 1.0
DOI 1.0 mg/kg + saline	30.2 ± 2.4
DOI 1.0 mg/kg + methiothepin 0.2 mg/kg	23.4 ± 1.7
DOI 1.0 mg/kg + methiothepin 1.0 mg/kg	20.7 ± 0.3 [a]
DOI 1.0 mg/kg + methiothepin 5.0 mg/kg (n = 7)	20.1 ± 0.1 [a]
Saline + saline (s.c. + i.p.)	24.7 ± 2.0
DOI 1.0 mg/kg + saline	32.4 ± 4.1 [b]
DOI 1.0 mg/kg + methysergide 1.0 mg/kg	24.4 ± 1.9
DOI 1.0 mg/kg + methysergide 5.0 mg/kg	27.3 ± 2.0
DOI 1.0 mg/kg + methysergide 15.0 mg/kg	21.4 ± 1.0 [b]
Group VI (n = 10)	
Saline s.c. + saline intrathalamically	30.1 ± 3.3
DOI 10 μg intrathalamically	34.0 ± 2.8
DOI 50 μg intrathalamically	30.5 ± 2.8
Ketanserin 20 mg/kg s.c.	21.1 ± 0.7 [a]
DOI 50 μg intrathalamically + ketanserin 20 mg/kg s.c.	21.1 ± 0.6 [b]
Group VII (n = 5)	
Saline intrahippocampally	41.2 ± 10.2
DOI 10 μg intrahippocampally	24.0 ± 2.0
DOI 50 μg intrahippocampally	27.6 ± 3.7
Saline intrahippocampally	23.2 ± 2.2

Movement periods were automatically registered and excluded from the high-voltage spindle data analysis by the magnet-coil movement-sensor detector of EEG cables which were bound to the rat's head. Recordings were made every third day in 6- to-10-month old male Han:Wistar rats. In groups I–V systemic, in group VI intrathalamic and in group VII intrahippocampal drug administrations were used. Values (minutes) represent group means ± S.E.M. Multivariable analysis of variance (MANOVA) followed by post-hoc Wilcoxon signed rank test was used for statistical analysis. [a] $P < 0.01$ vs. controls (saline), [b] $P < 0.05$ vs. controls (saline) (Wilcoxon signed rank test).

Methiothepin (i.p.) (Fig. 2, part b: high-voltage spindle total duration; Table 1: total recording time). There was a significant drug treatment effect on high-voltage spindle total duration ($F(3,30) = 12.10$ $P < 0.001$). All the doses of methiothepin increased high-voltage spindle total duration (0.1 mg/kg: $Z(2,9) = -2.67$, $P < 0.01$; 1.0 mg/kg: $Z(2,9) = -2.58$, $P < 0.01$; 5.0 mg/kg: $Z(0,11) = -2.93$, $P < 0.005$ vs. saline). Methiothepin at 5.0 mg/kg more effectively increased high-voltage spindle total duration than at 0.2 mg/kg ($Z(2,9) = -2.29$, $P < 0.02$), but not more effectively than at 1.0 mg/kg ($Z(3,9) = -1.41$, $P > 0.1$). There was no difference between methiothepin 0.2 and 1.0 mg/kg ($Z(3,8) = -1.87$, $P > 0.05$). There was also a significant treatment effect on total recording time ($F(3,30) = 8.43$, $P < 0.001$). Methiothepin 0.2 mg/kg had no effect on total recording time ($Z(8,3) = -0.84$, $P > 0.1$ vs. saline). The 1.0 mg/kg dose decreased total recording time vs. saline ($Z(7,2) = -2.31$, $P < 0.05$) and vs. the 0.2 mg/kg dose ($Z(9,2) = -2.00$, $P < 0.05$). The 5.0 mg/kg dose decreased total recording time vs. saline ($Z(10,1) = -2.71$, $P < 0.01$), and 0.2 mg/kg dose ($Z(8,2) = -2.44$, $P < 0.02$), but not vs. the 1.0 mg/kg dose ($Z(8,1) = -1.83$, $P > 0.05$).

Group III

Ketanserin (s.c.) (Fig. 3, part a: high-voltage spindle total duration; Table 1: total recording time). There was a significant drug treatment effect on high-voltage spindle total duration ($F(3,39) = 5.06$, $P < 0.01$). The high-

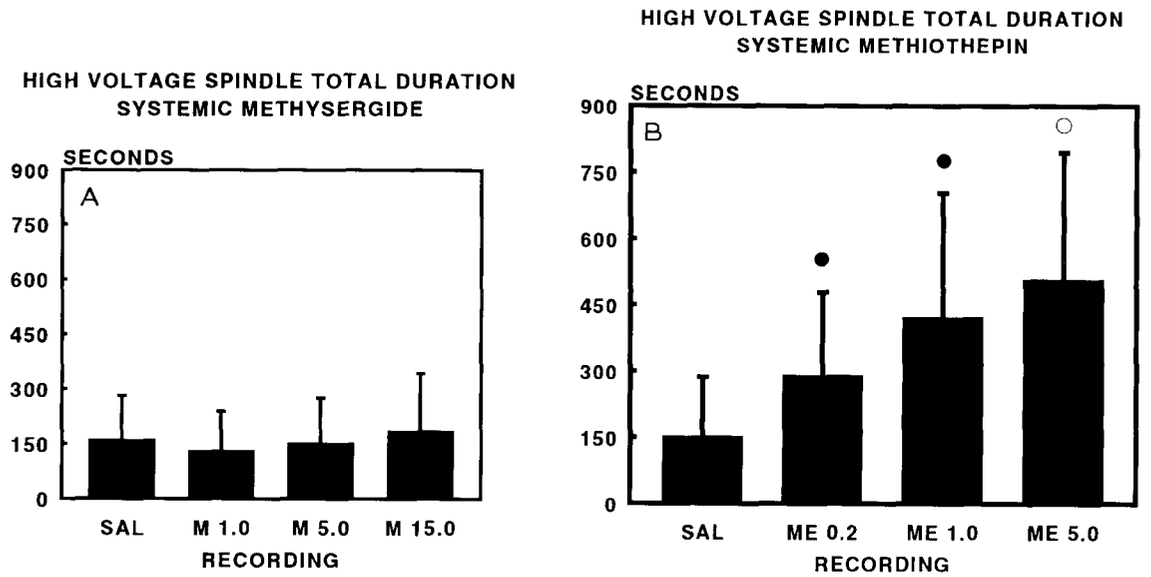

Fig. 2. Effects of systemic administration (i.p. 4.0 ml/kg, 30 min before recording session) of mixed 5-HT_1/5-HT_2 receptor antagonists, methysergide (part A), and methiothepin (part B), on the total duration (incidence × mean duration) of neocortical high-voltage spindles recorded during a 20-min cumulative behavioral waking-immobility period. The high-voltage spindle recordings were made every third day in 6-month-old male rats (n = 11). Values represent % group means ± S.E.M. of control (saline treated) values (100%). Abbreviations: SAL = saline; M1.0 = methysergide 1.0 mg/kg; M5.0 = methysergide 5.0 mg/kg; M15.0 = methysergide 15.0 mg/kg; ME0.2 = methiothepin 0.2 mg/kg; ME1.0 = methiothepin 1.0 mg/kg; ME5.0 = methiothepin 5.0 mg/kg. Part A: Multivariable analysis of variance (MANOVA) revealed no methysergide treatment effect on high-voltage spindle total duration. Part B: MANOVA followed by post-hoc analysis revealed that all the doses of methiothepin increased high-voltage spindle total duration, and that methiothepin at 5.0 mg/kg was more effective in increasing high-voltage spindle total duration than the other doses. • $P < 0.01$ vs. saline (Wilcoxon), ○ $P < 0.005$ vs. saline (Wilcoxon).

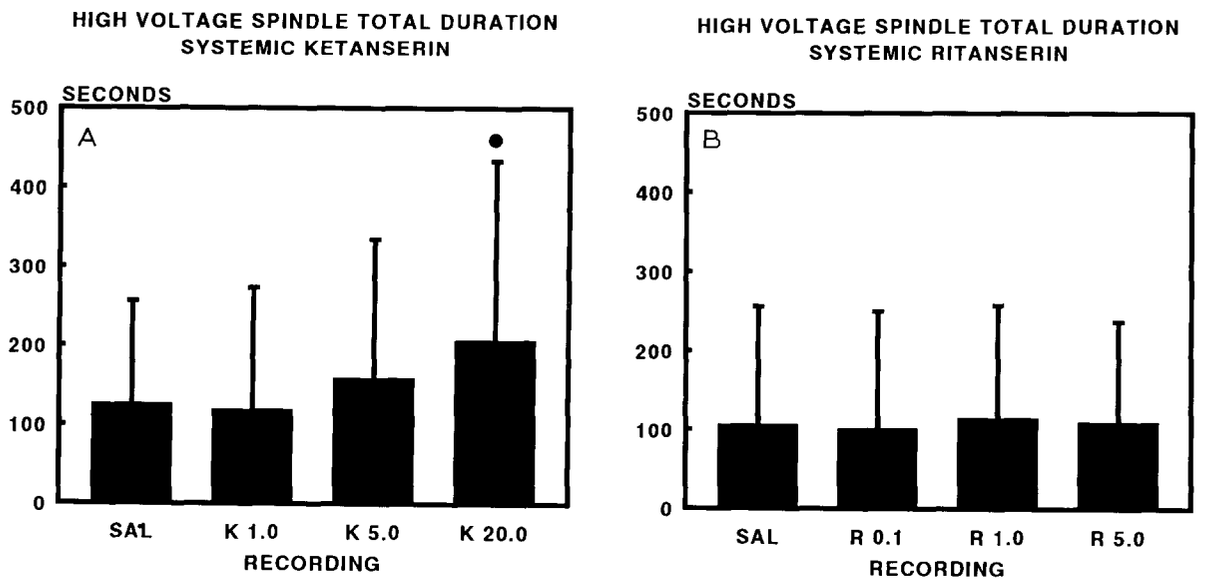

Fig. 3. Effects of systemic administration (s.c. 5.0 ml/kg, 30 min before recording session) of 5-HT_2 receptor antagonists, ketanserin (part A), and ritanserin (part B), on the total duration (incidence × mean duration) of neocortical high-voltage spindles recorded during a 20-min cumulative behavioral waking-immobility period. The high-voltage spindle recordings were made every third day in 10-month-old male rats (n = 14). Values represent % group means ± S.E.M. of control (saline treated) values (100%). Abbreviations: K1.0 = ketanserin 1.0 mg/kg; K5.0 = ketanserin 5.0 mg/kg; K20.0 = ketanserin 20.0 mg/kg; R0.1 = ritanserin 0.1 mg/kg; R1.0 = ritanserin 1.0 mg/kg; R5.0 = ritanserin 5.0 mg/kg. For other abbreviations see Fig. 2. Part A: Multivariable analysis of variance (MANOVA) followed by post-hoc analysis revealed that the highest dose of ketanserin increased high-voltage spindle total duration. • $P < 0.05$ vs. saline (Wilcoxon). Part B: Ritanserin had no effect on high-voltage spindle total duration.

est dose of ketanserin (20.0 mg/kg) increased high-voltage spindle total duration ($Z(5,9) = -2.04$, $P < 0.05$ vs. saline). Ketanserin at 1.0 mg/kg or 5.0 mg/kg had no effect on high-voltage spindle total duration ($Z(10,5) = -0.56$, $P > 0.1$, and $Z(6,9) = -11.45$, $P > 0.1$, respectively vs. saline). Furthermore, there was a significant treatment effect on total recording time ($F(3,39) = 3.98$, $P < 0.02$). Ketanserin at 1.0 and 5.0 mg/kg had no effect on total recording time ($Z(6,4) = -0.71$, $P > 0.1$ and $Z(6,3) = -0.47$, $P > 0.1$ vs. saline,

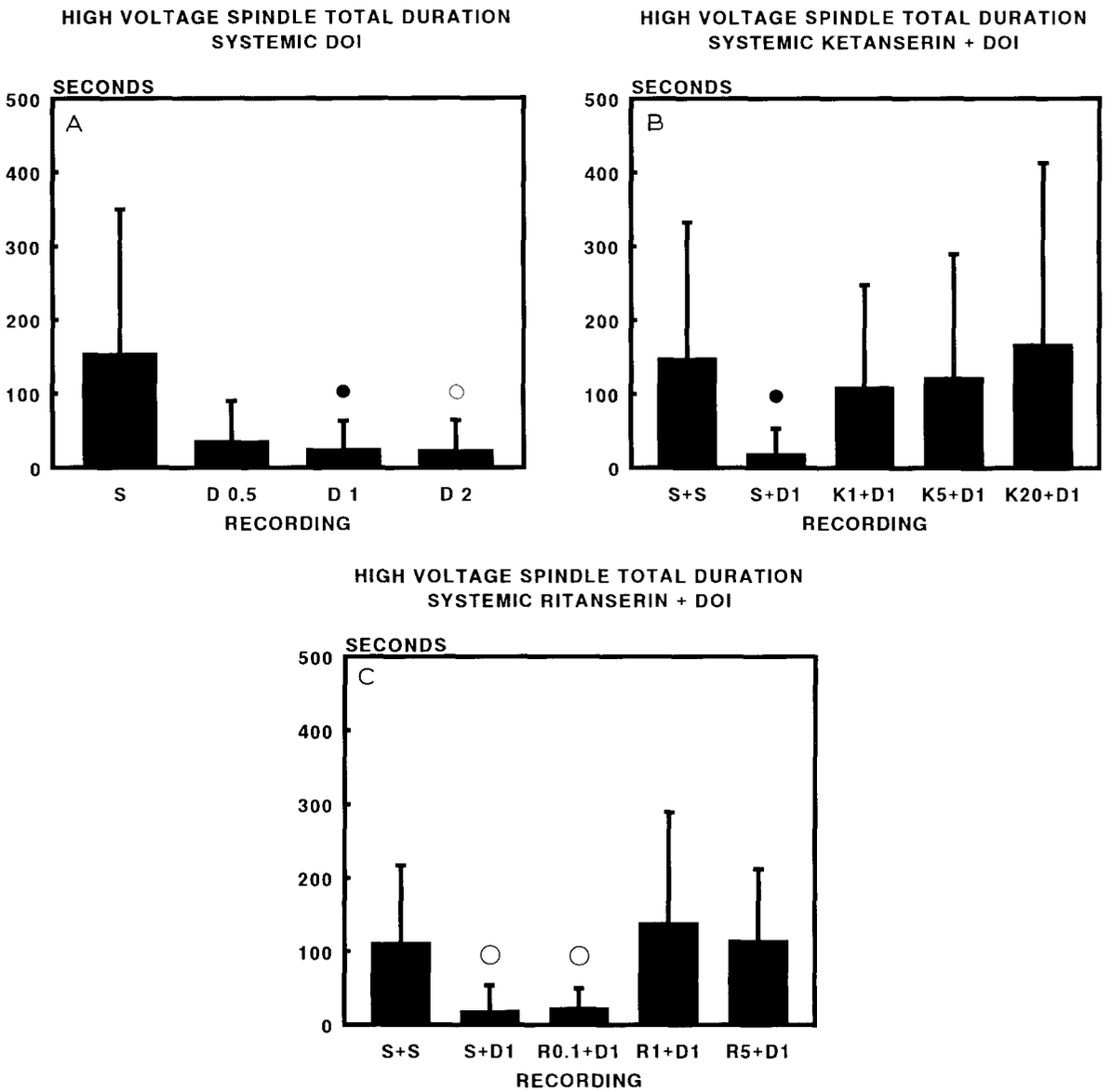

Fig. 4. Effects of systemic administration of a 5-HT_2 receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI; s.c. 2.0 ml/kg, 30 min before recording session) alone (part A), and when combined with 5-HT_2 receptor antagonists, ketanserin (s.c., 5.0 ml/kg, 30 min before recording session) (part B), or ritanserin (s.c., 5.0 ml/kg, 30 min before recording session) (part C), on the total duration (incidence × mean duration) of neocortical high-voltage spindles recorded during a 20-min cumulative behavioral waking-immobility period. The high-voltage spindle recordings were made every third day in 10-month-old male rats ($n = 13$ in part A and in part B; $n = 8$ in part C). Values represent % group mean ± S.E.M. of control (saline treated) values (100%). Abbreviations: S = saline; D0.5 = DOI 0.5 mg/kg; D1 = DOI 1.0 mg/kg; D2 = DOI 2.0 mg/kg; K1 = ketanserin 1.0 mg/kg; K5 = ketanserin 5.0 mg/kg; K20 = ketanserin 20.0 mg/kg; R0.1 = ritanserin 0.1 mg/kg; R1 = ritanserin 1.0 mg/kg; R5 = ritanserin 5.0 mg/kg. Part A: Multivariable analysis of variance (MANOVA) followed by post-hoc analysis revealed that the two highest doses of DOI decreased high-voltage spindle total duration, but there was no difference between these doses. • $P < 0.05$ vs. saline (Wilcoxon), ○ $P < 0.01$ vs. saline (Wilcoxon). Part B: The decrease in high-voltage spindle total duration induced by DOI 1.0 mg/kg was equally blocked by all doses of ketanserin. • $P < 0.005$ vs. saline (Wilcoxon). Part C: The decrease in high-voltage spindle total duration induced by DOI was equally antagonized by the two highest doses of ritanserin, but not by the lowest dose. ○ $P < 0.02$ vs. saline (Wilcoxon).

respectively), whereas ketanserin at 20.0 mg/kg significantly decreased recording time vs. saline ($Z(10,0) = -2.80$, $P < 0.01$) and the 5.0 mg/kg dose ($Z(8,1) = -2.43$, $P < 0.02$), but not vs. the 1.0 mg/kg dose ($Z(6,2) = -1.82$, $P > 0.05$).

Ritanserin (s.c.) (Fig. 3, part b: high-voltage spindle total duration; Table 1: total recording time). There was no significant drug treatment effect on high-voltage spindle total duration ($F(3,39) = 0.22$, $P > 0.1$) or total recording time ($F(3,39) = 2.22$, $P > 0.1$) seen with ritanserin.

Group IV

DOI (s.c.) (Fig. 4, part a: high-voltage spindle total duration; Table 1: total recording time). There was a significant drug treatment effect on high-voltage spindle total duration ($F(3,36) = 5.29$, $P < 0.005$). The two highest doses of DOI decreased high-voltage spindle total duration (1.0 mg/kg: $Z(9,4) = -1.99$, $P < 0.05$; 2.0 mg/kg: $Z(10,3) = -2.59$, $P < 0.01$ vs. saline), but there was no difference between these doses ($Z(8,5) = -0.77$, $P > 0.1$). The lowest dose of DOI (0.5 mg/kg) had no effect on high-voltage spindle total duration ($Z(9,4) = -1.64$, $P > 0.1$). Furthermore, there was a significant treatment effect on total recording time ($F(3,36) = 6.76$, $P = 0.001$). DOI 0.5 mg/kg had no effect ($Z(4,9) = -1.68$, $P > 0.05$ vs. saline), whereas 1.0 mg/kg ($Z(1,12) = -2.83$, $P < 0.005$) and 2.0 mg/kg ($Z(0,13) = -3.18$, $P < 0.002$) increased total recording time vs. saline. However, there was no difference between the effects of DOI 0.5 and 1.0 mg/kg ($Z(3,9) = -1.45$, $P > 0.1$), 0.5 and 2.0 mg/kg ($Z(4,9) = -1.78$, $P > 0.05$), or 1.0 and 2.0 mg/kg ($Z(7,6) = -0.49$, $P > 0.1$) on total recording time.

DOI (s.c.) + ketanserin (s.c.) (Fig. 4, part b: high-voltage spindle total duration; Table 1: total recording time). A significant drug treatment effect on high-voltage spindle total duration ($F(4,48) = 2.78$, $P < 0.05$) was observed. DOI at 1.0 mg/kg sifnificantly decreased high-voltage spindle total duration ($Z(11,2) = -2.90$, $P < 0.005$ vs. saline). All the doses of ketanserin blocked the decrease in high-voltage spindle total duration induced by DOI 1.0 mg/kg (ketanserin 1.0 mg/kg + DOI 1.0 mg/kg: $Z(9,4) = -1.12$, $P > 0.1$; ketanserin 5.0 mg/kg + DOI 1.0 mg/kg: $Z(6,6) = -0.63$, $P > 0.1$; ketanserin 20.0 mg/kg + DOI 1.0 mg/kg: $Z(4,8) = -0.94$, $P > 0.1$, vs. saline + saline). There was no dif-

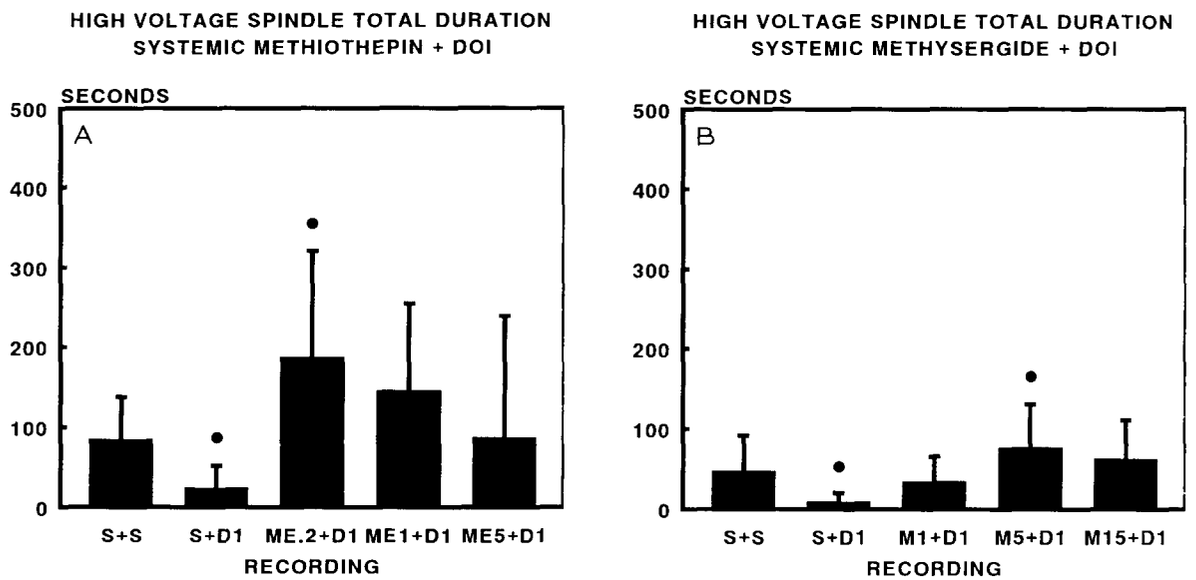

Fig. 5. Effects of systemic combined administration of a 5-HT_2 receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (s.c., 2.0 ml/kg, 30 min before recording session), and mixed 5-HT_1/5-HT_2 receptor antagonists, methiothepin (i.p., 4.0 ml/kg, 30 min before recording session) (part A), or methysergide (i.p., 4.0 ml/kg, 30 min before recording session) (part B), on the total duration (incidence × mean duration) of neocortical high-voltage spindles recorded during a 20-min cumulative behavioral waking-immobility period. The high-voltage spindle recordings were made every third day in 6-month-old male rats ($n = 9$ in part A, and $n = 7$ in part B). Values represent % group means ± S.E.M. of control (saline treated) values (100%). Abbreviations: ME.2 = methiothepin 0.2 mg/kg; ME1 = methiothepin 1.0 mg/kg; ME5 = methiothepin 5.0 mg/kg; M1 = methysergide 1.0 mg/kg; M5 = methysergide 5.0 mg/kg; M15 = methysergide 15.0 mg/kg. For other abbreviations see Fig. 4. Part A: Multivariable analysis of variance (MANOVA) followed by post-hoc analysis revealed that the two highest doses of methiothepin blocked the decrease in high-voltage spindle total duration induced by DOI and the lowest dose of methiothepin even increased high-voltage spindle total duration when combined with DOI. • $P < 0.05$ vs. saline (Wilcoxon). Part B: Methysergide at 1.0 and 15.0 mg/kg blocked the decrease in high-voltage spindle total duration induced by DOI, and when combined with DOI at 5.0 mg/kg even increased high-voltage spindle total duration. • $P < 0.05$ vs. saline (Wilcoxon).

ference between ketanserin 1.0 mg/kg and 5.0 mg/kg ($Z(4,8) = -0.78$, $P > 0.1$), 1.0 mg/kg and 20.0 mg/kg ($Z(6,7) = -0.66$, $P > 0.1$), or 5.0 mg/kg and 20.0 mg/kg ($Z(5,8) = -0.59$, $P > 0.1$) in blocking the effects of DOI 1.0 mg/kg. Furthermore, there was a significant treatment effect on total recording time ($F(4,48) = 16.54$, $P = 0.001$). DOI 1.0 mg/kg had no effect on total recording time ($Z(4,9) = -1.54$, $P > 0.1$ vs. saline). Combination of DOI 1.0 mg/kg and ketanserin 1.0 mg/kg decreased total recording time vs. saline ($Z(10,3) = -2.41$, $P < 0.02$) and vs. DOI 1.0 mg/kg alone ($Z(13,0) = -3.18$, $P < 0.002$). Combination of DOI 1.0 mg/kg and ketanserin 5.0 mg/kg decreased recording time vs. saline ($Z(10,2) = -2.35$, $P < 0.02$), vs. DOI 1.0 mg/kg alone ($Z(12,1) = -3.11$, $P < 0.002$), but not vs. DOI 1.0 mg/kg + ketanserin 1.0 mg/kg ($Z(7,2) = -1.07$, $P > 0.1$). Combination of DOI 1.0 mg/kg + ketanserin 20.0 mg/kg decreased recording time vs. saline ($Z(12,0) = -3.06$, $P < 0.005$), vs. DOI 1.0 mg/kg alone ($Z(13,0) = -3.18$, $P < 0.002$), but not vs. DOI 1.0 mg/kg + ketanserin 1.0 mg/kg ($Z(9,3) = -1.06$, $P > 0.1$) or DOI 1.0 mg/kg + ketanserin 5.0 mg/kg ($Z(7,6) = -0.03$, $P > 0.1$).

DOI (s.c.) + ritanserin (s.c.) (Fig. 4, part c: high-voltage spindle total duration; Table 1: total recording time). There was a significant drug treatment effect on high-voltage spindle total duration ($F(4,28) = 6.37$, $P < 0.01$). The decrease in high-voltage spindle total duration induced by DOI 1.0 mg/kg ($Z(8,0) = -2.52$, $P < 0.02$, vs. saline) was blocked by the two highest doses of ritanserin (ritanserin 1.0 mg/kg + DOI 1.0 mg/kg: $Z(3,5) = -1.12$, $P > 0.1$; ritanserin 5.0 mg/kg + DOI 1.0 mg/kg: $Z(3,4) = -0.25$, $P > 0.1$, vs. saline), but not by ritanserin at 0.1 mg/kg ($Z(8,0) = -2.52$, $P < 0.02$, vs. saline). Ritanserin 1.0 mg/kg and ritanserin 5.0 mg/kg more effectively blocked the effects of DOI 1.0 mg/kg than at 0.1 mg/kg ($Z(0,8) = -2.52$, $P < 0.02$, and $Z(1,7) = -2.38$, $P < 0.02$, respectively), but there was no difference between ritanserin 1.0 and 5.0 mg/kg ($Z(6,2) = -0.70$, $P > 0.1$). There was no significant treatment effect on total recording time ($F(4,28) = 1.33$, $P > 0.1$).

Group V

DOI (s.c.) + methiothepin (i.p.) (Fig. 5, part a: high-voltage spindle total duration; Table 1: total recording time). There was a significant drug treatment effect on high-voltage spindle total duration ($F(4,32) = 5.01$, $P < 0.005$). DOI 1.0 mg/kg significantly decreased high-voltage spindle total duration ($Z(8,1) = -2.19$, $P < 0.05$ vs. saline). The two highest doses of methiothepin blocked the decrease in high-voltage total duration induced by DOI 1.0 mg/kg (methiothepin 1.0 mg/kg + DOI 1.0 mg/kg: $Z(2,7) = -1.72$, $P > 0.05$, and methiothepin 5.0 mg/kg + DOI 1.0 mg/kg: $Z(8,1) = -1.60$, $P > 0.1$, respectively vs. saline + saline), and the combination of the lowest dose of methiothepin (0.2 mg/kg) + DOI 1.0 mg/kg further increased high-voltage spindle total duration vs. saline + saline ($Z(2,7) = -2.13$, $P < 0.05$). Methiothepin 0.2 mg/kg was more effective in blocking the decrease in high-voltage spindle total duration induced by DOI 1.0 mg/kg than methiothepin 5.0 mg/kg ($Z(8,1) = -2.55$, $P < 0.02$), but not more effective than methiothepin 1.0 mg/kg ($Z(6,3) = -1.83$, $P > 0.05$). There was no difference between methiothepin doses of 1.0 and 5.0 mg/kg in reversing the effects of DOI ($Z(7,2) = -1.48$, $P > 0.1$). Furthermore, there was a significant treatment effect on total recording time ($F(4,32) = 11.44$, $P < 0.001$). DOI 1.0 mg/kg had no effect on total recording time ($Z(1,6) = -1.86$, $P > 0.05$ vs. saline). Combination of DOI 1.0 mg/kg and methiothepin 0.2 mg/kg also had no effect on total recording time vs. saline ($Z(10,3) = -2.41$, $P < 0.02$), but decreased total recording time vs. DOI 1.0 mg/kg alone ($Z(8,1) = -2.31$, $P < 0.05$). Combination of DOI 1.0 mg/kg and methiothepin 1.0 mg/kg decreased recording time vs. saline ($Z(9,0) = -2.67$, $P < 0.01$), vs. DOI 1.0 mg/kg alone ($Z(9,0) = -2.67$, $P < 0.01$), and vs. DOI 1.0 mg/kg + methiothepin 0.2 mg/kg ($Z(5,0) = -2.02$, $P < 0.05$). Combination of DOI 1.0 mg/kg + methiothepin 5.0 mg/kg decreased recording time vs. saline ($Z(9,0) = -2.67$, $P < 0.01$), vs. DOI 1.0 mg/kg alone ($Z(9,0) = -2.67$, $P < 0.01$), and vs. DOI 1.0 mg/kg + methiothepine 0.2 mg/kg ($Z(5,0) = -2.02$, $P < 0.05$), but not vs. DOI 1.0 mg/kg + methiothepine 1.0 mg/kg ($Z(4,0) = -1.83$, $P > 0.05$).

DOI (s.c.) + methysergide (i.p.) (Fig. 5, part b: high-voltage spindle total duration; Table 1: total recording time). There was a significant drug treatment effect on high-voltage spindle total duration ($F(4,24) = 7.11$, $P < 0.001$ vs. saline). DOI 1.0 mg/kg significantly decreased high-voltage spindle total duration ($Z(6,1) = -2.20$, $P < 0.05$). Methysergide at 1.0 and 15.0 mg/kg blocked the decrease in high-voltage spindle total duration induced by DOI 1.0 mg/kg ($Z(5,2) = -1.77$, $P > 0.05$, and $Z(2,5) = -1.69$, $P > 0.05$, respectively vs. saline + saline), and 5.0 mg/kg methysergide further increased high-voltage spindle total duration vs. saline + saline when it was combined with DOI 1.0 mg/kg ($Z(1,6) = -2.20$, $P < 0.05$). Methysergide at 5.0 mg/kg more effectively blocked the effects of DOI 1.0 mg/kg than methysergide at 1.0 mg/kg ($Z(1,6) = -2.03$, $P < 0.05$), but not more effectively than the 15.0 mg/kg dose ($Z(1,6) = -1.86$, $P > 0.05$). Furthermore, there was a significant treatment effect on total recording time ($F(4,24) = 6.64$, $P = 0.001$). DOI 1.0 mg/kg significantly increased total recording time ($Z(0,7) = -2.37$, $P < 0.02$ vs. saline). Combination of DOI 1.0

mg/kg and methysergide 1.0 mg/kg had no effect on total recording time vs. saline ($Z(3,1) = -0.55$, $P > 0.1$), but decreased total recording time vs. DOI 1.0 mg/kg alone ($Z(7,0) = -2.37$, $P < 0.02$). Combination of DOI 1.0 mg/kg and methysergide 5.0 mg/kg had no effect on total recording time vs. saline ($Z(2,4) = -0.84$, $P > 0.1$), vs. DOI 1.0 mg/kg alone ($Z(5,2) = -1.35$, $P > 0.1$), or vs. DOI 1.0 mg/kg + methysergide 1.0 mg/kg ($Z(2,4) = -1.05$, $P > 0.1$). Combination of DOI 1.0 mg/kg + methysergide 15.0 mg/kg decreased recording time vs. saline ($Z(5,0) = -2.02$, $P < 0.05$), vs. DOI 1.0 mg/kg alone ($Z(7,0) = -2.37$, $P < 0.02$), and vs. DOI 1.0 mg/kg + methysergide 5.0 mg/kg ($Z(6,0) = -2.20$, $P < 0.05$), but not vs. DOI 1.0 mg/kg + methysergide 1.0 mg/kg ($Z(4,0) = -1.83$, $P > 0.05$).

Group VI

DOI intrathalamically + ketanserin systemically (s.c.) (Fig. 6, part a: high-voltage spindle total duration; Table 1: total recording time). Intrathalamic administration of saline did not affect high-voltage spindle total duration vs. baseline recording before the drug infusions ($Z(6,4) = -0.051$, $P > 0.1$). However, there was a significant drug treatment effect on high-voltage spindle total duration ($F(4,36) = 9.06$, $P < 0.001$). Intrathalamically administered DOI decreased high-voltage spindle total duration (DOI 10 μg: $Z(8,2) = -2.40$, $P < 0.02$; DOI 50 μg: $Z(10,0) = -2.80$, $P < 0.01$, vs. saline). Intrathalamical DOI at 50 μg more effectively decreased high-voltage spindle total duration than the 10 μg dose ($Z(8,2) = -1.99$, $P < 0.05$). When assessed from the polygraph charts with a ruler, the incidence, mean duration and total duration of high-voltage spindles did not differ between the right and left frontal cortices after intrathalamic DOI or saline administration into the right ventroposteromedial thalamic area, and there was no difference in the high-voltage spindle frequency or amplitude between the right and left frontal cortices. Systemic administration of ketanserin at 20 mg/kg alone increased high-voltage spindle total duration ($Z(1,9) = -2.60$, $P < 0.01$ vs. systemic saline) and blocked the decrease in high-voltage spindle total duration induced by intrathalamic DOI at the 50 μg dose ($Z(4,6) = -1.38$, $P > 0.1$ vs. systemic + intrathalamic saline). There was no difference in the high-voltage spindle total duration after intrathalamic saline administration recorded either before or after intrathalamic DOI recordings ($Z(7,3) = -0.76$, $P > 0.1$). Furthermore, there was also a significant treatment effect on total recording time ($F(4,36) = 10.46$, $P < 0.001$). Intrathalamic DOI 10 μg had no effect on total record-

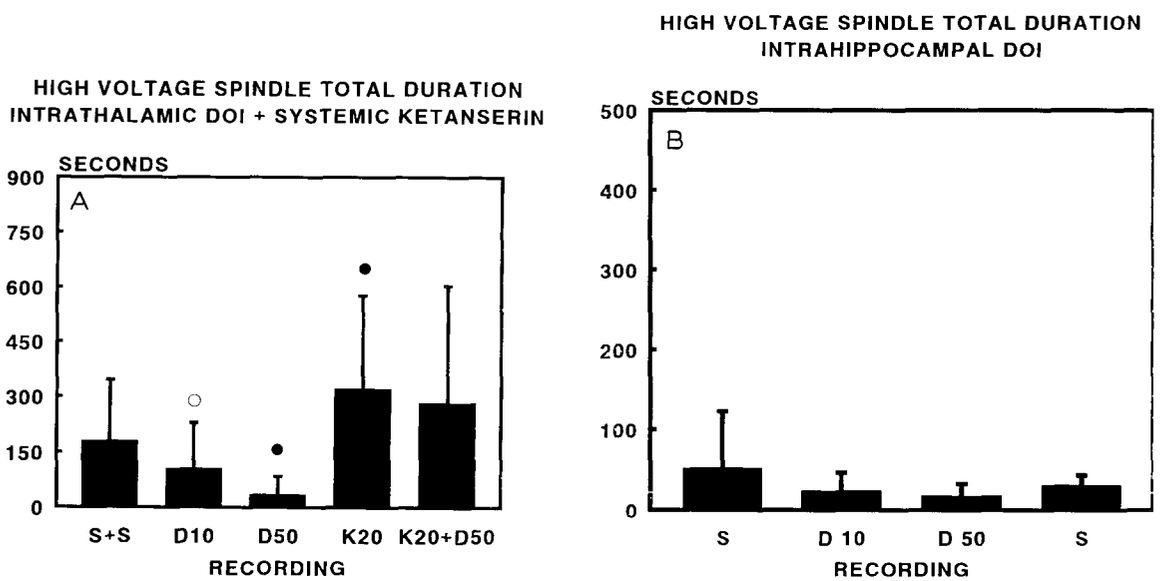

Fig. 6. Effects of intrathalamic (part A) and intrahippocampal (part B) administration of a 5-HT_2 receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (1.0 μl/rat, 10 min before recording), on the total duration (incidence × mean duration) of rat neocortical high-voltage spindles recorded during a 20-min cumulative behavioral waking-immobility period. The high-voltage spindle recordings were made every third day in 6-month-old male rats ($n = 10$ in part A, and $n = 5$ in part B). Values represent % group mean ± S.E.M. of control (saline treated) values (100%). Abbreviations: S + S = saline intrathalamically + saline s.c.; S = saline intrahippocampally; D10 = DOI 10 μg intrathalamically (part A) and intrahippocampally (part B); D50 = DOI 50 μg intrathalamically (part A) and intrahippocampally (part B); K20 = ketanserin 20 mg/kg s.c.; K20 + D50 = ketanserin 20 mg/kg s.c. + DOI 50 μg intrathalamically. Part A: Multivariable analysis of variance (MANOVA) followed by post-hoc analysis revealed that intrathalamic administration of DOI dose dependently decreased high-voltage spindle total duration, and systemic administration of a 5-HT_2 receptor antagonist, ketanserin (s.c. 4.0 ml/kg, 30 min before recording), blocked the effect of intrathalamic DOI. $^{\circ}$ $P < 0.02$ vs. saline (Wilcoxon), $^{\bullet}$ $P < 0.01$ vs. saline (Wilcoxon). Part B: MANOVA revealed no drug treatment effect after intrahippocampal DOI infusion.

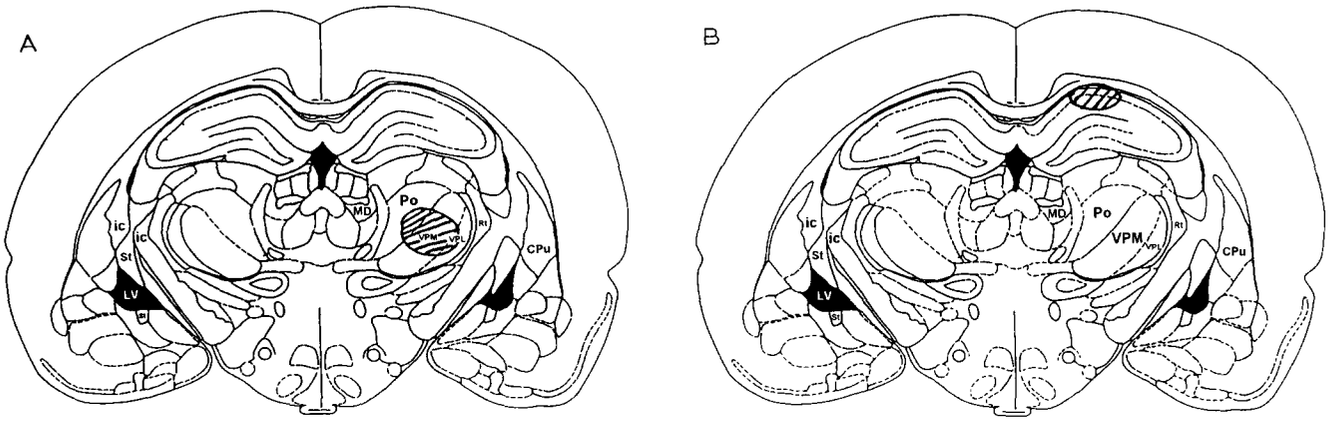

Fig. 7. A coronal reconstruction showing the placement of the cannulas which were implanted into the right ventromedial thalamic area ($n = 10$) (part A), or for control purposes into the right CA1 area of the hippocampus ($n = 5$) (part B) in 6-month-old male rats. The shaded area indicates the sites of the tips of the cannulas. Abbreviations: CPu = caudate putamen; ic = internal capsule; LV = lateral ventricle; MD = mediodorsal thalamic nucleus; Po = posterior thalamic nuclear group; Rt = reticular thalamic nucleus; st = stria terminalis; VPL = ventral posterolateral thalamic nucleus; VPM = ventral posteromedial thalamic nucleus.

ing time ($Z(2,8) = -1.89$, $P > 0.05$ vs. intrathalamic saline). Intrathalamic DOI 50 μg also had no effect on total recording time vs. intrathalamic saline ($Z(4,5) = -0.30$, $P > 0.1$) or DOI 10 μg ($Z(4,5) = -0.71$, $P > 0.1$). Systemic ketanserin 20.0 mg/kg decreased total recording time vs. intrathalamic + systemic saline ($Z(9,1) = -2.60$, $P < 0.01$), vs. intrathalamic DOI 10 μg ($Z(10,0) = -2.80$, $P < 0.01$), and vs. intrathalamic DOI 50 μg ($Z(10,0) = -2.80$, $P < 0.01$). Combination of intrathalamic DOI 50 μg + systemic ketanserin 20.0 mg/kg decreased total recording time vs. intrathalamic + systemic saline ($Z(8,1) = -2.25$, $P < 0.05$), vs. intrathalamic DOI 10 μg ($Z(10,0) = -2.80$, $P < 0.01$), and DOI 50 μg ($Z(10,0) = -2.80$, $P < 0.01$), but not vs. systemic ketanserin 20.0 mg/kg alone ($Z(1,3) = -0.37$, $P > 0.1$).

Group VII

DOI intrahippocampally (Fig. 6, part b: high-voltage spindle total duration; Table 1: total recording time). Intrahippocampal injection of saline did not affect high-voltage spindle total duration vs. baseline recording before drug infusions ($Z(2,3) = -0.54$, $P > 0.1$). Furthermore, there was no drug treatment effect on high-voltage spindle total duration after intrahippocampal injection of saline or DOI (10 and 50 μg) administrations ($F(2,8) = 1.53$, $P > 0.1$). There was no difference in high-voltage spindle total duration after intrahippocampal saline administration recorded either before or after intrahippocampal DOI recordings ($Z(2,3) = -0.40$, $P > 0.1$). The total recording time was not affected by intrahippocampal saline and DOI treatment ($F(2,8) = 0.73$, $P > 0.1$).

3.2. Histology

Histologycal analysis revealed that the tips of the cannulas were correctly located in the ventroposteromedial thalamic area in all the rats with intrathalamically implanted cannulas (Fig. 7, part a). Furthermore, in rats with intrahippocampal cannulas, the tips of the cannulas were located in hippocampal CA1 area (Fig. 7, part b).

4. Discussion

In recent years a number of different 5-HT receptor subtypes have been identified by pharmacological methods, and the heterogeneity of 5-HT receptors has been confirmed by the application of gene cloning techniques (Boess and Martin, 1994; Martin and Humphrey, 1994). The proposed criteria of the Serotonin Club Receptor Nomenclature Committee (see Humphrey et al., 1993; Martin and Humphrey, 1994) point to the existence of up to seven receptor classes for 5-HT (Humphrey et al., 1993; Martin and Humphrey, 1994). However, the understanding of the relationship of these receptor subclasses to physiological and behavioral functions has progressed much more slowly. Therefore, the present series of experiments was designed to investigate the role of 5-HT_1/5-HT_2 receptor subtypes in the modulation of rat thalamocortical oscillations as measured by neocortical high-voltage spindle activity.

When administered systemically, 8-OH-DPAT, a relatively selective 5-HT_{1A} receptor subtype agonist,

which may however also possess slight binding affinity for 5-ht$_7$ and perhaps 5-ht$_{5B}$ receptor subtypes (Boess and Martin, 1994), had no effect on neocortical high-voltage spindle activity of adult rats. In our preliminary studies, 8-OH-DPAT also failed to affect neocortical high-voltage spindle activity when about 80% of brain serotonin levels had been depleted by the serotonin synthesis inhibitor, *p*-chlorophenylalanine (400 mg/kg i.p. × 3), which also alone had no effect on neocortical high-voltage spindle activity (unpublished data), suggesting that neither pre- nor postsynaptic 5-HT$_{1A}$ receptors are significantly involved in the modulation of rat thalamocortical oscillations.

In contrast, systemic administration of a 5-HT$_2$ receptor agonist, DOI, significantly decreased neocortical high-voltage spindles. DOI is often regarded as a subtype selective 5-HT$_{2A}$ receptor agonist, but it may be equally active at 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors as well (Boess and Martin, 1994; Martin and Humphrey, 1994). The effect of DOI was blocked by the mixed 5-HT$_1$/5-HT$_2$ receptor antagonists, methiothepin and methysergide, as well as by 5-HT$_2$ receptor antagonists, ketanserin and ritanserin, emphasizing the involvement of 5-HT$_2$ receptors. Of the mixed 5-HT$_1$/5-HT$_2$ receptor antagonists that we used, methiothepin also significantly increased neocortical high-voltage spindles when it was administered alone. Interestingly, the best inhibition of the DOI-induced decrease in high-voltage spindles was achieved with the lowest dose of methiothepin tested. However, the higher doses of this antagonist were more effective in increasing high-voltage spindles on their own. Another mixed 5-HT$_1$/5-HT$_2$ receptor antagonist, methysergide, had no effect on neocortical high-voltage spindles when administered alone, but at all doses it was able to block the decrease in high-voltage spindle activity induced by DOI. The differences between the effects of methiothepin and methysergide, as well as the differences between the effects of different doses of methiothepin when administered alone or in combination with DOI, may be due to the fact that methiothepin is more effective than methysergide as an antagonist of the 5-HT autoreceptors, apparently a subtype (5-HT$_{1A}$) of the 5-HT$_1$ receptor group (Martin and Sanders-Bush, 1982), and that in addition to blocking 5-HT$_1$/5-HT$_2$ receptors, methiothepin, especially at higher doses, may also have anti-dopaminergic, as well as anti-histaminergic effects (Nelson et al., 1979), whereas methysergide may lack these effects (Leysen et al., 1981b). Therefore, the increase in high-voltage spindle activity observed with methiothepin in the present study may be related to its anti-dopaminergic effects; indeed intrastriatal injections of dopamine receptor blockers have been shown to increase the incidence of rat high-voltage spindles (Buzsáki et al., 1990c), and systemic injections of dopamine receptor antagonists increase rat spike-and-wave discharges (Warter et al., 1988). Furthermore, methiothepin may bind to 5-HT$_{1B}$, 5-ht$_6$ and 5-ht$_7$ receptors with higher affinity than methysergide. In contrast, methysergide may be more potent at 5-HT$_{1F}$, and 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors (Boess and Martin, 1994), although methiothepin may also have some affinity for 5-HT$_2$ receptor subtypes (Martin and Sanders-Bush, 1982; Peroutka, 1984). At 5-HT$_{1A}$, 5-ht$_{5A}$ and 5-ht$_{5B}$ receptor subtypes, these two agents appear to have the same potency (Boess and Martin, 1994). Thus, methiothepin and methysergide may not be regarded as subtype selective agents for 5-HT$_1$ or 5-HT$_2$ receptors (Boess and Martin, 1994).

Of the 5-HT$_2$ receptor antagonists, ketanserin, when administered alone at a relatively high dose (20.0 mg/kg), increased neocortical high-voltage spindle activity, and already at lower doses blocked the decrease in high-voltage spindles induced by DOI. However, at the dose of ketanserin that was required to increase high-voltage spindles in the present study (20.0 mg/kg), ketanserin may also have non-specific effects, especially binding to α_1-adrenoceptors and histamine (H$_1$) receptors (Leysen et al., 1981a). This might at least partially account for the observed increase in high-voltage spindle activity after the highest dose of ketanserin, since α_1-adrenoceptors have been shown to play an important role in the modulation of thalamic oscillations and related neocortical high-voltage spindles and spike-and-wave discharges (Micheletti et al., 1987; Buzsáki et al., 1990b; Riekkinen, Jr. et al., 1993d). Indeed, the increase in high-voltage spindle activity seen with the highest dose of ketanserin may be related to α_1-adrenoceptor blockade as another 5-HT$_2$ receptor antagonist, ritanserin, which in vivo does not bind to α-adrenergic sites (Leysen et al., 1985), had no effect on high-voltage spindle activity when administered alone, but, however, at the two highest doses blocked the decrease in high-voltage spindle activity seen after administration of DOI. Both ketanserin and ritanserin may display some selectivity for 5-HT$_{2A}$ receptors (Martin and Humphrey, 1994). Ketanserin may also display weak affinity for 5-HT$_{2C}$ receptors (30–50 times lower than its affinity for 5-HT$_{2A}$ receptors) and 5-HT$_{2B}$ receptors (Martin and Humphrey, 1994). Furthermore, ritanserin may bind to 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors with higher affinity than ketanserin and may also display some binding affinity for 5-ht$_6$ and 5-ht$_7$ receptors (Boess and Martin, 1994).

The results of systemic drug administrations suggested that 5-HT$_2$ receptors might play a role in the modulation of rat thalamocortical oscillations and related neocortical high-voltage spindles. However, when drugs are administered systemically, their peripheral effects cannot be excluded, and the site of action becomes a matter of speculation. It could be proposed that systemically infused serotonergic drugs may exert

their effects by affecting the behavioral or motor activity of the animal as an intermediate variable, rather than primarily affecting the thalamocortical system of the animals. Indeed, the discharge rates of the serotonergic neurons in the raphe nuclei in behaving animals are closely related to the activity of central motor systems, not in the sense that the raphe neurons would be motor or premotor themselves, but rather that their discharge rates covary with general levels and intensity of motor activity/behavioral state of the organism (Jacobs and Azmitia, 1992). Thus, as the discharge activity of the raphe serotonergic system is increased with the increase in behavioral arousal/motor activity, it has been proposed that the primary role of the serotonergic system in the brain may be to send information to target neurons regarding the level of motor activity/behavioral state of the organism (Jacobs and Azmitia, 1992). Furthermore, brain serotonin depletion in rats totally abolishes movement-but not immobility-related atropine-resistant (i.e. non-cholinergic) rhythmical slow activity in the hippocampus and low-voltage fast activity in the neocortex (Vanderwolf and Baker, 1986; Vanderwolf et al., 1989). Indeed, in the present study the recording time data (i.e. the total recording time needed to achieve a 20-min period of behavioral waking-immobility after the drug treatments) indicated that systemic treatments with 5-HT_1/5-HT_2 receptor subtype specific drugs did affect the behavioral or motor activity of the animals: of the mixed 5-HT_1/5-HT_2 receptor antagonists, methysergide had no effect, whereas methiothepine at the two highest doses used (1.0 and 5.0 mg/kg) significantly decreased total recording times, reflecting decreased behavioral/motor activity and induced obvious sedation/drowsiness. With respect to the 5-HT_2 receptor antagonists, ritanserin had no effect at all and only the highest (non-specific) dose (20.0 mg/kg) of ketanserin decreased total recording time. Systemic treatments with a 5-HT_2 receptor agonist, DOI, and a 5-HT_{1A} receptor agonist, 8-OH-DPAT, at higher doses significantly increased total recording times, reflecting increased behavioral or motor activity of the rats. Furthermore, DOI at the two highest doses used (1.0 and 2.0 mg/kg), in line with previous studies (Darmani et al., 1990), induced some head shakes. However, it is important to emphasize that the effects of drug treatment on neocortical high-voltage spindle activity reflect a relaxed behavioral waking-immobility state in the animals as we used a movement-sensor binding in the EEG cable magnet coil on the rat's heads which automatically excluded all the movement-related EEG epochs from the high-voltage spindle recordings. Thus, the obtained results reflect drug treatment effects on neocortical high-voltage spindle activity during quiet waking-immobility behavior per se, and not on behavioral/motor activity as an intermediate variable. However, to further control for the peripheral effects of the drugs we used also direct intrathalamic and control intrahippocampal infusions of a systemically effective drug, the 5-HT_2 receptor agonist, DOI.

Importantly, DOI decreased the neocortical high-voltage spindle activity of adult rats also when administered directly into the ventroposteromedial thalamus, and this effect was blocked by systemic administration of a relatively high dose of ketanserin, suggesting that activation of thalamic 5-HT_2 receptors may have suppressed the generation of thalamocortical oscillations and related neocortical high-voltage spindles. Furthermore, behavioral or motor activity as measured by total recording time was not affected and no head shakes were observed after intrathalamic DOI. It is unlikely that the effects of DOI on neocortical high-voltage spindle activity after intrathalamic infusion were due to the diffusion of the drug to other nearby brain structures after infusion. This is also indicated by the fact that infusion of DOI into a brain structure which is not thought to participate in the modulation of thalamocortical oscillations and related neocortical high-voltage spindles, namely the hippocampus, did not affect neocortical high-voltage spindle activity in the present study. No differences in high-voltage spindle incidence, mean or total duration, frequency or amplitude between the right and left frontal cortices after DOI or vehicle infusions to the right ventroposteromedial thalamic area were observed. This could be explained by the effects of drug infusion into the right ventroposteromedial thalamus being reflected in the network properties of nucleus reticularis thalamus – other thalamic nuclei – nucleus reticularis – circuitry (Buzsáki et al., 1990a,b,c; McCormick, 1992; Steriade et al., 1993) or direct anatomical connections between the right and left thalamic areas which have been described in the macaque monkey (Pare and Steriade, 1993), although these may not be as extensive in rats, as well as direct cortico-cortical connections (Vergnes et al., 1989). However, it is also important to note that in the present study we did not achieve a complete suppression of neocortical high-voltage spindle activity following intrathalamic DOI infusion, even at a relatively high dose (50 μg/1.0 μl/rat). This may be due to the fact that we used only unilateral intrathalamic DOI infusions. Therefore, the contralateral thalamocortical system and/or contralateral hemisphere may have been unaffected by intrathalamic DOI infusion and thus been able to produce thalamocortical oscillatory activity and related neocortical high-voltage spindles, which may then be immediately propagated to the whole of the cortex via the corpus callosum (Vergnes et al., 1989; Danober et al., 1994). Therefore, in order to fully understand the suppressant effects of intrathalamic DOI on neocortical high-voltage spindle activity, future studies with bilateral intrathalamic DOI infu-

sions should be performed. Furthermore, a relatively high systemic dose of ketanserin (20.0 mg/kg) was needed to block the high-voltage spindle activity decreasing effect seen after unilateral intrathalamic infusion of DOI. Ketanserin may itself have increased high-voltage spindle activity and may have bound to α_1-adrenoceptors and H_1 receptors (Leysen et al., 1981a). When ketanserin was used to block the effects of unilateral intrathalamic DOI infusion on high-voltage spindle activity, it may also have increased high-voltage spindles by affecting the untreated thalamocortical system or hemisphere. However, the observation that unilateral intrathalamic administration of DOI significantly decreased the neocortical high-voltage spindle activity of adult rats in the present study suggests that activation of 5-HT_2 receptors, possibly located in the thalamus, with a specific 5-HT_2 receptor agonist, DOI, may have been responsible for the decreasing effect on neocortical high-voltage spindle activity seen after intrathalamic drug infusion. Importantly, 5-HT_{2C} receptors (formerly 5-HT_{1C} receptors) (Humphrey et al., 1993; Boess and Martin, 1994; Martin and Humphrey, 1994) are expressed in the neurons of the rat thalamic sensory relay nuclei (Molineaux et al., 1989; Boess and Martin, 1994). Other 5-HT receptor subtypes which have been found to be expressed in the thalamus, as determined by Northern blot analysis of receptor mRNA, include 5-HT_{1A}, 5-HT_{1B} (only medial geniculate nucleus), 5-HT_{1F}, 5-HT_{2A}, 5-ht_{5A} and 5-ht_7 receptors (Boess and Martin, 1994).

In previous studies, no significant role for serotonin or different serotonin receptor subtypes in the modulation of rat thalamocortical oscillations, as measured by neocortical high-voltage spindles (Riekkinen, Jr. et al., 1991a) or spike-and-wave discharges (Marescaux et al., 1992), has been reported. For example, the increased availability of serotonin following injection of its precursor, 5-hydroxytryptophan, had no effect on spike-and-wave discharges (Marescaux et al., 1992), and inhibitors of serotonin reuptake, fluvoxamine at high doses slightly reduced and indalpine at high doses slightly increased spike-and-wave discharges (Marescaux et al., 1992), while alaproclate had no effect on the increase in high-voltage spindle activity occurring in aged rats (Riekkinen, Jr. et al., 1991a). Large depletions of serotonin by *p*-chlorophenylalanine had no effect on spike-and wave discharges (Marescaux et al., 1992) or high-voltage spindles (personal unpublished observation). Partial lesions of the nucleus raphe dorsalis alone had no effect on neocortical high-voltage spindles, but further aggravated the increase in high-voltage spindle activity induced by partial lesions of the cholinergic nucleus basalis (Riekkinen, Jr. et al., 1990a). Furthermore, in line with the present results in neocortical high-voltage spindles, methysergide and ritanserin had no effect and ketanserin at relatively high doses ($\geq$ 10 mg/kg) increased spike-and-wave discharges (Marescaux et al., 1992), and methysergide was ineffective in preventing the elevation in high-voltage spindle activity which is found in aged rats (Riekkinen, Jr. et al., 1991a). Finally, treatment with ondansetron, a 5-HT_3 receptor antagonist, did not affect the age-related increase in high-voltage spindles in rats (Riekkinen, Jr. et al., 1991a). However, as pharmacological and molecular biological techniques have indicated the existence of up to at least seven receptor subclasses for serotonin (Humphrey et al., 1993; Boess and Martin, 1994; Martin and Humphrey, 1994) further work will be needed to clarify the role(s) of other serotonin receptor subtypes in the modulation of rat thalamocortical oscillations and their related neocortical high-voltage spindles and spike-and-wave discharges.

In conclusion, the present results showing that activation of 5-HT_2 receptors, possibly located in the thalamus, with a specific 5-HT_2 receptor agonist, DOI, can modulate rat neocortical high-voltage spindle activity are in line with previous in vitro (Pape and McCormick, 1989; McCormick and Wang, 1991; McCormick, 1992; Steriade et al., 1993) and in vivo (Riekkinen, Jr. et al., 1990a; Marescaux et al., 1992) results, and support the view that the serotonergic system may complement the cholinergic, noradrenergic and dopaminergic systems (Levey et al., 1987; Micheletti et al., 1987; Buzsáki et al., 1988, 1990a,b,c; Warter et al., 1988; Riekkinen, Jr. et al., 1990b, 1991b, 1993a,b,c,d; Steriade and Buzsáki, 1990; McCormick, 1992; Danober et al., 1993, 1994; Steriade et al., 1993) in modulating rat thalamocortical oscillations, and consequently may modulate the transfer of information through the thalamus to the cortex and other structures.

Acknowledgements

This study was financially supported by the Finnish Academy of Sciences, the Farmos Research and Science Foundation and the Finnish Neurological Society. The authors wish to thank Mrs. Anna-Liisa Gidlund for histological analysis, and Ewen MacDonald, Ph.D., for revising the language of the manuscript.

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