ELSEVIER

European Journal of Pharmacology 299 (1996) 47–60

ejp

Suppression of neocortical high-voltage spindles by nicotinic acetylcholine and 5-HT_2 receptor stimulation

Pekka Jäkälä [a,b,*], Markus Björklund [a], Paavo Riekkinen Jr. [a,b]

[a] *University of Kuopio, Department of Neurology, P.O. Box 1627, FIN-70211 Kuopio, Finland*
[b] *Kuopio University Hospital, Department of Neurology, P.O. Box 1627, FIN-70211 Kuopio, Finland*

Received 22 May 1995; revised 27 November 1995; accepted 1 December 1995

Abstract

To investigate the roles of the nicotinic acetylcholine receptor and the serotonin (5-hydroxytryptamine; 5-HT) subtype 2 receptor in the modulation of rat thalamocortical oscillations, the effects of systemic (s.c.) administration of nicotine, a nicotinic acetylcholine receptor agonist, and 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a 5-HT_2 receptor agonist, on neocortical high-voltage spindle activity occurring during quiet waking-immobility behavior in aged (28 months of age) and adult (7 months of age) rats were studied. Nicotine 0.1 and 0.3 mg/kg alleviated the age-related increase of neocortical high-voltage spindles, whereas in adult rats only nicotine 0.3 mg/kg was effective. DOI 0.3, 1.0 and 2.0 mg/kg suppressed high-voltage spindles in both aged and adult rats. In aged rats, a combination of subthreshold doses of nicotine (0.03 mg/kg) and DOI (0.1 mg/kg) decreased neocortical high-voltage spindles, whereas in adult rats two different subthreshold dose combinations (nicotine 0.03 or 0.1 mg/kg + DOI 0.1 mg/kg) had no effect. *p*-Chlorophenylalanine (400 mg/kg/day i.p. for 3 consecutive days) treatment decreased brain serotonin concentration (> 80% reduction), but did not affect high-voltage spindles. However, in both aged and adult rats, *p*-chlorophenylalanine treatment blocked the decrease in high-voltage spindle activity produced by DOI 0.3 mg/kg, though not the decrease produced by higher doses of DOI (1.0 and 2.0 mg/kg). It is important that, in adult rats, *p*-chlorophenylalanine treatment was able to abolish the decrease in high-voltage spindle activity seen after a relatively high dose of nicotine (0.3 mg/kg). The results suggest that nicotinic acetylcholine and 5-HT_2 receptors may act in concert to suppress neocortical high-voltage spindling in rats, and that intact brain serotonergic systems may be important for some of the therapeutic effects of nicotine.

Keywords: Nicotinic acetylcholine receptor; 5-HT_2 receptor; Neocortical high-voltage spindle; Thalamocortical oscillation; (Rat)

1. Introduction

In waking-immobile rats, brief bursts of synchronized oscillatory high-voltage spindle activity may occur in the neocortical electroencephalograph (EEG) in some rats (Micheletti et al., 1987; Buzsáki et al., 1988, 1990; Sirviö et al., 1989; Riekkinen, Jr. et al., 1991a). Typically, neocortical high-voltage spindles occur only during low arousal and low vigilant states (drowsiness), being virtually absent during high vigilance states (Buzsáki et al., 1990). During neocortical high-voltage spindles, rhythmically active γ-aminobutyric acid (GABA)-containing nucleus reticularis thalamus neurons phasically hyperpolarize their thalamocortical target neurons and, in the absence of other depolarizing inputs, voltage- and time-dependent rebound Ca^{2+} spikes occur in a phase-locked manner in thalamocortical relay neurons (Steriade and Llinás, 1988; Buzsáki et al., 1990; McCormick, 1992; Steriade et al., 1993). The rhythmical bursts of thalamocortical neurons are transferred to the cortex also, where they induce excitatory postsynaptic potentials in cortical pyramidal neurons, thereby generating the EEG neocortical high-voltage spindles (Steriade et al., 1993). The transfer of information through the thalamus to the cortex and other structures may be disrupted during thalamic oscillatory activity (McCormick, 1992).

Factors that suppress rhythmic burst firing in nucleus reticularis neurons, and produce tonic depolarization or block hyperpolarization in thalamocortical relay neurons are regarded as anti-oscillatory factors (Buzsáki et al., 1990). The ascending subcortical neurotransmitter systems, e.g. cholinergic (Levey et al., 1987; Buzsáki et al., 1988; Riekkinen, Jr. et al., 1990, 1991b, 1992, 1993a, b, 1995; Steriade and Buzsáki, 1990; McCormick, 1992; Danober et

* Corresponding author. Tel.: 358-71-162014; fax: 358-71-162048.

SSDI 0014-2999(95)00833-0

al., 1993, 1994), noradrenergic (Micheletti et al., 1987; Buzsáki et al., 1990; Riekkinen, Jr. et al., 1991b; Jäkälä et al., 1992; McCormick, 1992), dopaminergic (Buzsáki et al., 1990; Warter et al., 1988), histaminergic (McCormick, 1992), and serotonergic (Riekkinen, Jr. et al., 1990; Marescaux et al., 1992; McCormick, 1992; Jäkälä et al., 1995) systems may suppress the generation of thalamocortical oscillations and facilitate the effective processing of information in thalamocortical systems.

Anatomical studies have shown that the thalamic nuclei receive both cholinergic and serotonergic (5-hydroxytryptamine; 5-HT) fibers that can regulate thalamic functioning (Steinbusch, 1981; McCormick, 1992; Wainer and Mesulam, 1990; Jacobs and Azmitia, 1992), and both in vitro and in vivo electrophysiological studies support a role for the muscarinic and nicotinic acetylcholine receptors and 5-HT receptors in the suppression of thalamocortical oscillations (Pape and McCormick, 1989; McCormick and Wang, 1991; McCormick, 1992; Riekkinen, Jr. et al., 1993a, b, 1995; Jäkälä et al., 1995). Therefore, as the number and duration of high-voltage spindles increases with age in rats (Buzsáki et al., 1988, 1990; Sirviö et al., 1989; Riekkinen, Jr. et al., 1991a), and as during aging, deficiences in both cholinergic (Wu et al., 1988; Riekkinen, Jr. et al., 1992) and serotonergic (Allen et al., 1983; Brunello et al., 1988) markers are observed in rat brain, it is possible that impaired functioning of subcortical modulatory systems, such as cholinergic and serotonergic systems, may contribute to aging-related electrophysiological abnormalities in thalamocortical systems (Buzsáki et al., 1988, 1990; Sirviö et al., 1989; Riekkinen, Jr. et al., 1991a). The aim of the present experiments was to study whether the nicotinic acetylcholine and 5-HT_2 receptor subtypes (1) interact in the modulation of rat thalamocortical oscillations as measured via neocortical high-voltage spindles and (2) suppress the age-related increase in high-voltage spindles. Therefore, we studied the effects of systemic single-dose and combined subthreshold dose injections of a nicotinic acetylcholine receptor agonist, nicotine, and a 5-HT_2 receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), on neocortical high-voltage spindle activity in rats. To study the possibility that combined deficiences in nicotinic acetylcholine and 5-HT_2 receptor-mediated functions would underlie the age-related increase in rat neocortical high-voltage spindle activity, both adult and aged rats were used. Furthermore, brain serotonin synthesis inhibition by *p*-chlorophenylalanine was used to study the effects of nicotine and DOI in aged and adult rats with dysfunctioning serotonergic systems.

2. Materials and methods

2.1. Animals

Male Han:Wistar rats (aged rats 28 months of age, $n = 16$ in the first group and $n = 9$ in the second group; adult rats 7 months of age, $n = 14$ in the first group and $n = 8$ in the second group) were used in the present study. 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 (Garratt et al., 1991; Jäkälä et al., 1993, 1995; Riekkinen, Jr. et al., 1993a, 1994; Riekkinen et al., 1993, 1994a, b; Riekkinen, Jr., 1994; Riekkinen, Jr. and Riekkinen, 1995). Nicotine tartrate (Sigma, USA) (0.03, 0.1 and 0.3 mg/kg) and (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) hydrochloride (Research Biochemicals International, USA) (0.1, 0.3, 1.0 and 2.0 mg/kg) were dissolved in saline and injected 2.0 ml/kg s.c. 25 min before recordings of neocortical high-voltage spindles. *p*-Chlorophenylalanine (Sigma, USA) was mixed in saline containing a 0.5% suspension of gum arabic (Sigma, USA) (mixed fresh every day) and injected i.p. (400 mg/kg/5.0 ml) on each of three consecutive days. For control purposes gum arabic was suspended in NaCl 0.9% (0.5% solution, mixed fresh every day) and injected i.p. (5.0 ml/kg) on each of three consecutive days.

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. A 2-week recovery period after implantation of the recording electrodes was allowed before any recordings were started.

2.4. High-voltage spindle recordings

Before recordings, the rats were placed twice into the recording cages for 10 min to adapt to the recording environment. To ascertain that the high-voltage spindle levels would be as constant as possible, five 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 high-voltage spindle total duration were observed between the fourth and the

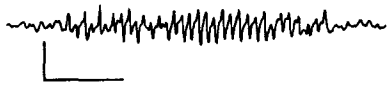

Fig. 1. Example of a typical neocortical high-voltage spindle episode recorded from the right active recording electrode above the frontal cortex (A = 1.0 mm and L = 2.0 mm relative to the bregma) during a period of behavioral waking-immobility (eyes open, head held up). The amplitude scale (vertical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

fifth baseline recording sessions (data not shown). To control for the circadian variations or arousal level, recordings after the test drug injections were made at the same time on the various recording days for individual animals. Recordings were made between 09:00–15:00 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., 1990, 1991a, b, 1992, 1993a, b, 1995; Jäkälä et al., 1992, 1995), (4) the time 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 recorded by the magnetic coil binding 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.

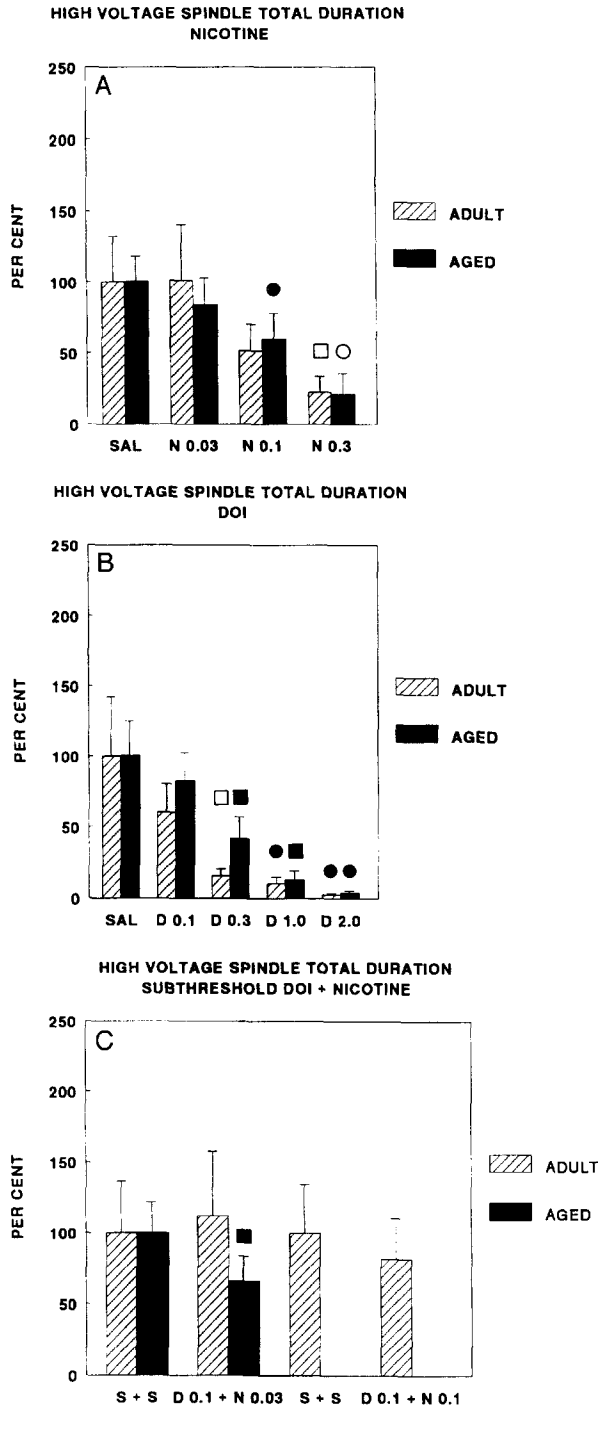

Fig. 2. Effects of systemic administration (s.c. 2.0 ml/kg, 25 min before recording session) of a nicotinic acetylcholine receptor agonist, nicotine (0.03, 0.1 and 0.3 mg/kg), and a 5-HT_2 receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (0.1, 0.3, 1.0 and 2.0 mg/kg) on the total duration (incidence × mean duration) of neocortical high-voltage spindles in aged (28 months of age, $n = 16$) and adult (7 months of age, $n = 14$) rats recorded during a 20-min cumulative behavioral waking-immobility period. The high-voltage spindle recordings were made every third day in a counterbalanced order. Values represent % group means ± S.E.M. of control (saline-treated) values (100%). Abbreviations: SAL = saline; N = nicotine (doses mg/kg); D = DOI (doses mg/kg). Part A: Multivariable analysis of variance (MANOVA) followed by post-hoc analysis revealed that the two highest doses of nicotine ($^{\bullet}$ $P < 0.01$, $^{\circ}$ $P < 0.002$ vs. saline) decreased high-voltage spindle total duration in aged rats ($F(3,45) = 17.43$, $P < 0.001$), whereas in adult rats only the highest dose of nicotine ($^{\square}$ $P < 0.05$ vs. saline) decreased high-voltage spindles ($F(3,39) = 3.90$, $P < 0.02$). Part B: The three highest doses of DOI ($^{\blacksquare}$ $P < 0.001$, and $^{\bullet}$ $P < 0.01$ vs. saline) significantly decreased neocortical high-voltage spindle activity in aged rats ($F(3,42) = 12.09$, $P < 0.001$) as well as in adult rats ($F(3,39) = 4.01$, $P < 0.02$) ($^{\square}$ $P < 0.05$, and $^{\bullet}$ $P < 0.01$ vs. saline). Part C: The combination of subthreshold doses of nicotine (0.03 mg/kg) and DOI (0.1 mg/kg) that were ineffective alone significantly decreased neocortical high-voltage spindle activity in aged rats ($Z(14,1) = -3,35$, $^{\blacksquare}$ $P < 0.001$ vs. saline + saline), whereas in adult rats two different subthreshold dose combinations had no effect (nicotine 0.03 mg/kg + DOI 0.1 mg/kg: $Z(4,9) = -0.91$, $P > 0.1$; nicotine 0.1 mg/kg + DOI 0.1 mg/kg: $Z(3,7) = -1.07$, $P > 0.1$ vs. saline, respectively).

Table 1
Effects of systemic administration (s.c. 2.0 ml/kg, 25 min before recording sessions) of a nicotinic acetylcholine receptor agonist, nicotine, and a 5-HT_2 receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), on the total recording times (the total time needed to achieve a cumulative 20-min period of quiet behavioral waking-immobility = immobility + movement periods) in aged (28 months of age) rats

Treatment	Total recording time (min)
($n = 16$)	
Saline	25.9 ± 0.75
Nicotine	
0.03 mg/kg	25.7 ± 1.19
0.1 mg/kg	25.8 ± 0.77
0.3 mg/kg	29.1 ± 1.78
MANOVA	$F(3,45) = 3.57$
P	> 0.05
($n = 15$)	
Saline	25.9 ± 0.83
DOI	
0.1 mg/kg	29.4 ± 1.34 [d]
0.3 mg/kg	36.2 ± 2.08 [b]
1.0 mg/kg	33.3 ± 1.87 [d]
MANOVA	$F(3,42) = 18.97$
P	< 0.001
($n = 9$)	
Saline	24.5 ± 0.60
DOI	
1.0 mg/kg	33.1 ± 2.97 [d]
2.0 mg/kg	39.4 ± 2.58 [c]
MANOVA	$F(2,16) = 16.40$
P	< 0.001
($n = 15$)	
Saline + saline	23.6 ± 0.80
DOI 0.1 mg/kg + nicotine 0.03 mg/kg	26.3 ± 1.31 [c]
Wilcoxon	$Z(3,11) = -2.73$
P	< 0.01
($n = 6$ in PCPA- and $n = 6$ in GA-treated rats)	
Saline before:	
– PCPA	24.3 ± 1.61
– GA	22.5 ± 0.94
Saline after:	
– PCPA	23.0 ± 0.80
– GA	22.7 ± 1.16
MANOVA	
Treatment effect	$F(1,10) = 1.13$
P	> 0.1
Group by treatment	$F(1,10) = 0.61$
P	> 0.1
($n = 6$ in PCPA- and $n = 6$ in GA-treated rats)	
Saline:	
– PCPA	23.0 ± 0.80
– GA	22.7 ± 1.16
Nicotine 0.1 mg/kg:	
– PCPA	22.8 ± 1.21
– GA	26.3 ± 1.97 [d]
MANOVA	
Treatment effect	$F(1,10) = 3.6$
P	> 0.05
Group by treatment	$F(1,10) = 4.31$
P	> 0.1
($n = 6$ in PCPA- and $n = 6$ in GA-treated rats)	
Saline:	
– PCPA	24.0 ± 1.12
– GA	25.7 ± 1.57
DOI 0.3 mg/kg:	
– PCPA	34.3 ± 4.11 [d]
– GA	34.5 ± 3.18 [d]
MANOVA	
Treatment effect	$F(1,10) = 22.05$
P	$= 0.001$
Group by treatment	$F(1,10) = 0.01$
P	> 0.1
($n = 7$)	
Saline:	
– PCPA	22.6 ± 1.31
DOI 1.0 mg/kg:	
– PCPA	29.9 ± 2.04 [d]
DOI 2.0 mg/kg:	
– PCPA	36.0 ± 2.04 [d]
MANOVA	$F(2,12) = 27.37$
P	< 0.001
($n = 6$ in PCPA- and $n = 6$ in GA-treated rats)	
Saline + saline:	
– PCPA	26.5 ± 1.43
– GA	24.2 ± 1.88
Nicotine 0.03 mg/kg + DOI 0.1 mg/kg:	
– PCPA	30.8 ± 3.13
– GA	33.2 ± 2.01
MANOVA	
Treatment effect	$F(1,10) = 3.84$
P	> 0.05
Group by treatment	$F(1,10) = 0.47$
P	> 0.1

The drug effects before and after *p*-chlorophenylalanine (PCPA) treatment (400 mg/kg/5.0 ml/day i.p. for three consecutive days) -induced brain serotonin depletion are shown. Gum arabic (GA) (0.5% solution) was used as a control treatment for *p*-chlorophenylalanine. Movement periods were recorded automatically and excluded from the high-voltage spindle total duration data analysis by magnet-coil movement-sensor detector of EEG cables which were bound on the rat's head. Multivariable analysis of variance (MANOVA) followed by the post-hoc Wilcoxon signed rank test was used for statistical analysis. Values represent means ± S.E.M. [a] $P < 0.001$, [b] $P < 0.002$, [c] $P < 0.01$ and [d] $P < 0.05$ vs. controls (saline) (Wilcoxon signed rank test).

Following the baseline recordings, the effects of nicotine and DOI on neocortical high-voltage spindle activity were tested. The effects of saline and different doses of nicotine (0.03, 0.1, 0.3 mg/kg) were tested in a counterbalanced order every third day in the first groups of aged and adult rats. Thereafter, a 10-day wash-out period was allowed. During the wash-out period, one aged rat lost its EEG electrode dental acrylic connection and was then excluded from further recordings. The effects of saline and different doses of DOI (0.1, 0.3, 1.0 mg/kg) were tested in a counterbalanced order every third day. After a 1-week

wash-out period, the effects of saline + saline and combinations of subthreshold doses of nicotine (0.03 mg/kg) and DOI (0.1 mg/kg) were tested in a counterbalanced order. A 3-day period was allowed between these recordings. In the second group of aged and adult rats, the effects of saline and higher doses of DOI (1.0 and 2.0 mg/kg) were tested in a counter-balanced order every third day. Furthermore, in this second group of adult rats the effects of saline + saline and another subthreshold combination of nicotine (0.1 mg/kg) and DOI (0.1 mg/kg) were tested in a counterbalanced order. A 3-day period was allowed between these recordings.

One week after this part of the study, in the first group, 8 aged and 7 adult rats were treated with *p*-chlorophenylalanine and, for control purposes, 7 aged and 7 adult rats received gum arabic treatment. Two aged *p*-chlorophenylalanine-treated rats showed signs of illness (i.e. did not eat their food pellets) after treatment and were excluded from further recordings. On the day following the last injection of *p*-chlorophenylalanine or gum arabic, a baseline recording without any injections was made. Thereafter, the neocortical high-voltage spindle recordings were made on consecutive days in the following order: (1) saline, (2) nicotine 0.1 mg/kg, (3) saline, (4) DOI 0.3 mg/kg, (5) saline + saline, (6) subthreshold doses of nicotine (0.03 mg/kg) + DOI (0.1 mg/kg). One aged and two adult gum arabic-treated rats and one adult *p*-chlorophenylalanine-treated rat lost their EEG electrode dental acrylic connections before the recordings were finished and were excluded from statistical analysis. In the second group of aged and adult rats, all the rats were treated with *p*-chlorophenylalanine. Thereafter, in aged rats the recordings were made in the following order: (1) saline, (2) DOI 1.0 mg/kg, (3) DOI 2.0 mg/kg every third day. In adult rats

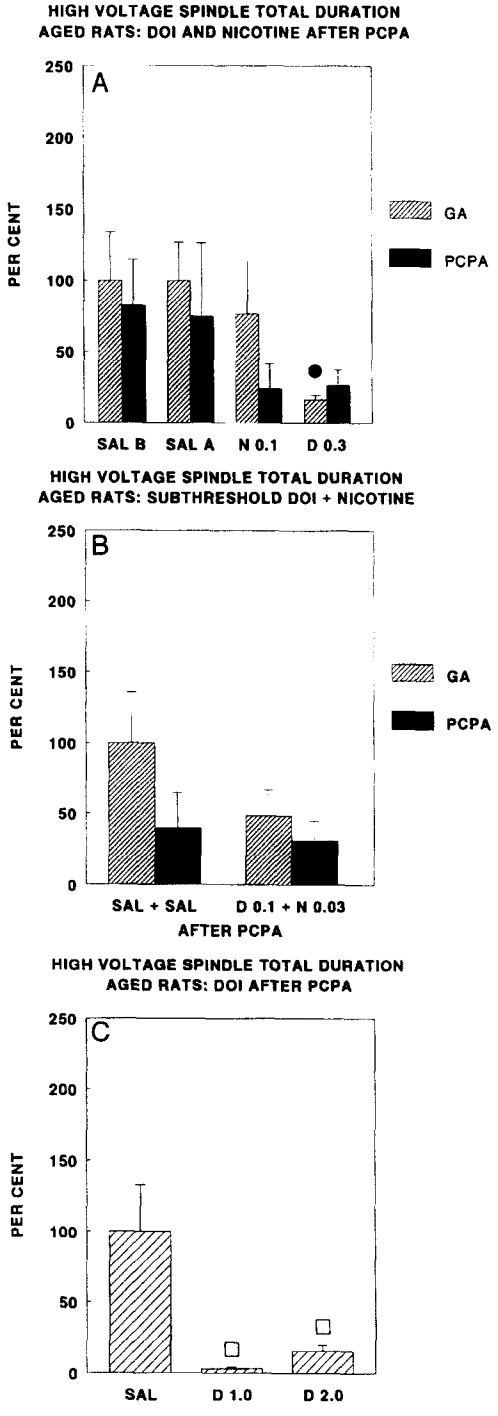

Fig. 3. Effects of *p*-chlorophenylalanine (PCPA) (400 mg/kg/5.0 ml/day i.p. for three consecutive days) treatment-induced brain serotonin synthesis inhibition on the effects of systemic administration (s.c. 2.0 ml/kg, 25 min before recording session) of a nicotinic acetylcholine receptor agonist, nicotine, and a 5-HT_2 receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) on the total duration (incidence × mean duration) of neocortical high-voltage spindles in aged (28 months of age, $n = 16$) rats recorded during a 20-min cumulative behavioral waking-immobility period. Gum arabic (GA) (0.5% solution) was used as a control treatment for *p*-chlorophenylalanine. The high-voltage spindle recordings were made every day. Values represent % group means ± S.E.M. of control (saline-treated) values (100%). Abbreviations: SAL A = saline before *p*-chlorophenylalanine or gum aragic treatment; SAL B = saline after *p*-chlorophenylalanine or gum arabic treatment. For other abbreviations see Fig. 2. Doses of nicotine and DOI are expressed as mg/kg. Part A: Multivariable analysis of variance revealed that *p*-chlorophenylalanine treatment had no effect on high-voltage spindle total duration in aged rats (MANOVA; Treatment effect: $F(1,10) = 2.24$, $P > 0.1$; Group by treatment effect: $F(1,10) = 1.49$, $P > 0.1$), and that nicotine (0.1 mg/kg) did not affect high-voltage spindle total duration in either *p*-chlorophenylalanine- or control-treated aged rats (Treatment effect: $F(1,10) = 4.23$, $P > 0.1$; Group by treatment effect: $F(1,10) = 0.61$, $P > 0.1$) whereas DOI (0.3 mg/kg) decreased high-voltage spindle total duration only in the control, but not in *p*-chlorophenylalanine-treated aged rats (Treatment effect: $F(1,19 = 12.17$, $P < 0.01$; Group by treatment effect: $F(1,10) = 5.61$, $P < 0.05$; $^{\bullet}$ $P < 0.01$). Part B: The combination of subthreshold doses of nicotine (0.03 mg/kg) and DOI (0.1 mg/kg) that alone were ineffective but in combination decreased high-voltage spindle total duration before *p*-chlorophenylalanine treatment, did not affect high-voltage spindle total duration in controls or *p*-chlorophenylalanine-treated rats (Treatment effect: $F(1,10) = 3.21$, $P > 0.1$; Group by treatment effect: $F(1,10) = 1.64$, $P > 0.1$). Part C: The highest doses of DOI (1.0 mg/kg: $P < 0.05$, and 2.0 mg/kg: $P < 0.05$ vs. saline, respectively; $^{\square}$ $P < 0.05$) were still capable of decreasing neocortical high-voltage spindle total duration in the group of *p*-chlorophenylalanine-treated rats ($F(2,12) = 9.68$, $P < 0.005$).

Table 2
Effects of systemic administration (s.c. 2.0 ml/kg, 25 min before recording sessions) of a nicotinic acetylcholine receptor agonist, nicotine, and a 5-HT_2 receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), on the total recording times (the total time needed to achieve a cumulative 20-min period of quiet behavioral waking-immobility = immobility + movement periods) in adult (7 months of age) rats

Treatment	Total recording time (min)
($n = 14$)	
Saline	28.4 ± 1.22
Nicotine	
0.03 mg/kg	27.5 ± 1.14
0.1 mg/kg	29.9 ± 1.41
0.3 mg/kg	35.1 ± 3.11 [d]
MANOVA	$F(3,39) = 5.58$
P	< 0.005
($n = 14$)	
Saline	29.0 ± 1.53
DOI	
0.1 mg/kg	29.2 ± 2.08
0.3 mg/kg	31.4 ± 2.44
1.0 mg/kg	32.5 ± 2.91
MANOVA	$F(3,39) = 1.05$
P	> 0.1
($n = 11$)	
Saline	25.4 ± 0.73
DOI	
1.0 mg/kg	34.0 ± 3.16 [d]
2.0 mg/kg	43.6 ± 1.71 [c]
MANOVA	$F(2,20) = 30.82$
P	< 0.001
($n = 14$)	
Saline + saline	27.7 ± 1.47
DOI 0.1 mg/kg + nicotine 0.03 mg/kg	24.7 ± 1.30
Wilcoxon	$Z(10,3) = -1.82$
P	> 0.05
($n = 10$)	
Saline + saline	28.9 ± 2.13
DOI 0.1 mg/kg + nicotine 0.1 mg/kg	32.9 ± 2.57
Wilcoxon	$Z(7,2) = -1.54$
P	> 0.1
($n = 6$ in PCPA- and $n = 4$ in GA-treated rats)	
Saline before:	
– PCPA	27.2 ± 2.01
– GA	25.8 ± 2.66
Saline after:	
– PCPA	22.2 ± 1.39 [d]
– GA	21.3 ± 1.10 [d]
MANOVA	
Treatment effect	$F(1,8) = 9.93$
P	< 0.02
Group by treatment	$F(1,8) = 0.47$
P	> 0.1
($n = 6$ in PCPA- and $n = 4$ in GA-treated rats)	
Saline:	
– PCPA	22.2 ± 1.39
– GA	21.3 ± 1.10
Nicotine 0.1 mg/kg:	
– PCPA	21.5 ± 0.67
– GA	21.8 ± 1.39
MANOVA	
Treatment effect	$F(1,8) = 0.01$
P	> 0.1
Group by treatment	$F(1,8) = 0.56$
P	> 0.1
($n = 8$)	
Saline:	
– PCPA	23.8 ± 0.98
Nicotine 0.3 mg/kg:	
– PCPA	25.4 ± 1.70
Wilcoxon	$Z(2,3) = -0.40$
P	> 0.1
($n = 6$ in PCPA- and $n = 4$ in GA-treated rats)	
Saline:	
– PCPA	22.2 ± 1.39
– GA	21.3 ± 1.10
DOI 0.3 mg/kg:	
– PCPA	36.5 ± 4.61 [d]
– GA	31.3 ± 5.20
MANOVA	
Treatment effect	$F(1,8) = 16.40$
P	< 0.005
Group by treatment	$F(1,8) = 0.52$
P	> 0.1
($n = 8$)	
Saline:	
– PCPA	26.0 ± 1.21
DOI 1.0 mg/kg:	
– PCPA	38.3 ± 1.63 [c]
DOI 2.0 mg/kg:	
– PCPA	37.6 ± 0.83 [d]
MANOVA	$F(2,14) = 27.7$
P	< 0.001
($n = 6$ in PCPA- and $n = 4$ in GA-treated rats)	
Saline + saline:	
– PCPA	24.8 ± 1.48
– GA	24.0 ± 0.70
Nicotine 0.03 mg/kg + DOI 0.1 mg/kg:	
– PCPA	26.8 ± 2.33
– GA	25.8 ± 0.50
MANOVA	
Treatment effect	$F(1,8) = 5.78$
P	< 0.05
Group by treatment	$F(1,8) = 0.03$
P	> 0.1
($n = 8$)	
Saline + saline:	
– PCPA	26.0 ± 1.21
DOI 0.1 mg/kg + nicotine 0.1 mg/kg:	
– PCPA	30.4 ± 3.21
Wilcoxon	$Z(6,2) = -1.54$
P	> 0.1

the following recordings were made every second day: (1) saline, (2) nicotine 0.3 mg/kg, (3) saline, (4) DOI 1.0 mg/kg, (5) DOI 2.0 mg/kg, (6) saline + saline, (7) nicotine 0.1 mg/kg + DOI 0.1 mg/kg.

2.5. Biochemistry

The rats were decapitated on the day following the last recordings. The brain was removed rapidly, dissected on ice and stored at −80°C. For assay, tissue samples were weighed and homogenized into distilled water (Potter, 10 times, 1000 rpm) (Braun, Germany). We had demonstrated (Jäkälä et al., 1993; Riekkinen et al., 1993, 1994b; Riekkinen, Jr. et al., 1994; Riekkinen, Jr. and Riekkinen, 1995) that the changes in concentrations of 5-HT, its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline, dopamine and its metabolite, homovanillic acid, induced by *p*-chlorophenylalanine (400 mg/kg/day i.p. on three consecutive days) treatment did not differ between frontal cortical, parieto-occipito cortical and hippocampal samples. Therefore, we used only frontal cortical samples for biochemical analysis. The method for determination of catecholamines, indoleamines and metabolites has been described previously in detail (Jäkälä et al., 1993).

2.6. Statistics

The multivariable analysis of variance (MANOVA) was used to analyse the overall drug treatment effects on neocortical high-voltage spindles. The post-hoc Wilcoxon signed rank test was used to analyse the differences between effects of various drug doses. High-voltage spindle incidences, mean durations and total durations (= incidence × mean duration), as well as total recording times (i.e. the total recording time needed to achieve a 20-min period of behavioral waking-immobility related EEG; movement periods were automatically excluded by the magnet coil movement sensor binding on the rat's head) were analyzed separately for each drug. The changes seen in rat neocortical high-voltage spindle activity were mainly due to changes in high-voltage spindle incidence and total duration, so that when high-voltage spindle incidence was decreased by the drug treatment, the high-voltage spindle total duration was correspondingly decreased, i.e. when a neocortical high-voltage spindle appeared, its mean duration was rather constant. Therefore, only the results of the drug effects on high-voltage spindle total duration are shown. Furthermore, the total recording times, which provide indirect information about the behavioral/motor activity of the rats after drug treatments, are reported. The one-way analysis of variance (ANOVA) followed by Duncan's post-hoc multiple group comparison was used in the statistical analysis of biochemical parameters. MANOVA and ANOVA were used to analyse the differences in drug treatment effects on high-voltage spindle total duration between aged and adult rats. $P < 0.05$ was accepted as significant.

Notes to Table 2:
The drug effects before and after *p*-chlorophenylalanine treatment (PCPA) (400 mg/kg/5.0 ml/day i.p. for three consecutive days) -induced brain serotonin depletion are shown. Gum arabic (GA) (0.5% solution) was used as a control treatment for *p*-chlorophenylalanine. Movement periods were recorded automatically and excluded from the high-voltage spindle total duration data analysis by magnet-coil movement-sensor detector of EEG cables which were bound on the rat's head. Multivariable analysis of variance (MANOVA) followed by the post-hoc Wilcoxon signed rank test was used in statistical analysis. Values represent means ± S.E.M. [c] $P < 0.01$ and [d] $P < 0.05$ vs. controls (saline) (Wilcoxon signed rank test).

3. Results

3.1. EEG measurements

3.1.1. Aged vs. adult rats

In aged rats, the high-voltage spindle total duration was significantly increased when compared to that of adult rats (aged rats 233.1 s ± S.E.M. 39.1 s vs. adult rats 46.0 s ± S.E.M 14.7 s; ANOVA: $F(1,29) = 19.2$, $P < 0.001$).

3.1.2. Aged rats

3.1.2.1. Nicotine (s.c.). Nicotine at 0.1 mg/kg and 0.3 mg/kg doses, but not at the 0.03 mg/kg dose significantly decreased the high-voltage spindle total duration vs. saline (Fig. 2, part A). Nicotine at 0.1 mg/kg and 0.3 mg/kg suppressed high-voltage spindles more effectively than at 0.03 mg/kg, and the 0.3 mg/kg dose was more effective than the 0.1 mg/kg dose. Nicotine at 0.03, 0.1 or 0.3 mg/kg had no effect on total recording time vs. saline treatment (Table 1).

3.1.2.2. DOI (s.c.). DOI 0.3 and 1.0 mg/kg decreased the high-voltage spindle total duration vs. saline, and there was no difference between these doses (Fig. 2, part B). The lowest dose of DOI (0.1 mg/kg) had no effect on the high-voltage spindle total duration. DOI at 0.1, 0.3 and 1.0 mg/kg increased total recording times vs. saline treatment (Table 1). Furthermore, in the second group of aged rats, DOI at high doses (1.0 mg/kg and 2.0 mg/kg) significantly suppressed the high-voltage spindle total duration (no difference between the doses) (Fig. 2, part B), and dose dependently increased total recording times (Table 1).

3.1.2.3. Combination of subthreshold doses of nicotine (s.c.) and DOI (s.c.). A combination of subthreshold doses of nicotine (0.03 mg/kg) and DOI (0.1 mg/kg), that were ineffective alone in aged rats (see above) significantly decreased the high-voltage spindle total duration (Fig. 2, part C), and also increased total recording time vs. saline + saline (Table 1).

3.1.2.4. p-Chlorophenylalanine (i.p.). *p*-Chlorophenylalanine treatment had no effect on the high-voltage spindle total duration (Fig. 3, part A) or total recording time

(Table 1) vs. gum arabic control treatment. Nicotine 0.1 mg/kg did not affect the high-voltage spindle total duration in either *p*-chlorophenylalanine-treated or control rats (Fig. 3, part A), and increased the total recording time in gum arabic-treated controls, but not in *p*-chlorophenylalanine-treated rats (Table 1). DOI 0.3 mg/kg significantly decreased the high-voltage spindle total duration in control rats, but not in *p*-chlorophenylalanine-treated rats (Fig. 3, part A), and significantly increased the total recording time in both gum arabic-treated controls and *p*-chlorophenylalanine-treated rats (Table 1). The combination of subthreshold doses of nicotine (0.03 mg/kg) and DOI (0.1 mg/kg) had no effect on the high-voltage spindle total duration (Fig. 3, part B), and did not affect the total recording time in either gum arabic-treated controls or *p*-chlorophenylalanine-treated rats (Table 1). However, higher doses of DOI (1.0 and 2.0 mg/kg) were still capable of decreasing the high-voltage spindle total duration in the second group of *p*-chlorophenylalanine-treated rats (Fig. 3, part C), and they also caused a dose-dependent increase in total recording time in these *p*-chlorophenylalanine-treated rats (Table 1).

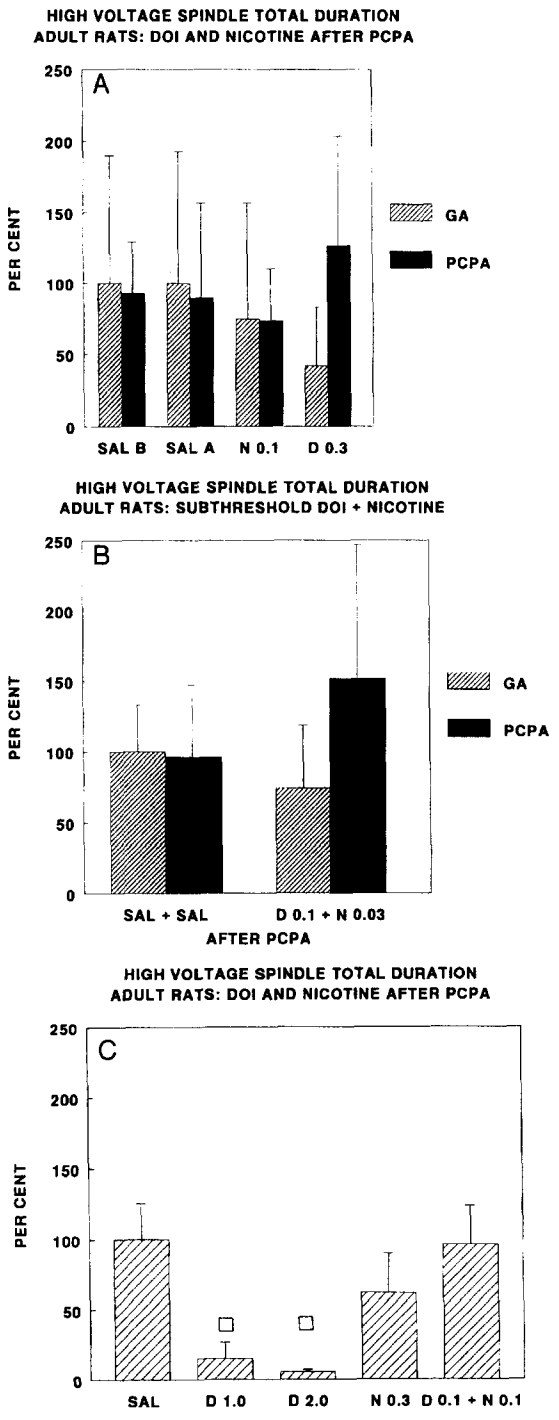

Fig. 4. Effects of *p*-chlorophenylalanine (PCPA) (400 mg/kg/5.0 ml/day i.p. for three consecutive days) treatment-induced brain serotonin synthesis inhibition on the effects of systemic administration (s.c. 2.0 ml/kg, 25 min before recording session) of a nicotinic acetylcholine receptor agonist, nicotine, and a 5-HT_2 receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) on the total duration (incidence × mean duration) of neocortical high-voltage spindles in adult (7 months of age, $n = 14$) rats recorded during a 20-min cumulative behavioral waking-immobility period. Gum arabic (GA) (0.5% solution) was used as a control treatment for *p*-chlorophenylalanine. The high-voltage spindle recordings were made every day. Values represent % group means ± S.E.M. of control (saline-treated) values (100%). For abbreviations see Figs. 2 and 3. Doses of nicotine and DOI are expressed as mg/kg. Part A: Multivariable analysis of variance (MANOVA) followed by post-hoc analysis revealed that *p*-chlorophenylalanine treatment had no effect on high-voltage spindle total duration (Treatment effect: $F(1,8) = 0.41$, $P > 0.1$; Group by treatment effect: $F(1,8) = 0.00$, $P > 0.1$), and that nicotine (0.1 mg/kg: Treatment effect: $F(1,8) = 0.70$, $P > 0.1$; Group by treatment effect: $F(1,8) = 0.03$, $P > 0.1$) and DOI (0.3 mg/kg: Treatment effect: $F(1,8) = 0.18$, $P > 0.1$; Group by treatment effect $F(1,8) = 3.80$, $P > 0.05$) did not affect high-voltage spindle total duration in control or *p*-chlorophenylalanine-treated rats. Part B: The combination of subthreshold doses of nicotine (0.03 mg/kg) and DOI (0.1 mg/kg) that alone and in combination were ineffective before *p*-chlorophenylalanine treatment, also did not affect high-voltage spindle total duration in control or *p*-chlorophenylalanine-treated rats (Treatment effect: $F(1,8) = 0.26$, $P > 0.1$; Group by treatment effect: $F(1,8) = 1.96$, $P > 0.1$). Part C: The highest doses of DOI (□ $P < 0.05$ vs. saline) were still capable of decreasing neocortical high-voltage spindle total duration in a group of *p*-chlorophenylalanine-treated rats ($F(2,14) = 15.3$, $P < 0.001$). On the other hand, a moderate dose of nicotine (0.3 mg/kg), which could decrease neocortical high-voltage spindle total duration before *p*-chlorophenylalanine treatment, did not affect neocortical high-voltage spindle activity in a group of adult rats after *p*-chlorophenylalanine treatment ($Z(2,6) = -0.98$, $P > 0.1$). Another combination of subthreshold doses of nicotine (0.1 mg/kg) and DOI (0.1 mg/kg) that alone and in combination were also ineffective before *p*-chlorophenylalanine treatment, still did not affect high-voltage spindle total duration after *p*-chlorophenylalanine treatment ($Z(3,4) = -0.59$, $P > 0.1$).

3.1.3. Adult rats

3.1.3.1. Nicotine (s.c.). Nicotine 0.3 mg/kg, but not 0.1 or 0.03 mg/kg, significantly decreased the high-voltage spindle total duration vs. saline in adult rats (Fig. 2, part A). Furthermore, nicotine at the highest dose (0.3 mg/kg) significantly increased the total recording time vs. saline treatment or other nicotine doses (Table 2).

3.1.3.2. DOI (s.c.). DOI 0.3 mg/kg decreased the high-voltage spindle total duration vs. saline, whereas the doses of 0.1 and 1.0 mg/kg had no effect, but there was no significant difference between the effects of 0.3 mg/kg and 1.0 mg/kg doses of DOI (Fig. 2, part B). None of these doses of DOI had any effect on the total recording time vs. saline treatment (Table 2). In the second group of adult rats both high doses of DOI (1.0 and 2.0 mg/kg) suppressed neocortical high-voltage spindle total duration (no difference between the doses) (Fig. 2, part B), and dose dependently increased the total recording time vs. saline treatment (Table 2).

3.1.3.3. Combination of subthreshold doses of nicotine (s.c.) and DOI (s.c.). The combination of subthreshold doses of nicotine and DOI (nicotine 0.03 mg/kg + DOI 0.1 mg/kg) that were ineffective alone (see above), had no effect on high-voltage spindle total duration (Fig. 2, part C) or total recording time (Table 2) vs. saline + saline treatment. Furthermore, in the second group of adult rats, another subthreshold dose combination of nicotine (0.1 mg/kg) + DOI (0.1 mg/kg) had no effect on high-voltage spindle total duration (Fig. 2, part C) or total recording time (Table 2) vs. saline + saline treatment.

3.1.3.4. p-Chlorophenylalanine (i.p.). *p*-Chlorophenylalanine treatment had no effect on high-voltage spindle total duration (Fig. 4, part A) or total recording time (Table 2) vs. gum arabic control treatment. Nicotine 0.1 mg/kg or DOI 0.3 mg/kg did not affect high-voltage spindle total duration in either *p*-chlorophenylalanine-treated or control rats (Fig. 4, part A). Nicotine 0.1 mg/kg did not affect the total recording time in either gum arabic-treated controls or *p*-chlorophenylalanine-treated rats, whereas DOI 0.3 mg/kg increased total recording time in *p*-chlorophenylalanine-treated rats, but not in gum arabic-treated controls (Table 2). Nicotine at the higher dose (0.3 mg/kg) had no effect on high-voltage spindle total duration (Fig. 4, part C) and did not increase the total recording time (Table 2) in the second group of *p*-chlorophenylalanine-treated rats. High doses of DOI (1.0 and 2.0 mg/kg) significantly decreased high-voltage spindle total duration (Fig. 4, part C) and increased total recording times (Table 2) in these *p*-chlorophenylalanine-treated rats (no difference between the doses). The combination of subthreshold doses of nicotine and DOI (nicotine 0.03 mg/kg + DOI 0.1 mg/kg) had no effect on high-voltage spindle total duration (Fig. 4, part B) or total recording time (Table 2) in either gum arabic-treated control or *p*-chlorophenylalanine-treated rats. Furthermore, another subthreshold dose combination of nicotine (0.1 mg/kg) + DOI (0.1 mg/kg) did not affect high-voltage spindle total duration (Fig. 4, part C) or total recording time (Table 2) in the second group of *p*-chlorophenylalanine-treated rats.

3.1.4. Group (aged vs. adult rats) by drug-treatment interactions

There were significant group (aged vs. adult rats) interactions for drug treatment for (a) the effects of nicotine (saline vs. 0.03, 0.1 and 0.3 mg/kg of nicotine) on high-voltage spindle total duration (MANOVA: $F(3,84) = 7.38$, $P < 0.001$), and (b) the effects of a subthreshold dose combination of nicotine (0.03 mg/kg) + DOI (0.1 mg/kg) vs. saline + saline on high-voltage spindle total duration (MANOVA: $F(1,27) = 18.95$, $P < 0.001$). No other group interactions for drug treatment were found in high-voltage spindle total duration analyses (data not shown; $P > 0.05$ for all).

3.2. Biochemistry

3.2.1. Aged rats

p-Chlorophenylalanine treatment significantly decreased the frontal cortical 5-HT ($F(1,11) = 844.7$, $P < 0.001$; 84% reduction) and 5-HIAA ($F(1,11) = 1189.6$, $P < 0.001$; 90.3% reduction) concentrations vs. gum arabic control treatment. The concentrations of noradrenaline ($F(1,11) = 0.132$, $P > 0.1$; 1.5% reduction), dopamine ($F(1,11) = 2.59$, $P > 0.1$; 5.2% reduction) and homovanillic acid ($F(1,11) = 0.006$, $P > 0.1$; 0.4% increase) were not significantly affected.

3.2.2. Adult rats

p-Chlorophenylalanine treatment significantly decreased the frontal cortical 5-HT ($F(1,10) = 17.59$, $P < 0.005$; 84.7% reduction) and 5-HIAA ($F(1,10) = 141.6$, $P < 0.001$; 92.5% reduction) concentrations vs. arabic gum control treatment. The concentrations of noradrenaline ($F(1,10) = 0.01$, $P > 0.1$; 1.8% reduction), dopamine ($F(1,9) = 0.10$, $P > 0.1$; 11.2% increase) and homovanillic acid ($F(1,10) = 3.69$, $P > 0.05$; 26.1% reduction) were non-significantly affected.

4. Discussion

An interesting finding was that the nicotinic acetylcholine receptor agonist, nicotine, alleviated the age-related increase in rat neocortical high-voltage spindle activity. Nicotine has been shown to decrease neocortical high-voltage spindle activity (Radek, 1993; Riekkinen, Jr. et al., 1993a) and spike-and-wave discharges (Danober et al., 1993) in adult rats, and brief spindle episodes in mice (Ryan, 1985). Furthermore, a nicotinic acetylcholine receptor antagonist, mecamylamine, was previously shown to dose dependently increase rat high-voltage spindles, and at a subthreshold dose, ineffective alone, to block the high-voltage spindle activity-suppressing effect of nicotine in adult rats (Riekkinen, Jr. et al., 1993a). In the present

study, lower doses of nicotine were required in aged rats than in adult rats to decrease neocortical high-voltage spindles, suggesting that aged rats may be more sensitive to the high-voltage spindle activity-suppressing effects of nicotine. Note that activation of presynaptic nicotinic acetylcholine receptors may increase the release of acetylcholine (Beani et al., 1989), and the release of acetylcholine has been shown to be decreased in the cerebral cortex of aged rats (Wu et al., 1988). Furthermore, the loss of cholinergic neurons in the nucleus basalis during aging correlates well with the observed increase in neocortical high-voltage spindle activity (Riekkinen, Jr. et al., 1992). The present results showing that aged rats may be more sensitive to the high-voltage spindle activity-suppressing effects of nicotine suggest that deficient nicotinic acetylcholine receptor stimulation may contribute to the age-related increase in neocortical high-voltage spindle activity in rats. However, the possibility that the pharmacokinetics of nicotine or the levels of nicotine in brain tissue differ between 7-month-old and very old (28-month-old) rats may also be a contributory factor that must not be excluded in the interpretation of the results. In future studies this issue could be investigated, for example, by comparing the efficacy of intracerebroventricular infusions of nicotine or other nicotinic acetylcholine receptor subtype-specific drugs to suppress high-voltage spindles in adult and aged rats.

Results obtained with slicing techniques also support a role for the nicotinic acetylcholine receptors in the modulation of thalamocortical neurotransmission: in thalamic slice preparations, the fast excitatory response of thalamocortical relay neurons in guinea-pig and cat lateral geniculate nuclei seen after the application of acetylcholine is associated with substantial increases in membrane cationic conductance, and this is mimicked by application of nicotinic receptor agonists (McCormick, 1992). This suggests that activation of nicotinic receptors in thalamocortical neurons results in fast excitation, which may be associated with high-fidelity transfer of information through the thalamus (McCormick, 1992).

The site of action of nicotinic drugs in modulating thalamically generated high-voltage spindles may involve both pre- and postsynaptic nicotinic receptors. Nicotine (and acetylcholine) acting on presynaptic nicotinic receptors may increase the release of acetylcholine (Beani et al., 1989) in areas important for the modulation of high-voltage spindle activity (Buzsáki et al., 1988; McCormick, 1992; Steriade and Buzsáki, 1990). Therefore, it is possible that nicotine may directly stimulate nicotinic and indirectly stimulate muscarinic postsynaptic receptors (Krnjevic et al., 1971; McCormick, 1992). Indeed, previous studies have emphasized the importance of the muscarinic acetylcholine receptors in the modulation of rat thalamocortical oscillations and their related neocortical high-voltage spindles. For example, systemic administration of low to moderate doses of a muscarinic receptor antagonist, scopolamine, increases the incidence of rat high-voltage spindles (Riekkinen, Jr. et al., 1990, 1991a, b), and spike-and-wave discharges (Danober et al., 1993). Also, systemic administration of muscarinic receptor agonists, oxotremorine (predominantly a muscarinic M_2 receptor agonist), pilocarpine (a mixed muscarinic M_1/M_2 receptor agonist) and AF-102B (predominantly a muscarinic M_1 receptor agonist) suppresses rat high-voltage spindles in a dose-dependent manner and induces arousal-like cortical EEG activity (Riekkinen, Jr. et al., 1991b, 1993b). Furthermore, tetrahydroaminoacridine, a cholinesterase inhibitor, also possessing agonistic activity at muscarinic M_1 and M_2 and nicotinic acetylcholine receptors, effectively suppresses neocortical high-voltage spindles in both aged and adult rats (Riekkinen, Jr. et al., 1991b), and to some extent alleviates the increase in high-voltage spindle activity induced by partial lesions of the cholinergic nucleus basalis (Riekkinen, Jr. et al., 1991b). Some of the effects of nicotinic acetylcholine receptor-active drugs to modulate high-voltage spindle activity may also be mediated via heterosynaptic receptors. Indeed, the activity of many noncholinergic neurotransmitter systems, such as the noradrenergic (Joseph et al., 1990) and serotonergic (Ribeiro et al., 1993) systems, have been found to be modulated by nicotine. It could also be argued that the peripheral side-effects of nicotine might partially account for its ability to suppress high-voltage spindle activity. However, 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 drug treatment) indicated that systemic treatment with nicotine either did not affect or only slightly increased the motor/behavioral activity of the rats. In addition, we had found that administration of a peripherally acting nicotinic receptor antagonist, hexamethonium, at a relatively high dose (1.0 mg/kg) did not cause any changes in neocortical high-voltage spindle activity in adult rats (Riekkinen, Jr. et al., 1993a). It is also important to emphasize that, in the present study the drug treatment effects on neocortical high-voltage spindle activity reflect a behavioral waking-immobility state in the animals since we used a movement-sensor binding in the EEG cable magnet coil on the rat's head which automatically excluded all the movement-related EEG epochs from the high-voltage spindle recordings. Therefore, it is likely that at the doses used in the present study (0.03–0.3 mg/kg), the peripheral side-effects of nicotine were not responsible for its high-voltage spindle activity-suppressing effects, and the results reflect the effects of the drug treatment on neocortical high-voltage spindle activity during quiet waking-immobility behavior itself, and not on behavioral/motor activity as an intermediate variable.

Another noteworthy finding of the present study was that DOI, a relatively specific 5-HT_2 receptor agonist (Boess and Martin, 1994; Martin and Humphrey, 1994), significantly decreased neocortical high-voltage spindles in aged rats. DOI is often regarded as a subtype-selective

5-HT_{2A} receptor agonist, but may be equally active at 5-HT_{2B} and 5-HT_{2C} receptors (Boess and Martin, 1994; Martin and Humphrey, 1994). In line with the present results, in our previous study, DOI 0.5, 1.0 and 2.0 mg/kg decreased high-voltage spindle activity in adult rats (Jäkälä et al., 1995). In the present study moderate to high doses of DOI (0.3, 1.0 and 2.0 mg/kg) were required to decrease neocortical high-voltage spindles in both aged and adult rats. Interestingly, radioligand-binding studies have demonstrated diminished populations of serotonin receptors, including 5-HT_2 receptors in the rat brain as the animal gets older (Allen et al., 1983; Brunello et al., 1988). There is also increased turnover of serotonin and a reduction in serotonin high-affinity uptake (Brunello et al., 1988). Thus, it is possible that aged and adult rats could differ in their response to 5-HT_2 receptor subtype-specific drugs. However, at the doses used in the present study (0.1–2.0 mg/kg) no significant differences were seen between aged and adult rats in their response to DOI alone to suppress high-voltage spindle activity.

It is likely that 5-HT_2 receptor stimulation is important for the DOI-induced suppression of thalamically generated neocortical high-voltage spindles. Previously, DOI at a high dose (2.0 mg/kg) had no effect on cortical acetylcholine release in freely moving guinea pigs (Bianchi et al., 1990), and the behavioral effects of DOI (2.0 mg/kg) were completely antagonized by ketanserin, a 5-HT_2 receptor antagonist (Leysen et al., 1981) in rats (Riekkinen et al., 1994a; Riekkinen, Jr., 1994). However, in another study, DOI at 0.2 and 0.5 mg/kg doses caused a 50% reduction in hippocampal release of noradrenaline in freely moving rats (Done and Sharp, 1992), and at a very high dose (5.0 mg/kg), it also slightly increased dopaminergic activity in the rat limbic forebrain (Nissbrandt et al., 1992). Therefore, there is a possibility that, at the doses used in the present study (0.1–2.0 mg/kg), some of the high-voltage spindle activity-suppressing effects of DOI were mediated via modulation of cholinergic, noradrenergic or dopaminergic activity.

Results of slicing technique studies also support a role for 5-HT_2 receptors in the modulation of thalamocortical neurotransmission: in slice preparations from guinea-pig nucleus reticularis neurons and cat perigeniculate nucleus, application of serotonin leads to pronounced and prolonged excitation associated with the occurrence of single spike activity, and it is important that this excitatory response to serotonin application is specifically mimicked by 5-HT_2 receptor agonists and blocked by 5-HT_2 receptor antagonists (McCormick and Wang, 1991). Thus, the present results showing that systemic administration of DOI significantly decreases neocortical high-voltage spindle activity in both aged and adult rats are in good agreement with results of in vitro studies, and suggest that activation of 5-HT_2 receptors may be sufficient to reduce thalamocortical oscillations and their related neocortical high-voltage spindles. However, when drugs are administered systemically, peripheral effects cannot be excluded, and the site of action becomes a matter of speculation. Indeed, the discharge activity of the raphe serotonergic system is increased in accordance with increases in behavioral arousal/motor activity (Jacobs and Azmitia, 1992). In the present study, systemic treatment with DOI increased the motor activity of the animals as indicated by the increase in total recording times. Furthermore, DOI at the two highest doses used (1.0 and 2.0 mg/kg), in line with previous observations (Darmani et al., 1990), induced head shakes in some of the rats. Note that, in our previous study, DOI decreased neocortical high-voltage spindle activity in adult rats not only after systemic injections, but also when administered unilaterally directly into the ventroposteromedial thalamic area (Jäkälä et al., 1995), and this effect could be blocked by systemic administration of ketanserin, a 5-HT_2 receptor antagonist (Leysen et al., 1981). This indicates that activation of 5-HT_2 receptors, possibly located in the thalamus, with a specific 5-HT_2 receptor agonist (DOI) may suppress the generation of thalamocortical oscillations and their related neocortical high-voltage spindles (Jäkälä et al., 1995). In support of this interpretation are the observations that the thalamic nuclei which are associated with sensory transmission receive serotonergic innervation (Steinbusch, 1981; Jacobs and Azmitia, 1992), and that 5-HT_{2C} receptors (former 5-HT_{1C} receptors) (Boess and Martin, 1994; Martin and Humphrey, 1994) are expressed in the neurons of the rat thalamic sensory relay nuclei (Molineaux et al., 1989).

Interestingly, combined treatment with subthreshold doses of the nicotinic acetylcholine and 5-HT_2 receptor agonists significantly decreased high-voltage spindles in aged rats but not in adult rats. Different theories may explain this synergistic effect of nicotine and DOI treatment on neocortical spindling activity of aged rats. First, nicotine and DOI may act via independent mechanisms to suppress neurophysiological phenomena that increase high-voltage spindle activity. Indeed, anatomical studies have shown that nicotinic acetylcholine and 5-HT_2 receptors are distributed at different forebrain sites, such as cortex, basal ganglia and thalamus (Molineaux et al., 1989; Wada et al., 1989; Wainer and Mesulam, 1990; Jacobs and Azmitia, 1992; McCormick, 1992), suggesting that systemically injected nicotine and DOI may act via several brain areas that regulate thalamocortical oscillations. Therefore, it is possible that nicotine and DOI acted at different anatomical levels of the thalamocortical circuitry to inhibit high-voltage spindling. However, an equally plausible theory is that nicotine and DOI also share some brain nuclei, such as the reticular or relay nuclei of the thalamus, that could mediate the high-voltage spindle activity-suppressing effects of these drugs. Indeed, earlier in vitro electrophysiological evidence suggests that activation of nicotinic acetylcholine and 5-HT_2 receptors may decrease hyperpolarization of thalamocortical relay neurons and prevent the activation of Ca^{2+}-mediated spiking and generation of

oscillatory firing (McCormick and Wang, 1991; McCormick, 1992). Further, we have described that infusion of DOI (Jäkälä et al., 1995) or nicotine (Riekkinen, Jr. et al., 1995) into the ventroposteromedial thalamic area sensory relay nuclei suppresses high-voltage spindles, indicating that activation of nicotinic acetylcholine and 5-HT_2 receptors located in this thalamic area suppresses thalamic oscillations and that peripherally injected drugs may act via the thalamus to decrease spindling.

Partial raphe dorsalis lesions alone do not affect neocortical high-voltage spindle activity, but the lesion aggravates the increase of high-voltage spindle activity induced by partial lesions of the cholinergic nucleus basalis magnocellularis (Riekkinen, Jr. et al., 1990). Anatomical studies provide further evidence for the sites of possible interaction between the cholinergic and serotonergic systems in the modulation of thalamocortical oscillations and their related neocortical high-voltage spindles: the basal forebrain area containing cortically and thalamically projecting cholinergic neurons is innervated by the raphe dorsalis, and the brainstem serotonergic cell groups receive cholinergic inputs from the basal forebrain (Zaborsky et al., 1991; Jacobs and Azmitia, 1992). Moreover, the cortical areas and the thalamic reticular nucleus, as well as thalamocortical relay nuclei, receive inputs from both the basal forebrain and brainstem cholinergic neurons and raphe dorsalis (Steinbusch, 1981; Wainer and Mesulam, 1990; Jacobs and Azmitia, 1992). Therefore, the cholinergic and serotonergic systems could interact at the cell body level, thalamus or cortex. Future studies with local microinfusions of e.g. nicotine and DOI should elucidate the sites of possible interaction. The finding that aged rats were more sensitive to nicotinic acetylcholine and 5-HT_2 receptor stimulation to decrease high-voltage spindles, suggests that combined dysfunction of nicotinic acetylcholine and 5-HT_2 receptor-mediated functions (Allen et al., 1983; Brunello et al., 1988; Wu et al., 1988) may be at least partially responsible for the age-related increase seen in neocortical high-voltage spindles in rats (Buzsáki et al., 1988, 1990; Sirviö et al., 1989; Riekkinen, Jr. et al., 1991a). However, the earlier mentioned possibility that nicotine or DOI pharmacokinetics, and levels of nicotine or DOI in brain tissue differ between 7- and 28-month-old rats may also be a contributory factor complicating the interpretation of these results.

p-Chlorophenylalanine treatment produced a significant reduction in the frontal cortical concentrations of both serotonin and its major metabolite, 5-HIAA, in both adult and aged rats, and slightly but non-significantly affected the noradrenergic and dopaminergic systems. Unfortunately, the thalamic concentrations of monoamines and metabolites were not determined. However, in previous studies the changes in monoamine and metabolite concentrations caused by *p*-chlorophenylalanine treatment were of the same magnitude in the frontal cortical, parieto-occipito cortical and hippocampal samples (Jäkälä et al., 1993; Riekkinen et al., 1993, 1994b; Riekkinen, Jr. et al., 1994; Riekkinen, Jr. and Riekkinen, 1995), suggesting that *p*-chlorophenylalanine treatment may reduce serotonin and 5-HIAA concentrations equally throughout the brain, probably including the thalamus. Interestingly, *p*-chlorophenylalanine treatment alone neither affected behavioral/motor activity of the rats since there were no changes in the total recording times, nor had any effect on neocortical high-voltage spindle activity in either adult or aged rats. However, in both aged and adult rats, *p*-chlorophenylalanine treatment abolished the high-voltage spindle activity suppressing effects of a moderate dose of DOI (0.3 mg/kg), but not that produced by higher doses of DOI (1.0 and 2.0 mg/kg). This indicates that endogenous serotonin and DOI 0.3 mg/kg act in concert to regulate high-voltage spindle activity. However, at high doses, DOI itself may stimulate 5-HT_2 receptors so effectively that no endogenous serotonin is required for suppression of high-voltage spindle activity.

An interesting and novel finding was that, in adult rats, *p*-chlorophenylalanine abolished the decrease in high-voltage spindle activity seen after a moderately high dose of nicotine (0.3 mg/kg). It could be argued that this relatively high dose of nicotine was insufficient and that, at higher doses, nicotine would have decreased high-voltage spindle activity even after *p*-chlorophenylalanine treatment. However, nicotine at a slightly higher dose than used in the present study causes marked peripheral and cental side-effects and therefore we did not test the effects of higher doses (Riekkinen, Jr. et al., 1993a, 1994; Riekkinen, Jr. and Riekkinen, 1995). Previous behavioral studies support the view that intact serotonergic systems are important for the therapeutic effects of nicotine. First, Riekkinen Jr. et al. found that *p*-chlorophenylalanine treatment blocked the improving effects of nicotine on passive avoidance and water maze spatial navigation behavior of medial-septal (Riekkinen, Jr. et al., 1994) and nucleus-basalis (Riekkinen, Jr. and Riekkinen, 1995) -lesioned rats. Second, Ribeiro et al. found that systemically injected nicotine increases serotonin release (Ribeiro et al., 1993). Therefore, the present and previous results indicate that nicotinic acetylcholine and serotonergic systems may interact in the regulation of behavioral and electrophysiological functions, including thalamocortical arousal, and that the integrity of 5-HT projections may be a prerequisite for some of the therapeutic effects of nicotine.

The expression of high-voltage spindles has been shown to be genetically determined (Buzsáki et al., 1990). For example, in Fisher 344 rats high-voltage spindles were present in 87.5% of the 3-month-old and in 100% of the older (12 months or older) rats. Furthermore, the mean incidence of high-voltage spindles showed an about 3-fold increase at the age of 12 months and an about 8-fold increase at the age of 26 months or over when compared to 3-month-old Fisher 344 rats (Buzsáki et al., 1990). On the other hand, in two other rat strains, the Buffalo and

random-bred Sprague-Dawley rats, the naturally occurring high-voltage spindles were completely absent in 3-month-old rats, and did not appear until a later age (in 58.3% of 12-month-old rats and in 71.4% of over 26-month-old rats) (Buzsáki et al., 1990). There are no quantitative data for our Han:Wistar rats regarding the increased expression of high-voltage spindles during aging. In one previous study high-voltage spindles were present in all 17- to 22-month-old male Han:Wistar rats, but were absent in all the 4-month-old rats (Sirviö et al., 1989). Therefore, the increased expression of high-voltage spindles in Han:Wistar rats also seems to be age-related, but it is not known whether it is necessarily related with very old (e.g. 28 months) age. Thus, there is a possibility that attenuation of high-voltage spindles in 12- to 14-month-old Han:Wistar rats may not necessarily differ neuropharmacologically from a similar effect in 28-month-old rats. This issue could be investigated for example by doing follow-up pharmaco-EEG at different ages in the same group of rats. However, it must also be kept in mind that when studying the possibility of changes in the neuropharmacological modulation of high-voltage spindles during aging, 'aged' rats, i.e. 2 years of age or over, should also be used.

In conclusion, the present results showing that nicotinic acetylcholine and 5-HT_2 receptor agonists cause a decrease in rat neocortical high-voltage spindle activity are in good agreement with results of previous in vitro (Pape and McCormick, 1989; McCormick and Wang, 1991; McCormick, 1992; Steriade et al., 1993) and in vivo (Danober et al., 1993; Radek, 1993; Riekkinen, Jr. et al., 1993a, 1995; Jäkälä et al., 1995) studies. The results suggest that joint activation of nicotinic acetylcholine and 5-HT_2 receptor subtypes may suppress rat thalamocortical oscillations, and consequently maintain effective processing of information in thalamocortical systems. Further, as *p*-chlorophenylalanine-induced brain serotonin depletion in adult rats abolished the high-voltage spindle activity-suppressing effect of a relatively high dose of nicotine, we suggest that intact brain serotonergic systems may be important for some of the therapeutic effects of nicotine. These data may also be of some clinical relevance, suggesting that combination drug therapies, aimed at stimulating the nicotinic acetylcholine and 5-HT_2 receptors, could offer an approach for the normalization of deteriorating arousal functions related to e.g. Alzheimer's disease, and a variety of other clinical disorders.

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. Arja Parkkali for biochemical analyses, and Ewen MacDonald, Ph.D., for revising the language of the manuscript.

References

Allen, S.S., J.S. Benton, M.J. Goodhardt, E.A. Hann, N.R. Sims, C.C.T. Smith, J.A. Spillane, D.M. Bowen and A.N. Davison, 1983, Biochemical evidence of selective nerve cell changes in the normal ageing human and rat brain, J. Neurochem. 41, 256.

Beani, L., C. Bianchi, L. Ferraro, L. Nilsson, A. Nordberg, L. Romanelli, P. Spalluto, A. Sundwall and E. Tanganelli, 1989, Effects of nicotine on the release of acetylcholine and amino acids in the brain, in: Nicotine Receptors in the CNS: Their Role in Synaptic Transmission, eds. A. Nordberg, K. Fuxe, B. Holmstedt and A. Sundwall (Elsevier, Amsterdam) p. 149.

Bianchi, C., A. Siniscalchi and L. Beani, 1990, 5-HT_{1A} agonists increase and 5-HT_3 agonists decrease acetylcholine efflux from cerebral cortex of freely-moving guinea-pigs, Br. J. Pharmacol. 101, 448.

Boess, F.G. and I.L. Martin, 1994, Molecular biology of 5-HT receptors, Neuropharmacology 33(3/4), 275.

Brunello, N., M. Riva, A.C. Rovescalli, R. Galimberti and G. Racasni, 1988, Age-related changes in rat serotonergic and adrenergic systems and in receptor responsiveness to subchronic desipramine treatment, Pharmacol. Toxicol. 63, 150.

Buzsáki, G., R.G. Bickford, G. Ponomaref, L.J. Thal, R. Mandel and F.H. Gage, 1988, Nucleus basalis and thalamic control of neocortical activity in the freely moving rat, J. Neurosci. 8(11), 4007.

Buzsáki, G., I. Laszlovsky, A. Lajtha and C. Vadasz, 1990, Spike-and-wave neocortical patterns in rats: genetic and aminergic control, Neuroscience 38(2), 323.

Danober, L., A. Depaulis, C. Marescaux and M. Vergnes, 1993, Effects of cholinergic drugs on genetic absence seizures in rats, Eur. J. Pharmacol, 234(2–3), 263.

Danober, L., M. Vergnes, A. Depaulis and C. Marescaux, 1994, Nucleus basalis lesions suppress spike and wave discharges in rats with spontaneous absence-epilepsy, Neuroscience 59(3), 531.

Darmani, N.A., B.R. Martin, U. Pandey and R.A. Glennon, 1990, Do functional relationships exist between 5-HT_{1A} and 5-HT_2 receptors, Pharmacol. Biochem. Behav. 36, 901.

Done, C.J.G. and T. Sharp, 1992, Evidence that 5-HT_2 receptor activation decreases noradrenaline release in rat hippocampus in vivo, Br. J. Pharmacol. 107, 240.

Garratt, J.C., E.J. Kidd, I.K. Wright and C.A. Marsden, 1991, Inhibition of 5-hydroxytryptamine neuronal activity by the 5-HT agonist, DOI, Eur. J. Pharmacol. 199, 349.

Jacobs, B.L. and E.C. Azmitia, 1992, Structure and function of the brain serotonin system, Physiol. Rev. 72, 165.

Jäkälä, P., T. Viitamaa, J. Sirviö, P. Riekkinen, Jr., J. Salonen, A. Haapalinna, R. Virtanen and P. Riekkinen, 1992, Continuous α_2-adrenoceptor blockade by atipamezole decreases neocortical high-voltage spindle activity in rats, Eur. J. Pharmacol. 221, 351.

Jäkälä, P., J. Sirviö, P. Riekkinen, Jr. and P. Riekkinen, 1993, Effects of *p*-chlorophenylalanine and methysergide on the performance of a working memory task, Pharmacol. Biochem. Behav. 44(2), 411.

Jäkälä, P., J. Sirviö, E. Koivisto, M. Björklund, J. Kaukua and P. Riekkinen, Jr., 1995, Modulation of rat neocortical high-voltage spindle activity by 5-HT_1/5-HT_2 receptor subtype specific drugs, Eur. J. Pharmacol. 282, 39.

Joseph, M.H., S.L. Peters, A. Prior, S.N. Mitchell, M.P. Brazell and J.A. Gray, 1990, Chronic nicotine administration increases tyrosine hydroxylase selectivity in rat hippocampus, Neurochem. Int. 16, 269.

Krnjevic, K., R. Pumain and L. Renaud, 1971, The mechanism of excitation by acetylcholine in the cerebral cortex, J. Physiol. (London) 215, 447.

Levey, A., A.E. Hallanger and B.H. Wainer, 1987, Cholinergic nucleus basalis may influence the cortex via the thalamus, Neurosci. Lett. 74, 7.

Leysen, J.E., C.J.E. Niemegeers, J.M. Nueten and P.M. Laduron, 1981, [^{3}H]Ketanserin (R 41 468), a selective ^{3}H-ligand for serotonin$_2$

receptor binding sites. Binding properties, brain distribution, and functional role, Mol. Pharmacol. 21, 301.

Marescaux, C., M. Vergnes and A. Depaulis, 1992, Neurotransmission in rats with spontaneous generalized nonconvulsive epilepsy, in: Neurotransmission in Epilepsy, eds. G. Avanzini, J. Engel, Jr., R. Fariello and U. Heinemann, Epilepsy Res. Suppl. 8, 335.

Martin, G.R. and P.P.A. Humphrey, 1994, Receptors for 5-hydroxytryptamine: current perspectives on classification and nomenclature, Neuropharmacology 33(4), 261.

McCormick, D.A., 1992, Neurotransmitter actions in the thalamus and cerebral cortex, J. Clin. Neurophysiol. 9(2), 212.

McCormick, D.A. and Z. Wang, 1991, Serotonin and noradrenaline excite GABAergic neurons of the guinea-pig and cat nucleus reticularis thalami, J. Physiol. 442, 235.

Micheletti, G., J.-M. Warter, C. Marescaux, A. Depaulis, I. Tranchant, L. Rumbach and M. Vergnes, 1987, Effects of drugs affecting noradrenergic transmission in rats with spontaneous petit mal seizures, Eur. J. Pharmacol. 135, 397.

Molineaux, S., T. Jessell, R. Axel and D. Julius, 1989, 5-HT_{1c} receptor is a prominent serotonin receptor subtype in the central nervous system, Proc. Natl. Acad. Sci. USA 86, 6793.

Nissbrandt, H., N. Waters and S. Hjorth, 1992, The influence of serotonergic drugs on dopaminergic neurotransmission in rat substantia nigra, striatum and limbic forebrain in vivo, Naunyn-Schmied. Arch. Pharmacol. 346, 12.

Pape, H.-C. and D.A. McCormick, 1989, Noradrenaline and serotonin selectively modulate thalamic burst firing by enhancing a hyperpolarization-activated cation current, Nature 340, 715.

Radek, R.J., 1993, Effects of nicotine on cortical high voltage spindles in rats, Brain Res. 625(1), 23.

Ribeiro, E.B., R.L. Bettiger, M. Bogdanov and R. Wurtman, 1993, Effects of systemic nicotine on serotonin release in rat brain, Brain Res. 621, 311.

Riekkinen, M., J. Sirviö and P. Riekkinen, Jr., 1993, Pharmacological consequencies of nicotinergic plus serotonergic manipulations, Brain Res. 662, 139.

Riekkinen, M., J. Sirviö and P. Riekkinen, Jr., 1994a, Treatment with a 5-$HT_{2A/2C}$ receptor agonist disrupts initial development of spatial navigation strategies in rats, Soc. Neurosci. Abstr. p. 305.

Riekkinen, M., R. Tolonen and P. Riekkinen, Jr., 1994b, Interaction between 5-HT_{1A} and nicotinic cholinergic receptors in the regulation of water maze navigation behavior, Brain Res. 649, 174.

Riekkinen, Jr., P., 1994, 5-HT_{1A} and muscarinic acetylcholine receptors jointly modulate passive avoidance behavior, Eur. J. Pharmacol. 262, 77.

Riekkinen, Jr., P. and M. Riekkinen, 1995, Effects of tetrahydroaminoacridine and nicotine in nucleus basalis and serotonin-lesioned rats, Eur. J. Pharmacol. 279(1), 65.

Riekkinen, Jr., P., J. Sirviö, R. Miettinen and P. Riekkinen, 1990, Interaction between raphe dorsalis and nucleus basalis magnocellularis in the regulation of high-voltage spindle activity in rat neocortex, Brain Res. 526, 31.

Riekkinen, Jr., P., J. Sirviö and P. Riekkinen, 1991a, Non-reversal of scopolamine- or age-related EEG changes by ondansetron, methysergide or alaproclate, Psychopharmacology 103, 567.

Riekkinen, Jr., P., J. Sirviö, A. Valjakka, M. Riekkinen, R. Lammintausta and P. Riekkinen, 1991b, Effects of atipamezole and tetrahydroaminoacridine on nucleus basalis lesion-induced EEG changes, Brain Res. Bull. 27, 231.

Riekkinen, Jr., P., M. Riekkinen, J. Sirviö, R. Miettinen and P. Riekkinen, 1992, Loss of cholinergic neurons in the nucleus basalis induces neocortical electroencephalographic and passive avoidance deficits, Neuroscience 47(4), 823.

Riekkinen, Jr., P., M. Riekkinen and J. Sirviö, 1993a, Effects of nicotine on neocortical electrical activity in rats, J. Pharmacol. Exp. Ther. 267(2), 776.

Riekkinen, Jr., P., M. Riekkinen, A. Fisher, T. Ekonsalo and J. Sirviö, 1993b, Effects of muscarinic receptor agonists and anticholinesterase drugs on high-voltage spindles and slow waves, Eur. J. Pharmacol. 240, 1.

Riekkinen, Jr., P., J. Sirviö and M. Riekkinen, 1994, Serotonin depletion decreases the therapeutic effect of nicotine, but not THA, in medial septal-lesioned rats, Brain Res. 662, 95.

Riekkinen, Jr., P., J. Kuitunen and M. Riekkinen, 1995, Effects of thalamic and nucleus basalis infusions of nicotine on cortical EEG, NeuroReport 6, 1625.

Ryan, L.J., 1985, Cholinergic regulation of neocortical spindling in DBA/2 mice, Exp. Neurol. 89, 372.

Sirviö, J., A. Pitkänen, A. Pääkkönen, J. Partanen and P. Riekkinen, 1989, Brain cholinergic enzymes and cortical EEG activity in young and adult rats, Comp. Biochem. Physiol. 94C(1), 277.

Steinbusch, H.W.M., 1981, Distribution of serotonin-immunoreactivity in the central nervous system of the rat – cell bodies and terminals, Neuroscience 6, 557.

Steriade, M. and G. Buzsáki, 1990, Parallel activation of thalamic and cortical neurons by brainstem and forebrain cholinergic systems, in: Brain Cholinergic Systems, eds. M. Steriade and D. Biesold (Oxford University Press, Oxford) p. 3.

Steriade, M. and R.R. Llinás, 1988, The functional stages of the thalamus and associated neuronal interplay, Physiol. Rev. 68, 649.

Steriade, M., D.A. McCormick and T.J. Sejnowski, 1993, Thalamocortical oscillations in the sleeping and aroused brain, Science, 262(5134), 679.

Wada, E., K. Wada, J. Baulter, E. Deneris, S. Heinemann, J. Patrick and L.W. Swanson, 1989, Distribution of alpha2, alpha3, alpha4 and beta2 neuronal nicotinic receptor subunit mRNA in the central nervous system: a hybridization histochemical study in the rat, J. Comp. Neurol. 284, 314.

Wainer, B.H. and M.-M. Mesulam, 1990, Ascending cholinergic pathways in the rat brain, in: Brain Cholinergic Systems, eds. M. Steriade and D. Biesold (Oxford University Press, Oxford) p. 65.

Warter, J.-M., M. Vergnes, A. Depaulis, C. Tranchant, L. Rumbach, G. Micheletti and C. Marescaux, 1988, Effects of drugs affecting dopaminergic transmission in rats with spontaneous petit mal-like seizures, Neuropharmacology 27(3), 269.

Wu, C.F., R. Bertorelli, M. Sacconi, G. Pepeu and S. Consolo, 1988, Decrease of brain acetylcholine release in aging freely-moving rats detected by microdialysis, Neurobiol. Aging 9(4), 357.

Zaborsky, L., W.E. Cullinan and A. Braun, 1991, Afferents to basal forebrain cholinergic projection neurons: an update, in: Basal Forebrain: Anatomy to Function, eds. C.T. Napier, P.W. Kaliwas and I. Hanin (Plenum Press, New York).