ELSEVIER

European Journal of Pharmacology 324 (1997) 147–151

ejp

Effects of the dopamine D_3 receptor agonist, *R*(+)-7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin, on memory processes in mice

Makoto Ukai *, Tomoko Tanaka, Tsutomu Kameyama

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Meijo University, Nagoya 468, Japan

Received 19 September 1996; revised 17 January 1997; accepted 24 January 1997

Abstract

The putative dopamine D_3 receptor agonist, *R*(+)-7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (*R*(+)-7-OH-DPAT) (0.1–100 μg/kg, s.c.), administered before training, immediately after training, and before retention significantly shortened step-down latency of passive avoidance learning, indicating the amnesic effects of *R*(+)-7-OH-DPAT. Neither the dopamine D_1 receptor antagonist, *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine maleate (*R*(+)-SCH23390) (2.5 and 5 μg/kg, i.p.), nor the dopamine D_2 receptor antagonist, *S*(−)-sulpiride (10 and 30 mg/kg, i.p.), markedly influenced the *R*(+)-7-OH-DPAT (10 and 100 μg/kg, s.c.)-induced amnesia. In addition, only a 1000 μg/kg dose of *R*(+)-7-OH-DPAT decreased locomotor activity; 1 and 100 μg/kg doses of the drug were ineffective. These results suggest that the amnesic effects of the dopamine D_3 receptor agonist, *R*(+)-7-OH-DPAT, are not mediated via dopamine D_1 or D_2 receptors in the brain. © 1997 Elsevier Science B.V.

Keywords: *R*-(+)-7-OH-DPAT (*R*-(+)-7-hydroxy-*N*,*N*-(di-*n*-propyl-2-amino)tetralin); Dopamine D_3 receptor; Passive avoidance learning; Spontaneous locomotor activity; Memory; (Mouse)

1. Introduction

Several lines of evidence have suggested that learning and memory can be modified by stimulation of central dopaminergic systems in laboratory animals (Bracs et al., 1984; Kesner et al., 1981; Buresova and Bures, 1982; Ichihara et al., 1988; Levin and Bowman, 1986; Levin et al., 1989; Mattingly, 1986). Dopamine receptors are currently classified into five subtypes, that is D_1 through D_5 receptors, and both dopamine D_1 and D_2 receptors reportedly play a major role in dopaminergic neurotransmission (Kebabian and Calne, 1979). It is suggested that dopamine D_1 and D_2 receptors are particularly involved in learning and memory.

In contrast, dopamine D_3 receptors are distributed with highest densities in the limbic system, i.e., the islands of Calleja, olfactory tubercles and nucleus accumbens (Sokoloff et al., 1990). Results of various pharmacological studies indicate that activation of dopamine D_3 receptors is associated with hypomotility (Daly and Waddington, 1993; Waters et al., 1993; Svensson et al., 1994a,b), induction of yawning (Damsma et al., 1993), hypothermia (Millan et al., 1994) and hypertension (Van de Buuse, 1993). However, the involvement of dopamine D_3 receptors in learning and memory is unclear.

R(+)-7-Hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (*R*(+)-7-OH-DPAT), a dopamine D_3 receptor agonist, and its racemate have been used to elucidate the function of dopamine D_3 receptors. Although *R*(+)-7-OH-DPAT has been reported to be 64- to 220-fold selective for dopamine D_3 receptors compared with dopamine D_2 receptors (Damsma et al., 1993; Baldessarini et al., 1993), it is possible that dopamine D_1 and D_2 receptors play a role in the effects of *R*(+)-7-OH-DPAT.

The present study was designed to determine the effects of *R*(+)-7-OH-DPAT on passive avoidance learning, and these effects of *R*(+)-7-OH-DPAT were characterized by using the dopamine D_1 receptor antagonist, *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine maleate (*R*(+)-SCH 23390), and the dopamine D_2 receptor antagonist, *S*(−)-sulpiride. In addition, we examined the effects of *R*(+)-7-OH-DPAT on spontaneous locomotor activity which often affects learning behavior of mice.

* Corresponding author. Tel.: (81-52) 832-1781; Fax: (81-52) 834-8780; e-mail: ukai@meijo-u.ac.jp

PII S0014-2999(97)00075-7

2. Materials and methods

2.1. Animals

Male *ddY* mice (Nihon SLC, Hamamatsu, Japan) aged 6 or 7 weeks were used. The animals were housed under standard conditions (23 ± 1°C, 50 ± 10% humidity, light-dark cycle with the light on between 8:00 and 20:00 h) with free access to water and food. The mice were used for the experiments following adaptation to laboratory conditions for at least 4 days, and were naive to each of the tests including passive avoidance learning and spontaneous locomotor activity. The experiments were conducted between 10:00 and 18:00 h in a sound-attenuated room.

2.2. Passive avoidance learning

The passive avoidance apparatus consisted of a Plexiglas inner box ($30 \times 30 \times 40$ cm high) with a grid floor and a sound-attenuated wooden outer box ($35 \times 40 \times 90$ cm) with a 15-W light. The grid floor consisted of 30 parallel steel rods (0.3 cm in diameter) set 1 cm apart. A wooden platform ($4 \times 4 \times 4$ cm) was placed in the center of the grid floor (Kameyama et al., 1986). In the training period, each mouse was placed gently onto the wooden platform. When the mouse stepped down from the platform and placed all its paws on a grid floor, an intermittent electroshock (60 V, DC, 0.5 s, 1 Hz) was delivered within 3–15 s after placement. Even a higher dose (1000 μg/kg) of *R*(+)-7-OH-DPAT administered prior to training did not markedly affect step-down latencies in the training trials. Namely, all the mice used stepped down within 3–15 s in the training period. The retention tests were done 24 h after training. Each mouse was again placed onto the platform and the step-down latency was measured. An upper cut-off time was set at 300 s.

2.3. Spontaneous locomotor activity

The spontaneous locomotor activity of the mice was measured with animal movement analyzing systems (Scanet SV-10, Toyo Sangyo, Toyama, Japan). These systems were equipped with 144 pairs of photosensors (*x*-axis: 88 pairs, *y*-axis: 56 pairs) set at a 5 mm interval, covering a measurement area of 480×300 mm (Ukai et al., 1994). Prior to the start of recording, the animals (one animal per testing cage) were placed in Plexiglas cages ($400 \times 200 \times 200$ mm). Two sizes of horizontal activity were observed as follows. Movement 1 depicts all sizes of horizontal activity, while movement 2 only depicts larger sizes of horizontal activity. Motility of 5-mm distance was taken as one activity count in movement 1. Motility of less than a 60-mm distance did not constitute a count in movement 2. When the mice covered more than a 60-mm distance, for example 75 mm, an activity count of 15 was reached, because photosensors were arranged at a 5-mm interval. The exact distance of diagonal movements not parallel to the alignment of photosensors could be automatically measured through the photosensors. One unit of movement without halt was recorded on the counters of the systems. Vertical activity gave a measure of the number of rearings. Five sets of Scanet SV-10 were connected to a personal computer (PC-9801 RX, Nihondenki, Tokyo, Japan).

2.4. Drugs and treatments

R(+)-7-OH-DPAT and *R*(+)-SCH 23390 (Research Biochemicals International, Natick, MA, USA) were dissolved in 0.9% saline and distilled water, respectively, while *S*(−)-sulpiride (Research Biochemicals International) was dissolved in 8.5% lactic acid and 1.0 N sodium hydroxide in a 3:2 ratio. *R*(+)-7-OH-DPAT, *R*(+)-SCH 23390 and *S*(−)-sulpiride were administered s.c., i.p. and i.p. in a volume of 10 ml/kg, respectively.

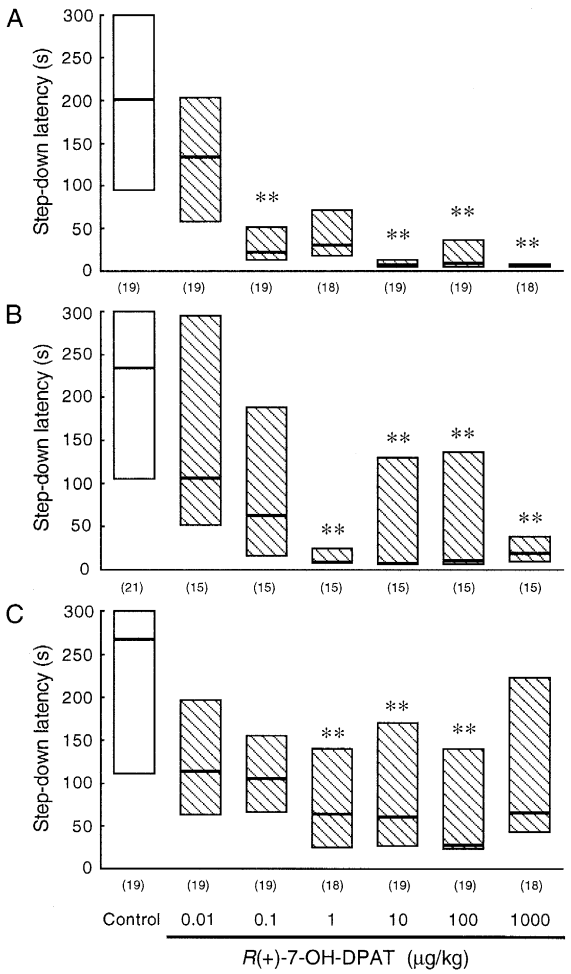

Fig. 1. Effects of *R*(+)-7-OH-DPAT on step-down latency of passive avoidance learning in mice. *R*(+)-7-OH-DPAT (s.c.) was administered at different time schedules (A, 30 min before training; B, immediately after training; C, 30 min before retention). Data were expressed as the median and interquartile ranges, which are the distances between the first quartile (25th percentile) and the third percentile (75th percentile). The number of mice used is shown in parentheses. $^{**}P < 0.01$ vs. control.

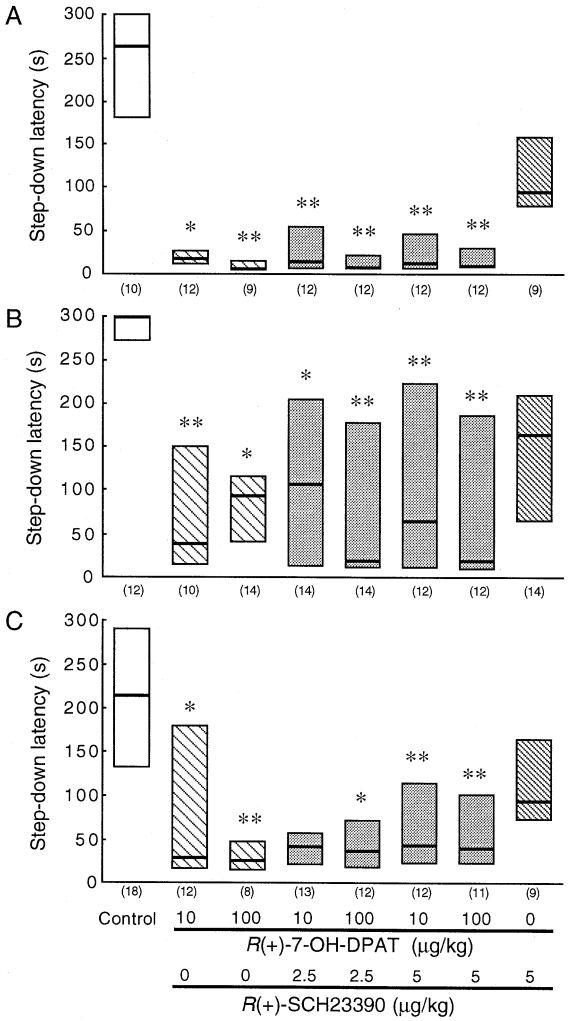

Fig. 2. Effects of $R(+)$-7-OH-DPAT and its combination with $R(+)$-SCH 23390 on step-down latency of passive avoidance learning in mice. $R(+)$-7-OH-DPAT (s.c.) was administered at different time schedules (A, 30 min before training; B, immediately after training; C, 30 min before retention). $R(+)$-SCH 23390 (i.p.) was administered 30 min before administration of $R(+)$-7-OH-DPAT. Data were expressed as the median and interquartile ranges, which are the distances between the first quartile (25th percentile) and the third percentile (75th percentile). The number of mice used is shown in parentheses. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control.

In passive avoidance learning, $R(+)$-7-OH-DPAT was given 30 min before training, immediately after training or 30 min before retention was tested. $R(+)$-SCH 23390 and $S(-)$-sulpiride were given 30 min before administration of $R(+)$-7-OH-DPAT. For the measurement of locomotor activity, individual mice were placed into testing cages 30 min after administration of $R(+)$-7-OH-DPAT and activity was observed for 10 min.

2.5. Statistical analysis

The results of passive avoidance learning were expressed in terms of medians and interquartile ranges (Q1–Q3), since an upper cut-off time was set. The results of locomotor activity were expressed as means ± S.E.M. Statistical comparisons for the results of passive avoidance learning were made with a Kruskal-Wallis test, and for those for locomotor activity were made with one-way analysis of variance (ANOVA). Further statistical analyses for individual groups were done with Bonferroni's test. A P value less than 0.05 was accepted as showing statistical significance for all evaluations.

3. Results

3.1. Effects of R(+)-7-OH-DPAT on passive avoidance learning

$R(+)$-7-OH-DPAT (0.1–1000 μg/kg) administered before training ($H(6) = 58.43$, $P < 0.01$), immediately af-

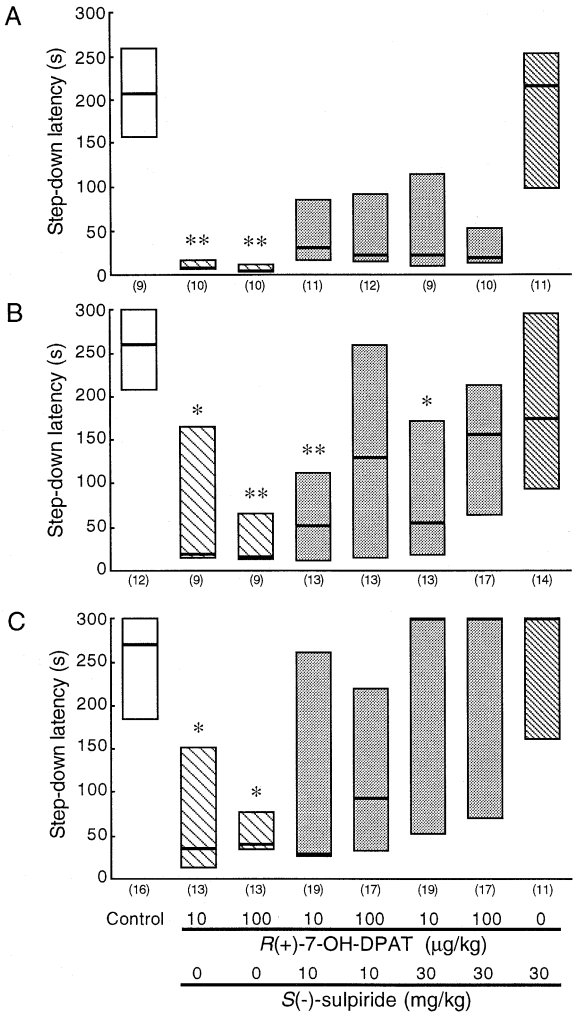

Fig. 3. Effects of $R(+)$-7-OH-DPAT and its combination with $S(-)$-sulpiride on step-down latency of passive avoidance learning in mice. $R(+)$-7-OH-DPAT (s.c.) was administered at different time schedules (A, 30 min before training; B, immediately after training; C, 30 min before retention). $S(-)$-Sulpiride (i.p.) was administered 30 min before administration of $R(+)$-7-OH-DPAT. Data were expressed as the median and interquartile ranges, which are the distances between the first quartile (25th percentile) and the third percentile (75th percentile). The number of mice used is shown in parentheses. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control.

ter training ($H(6) = 39.11$, $P < 0.01$) and before retention ($H(6) = 22.45$, $P < 0.01$) significantly shortened step-down latency (Fig. 1). *R*(+)-SCH 23390 (2.5 and 5 μg/kg) had no significant effects on the decrease in step-down latency induced by *R*(+)-7-OH-DPAT (10 and 100 μg/kg) injected before training ($H(7) = 39.04$, $P < 0.01$), immediately after training ($H(7) = 29.23$, $P < 0.01$) or before retention ($H(7) = 30.20$, $P < 0.01$) (Fig. 2). Although *S*(−)-sulpiride (10 and 30 mg/kg) showed a tendency to reverse the decrease in step-down latency induced by *R*(+)-7-OH-DPAT (10 and 100 μg/kg) injected before training ($H(7) = 33.12$, $P < 0.01$), immediately after training ($H(7) = 28.03$, $P < 0.01$) or before retention ($H(7) = 31.44$, $P < 0.01$), the effects of *S*(−)-sulpiride were not significant (Fig. 3). In addition, *R*(+)-SCH 23390 (5 μg/kg) or *S*(−)-sulpiride (30 mg/kg) alone failed to affect the step-down latency of passive avoidance learning in normal mice (Figs. 2 and 3).

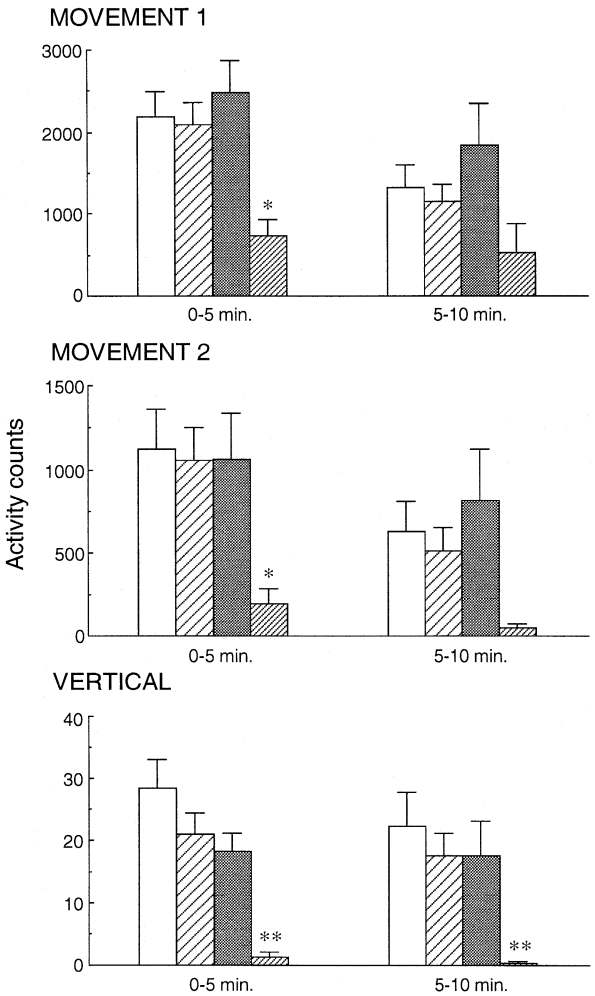

Fig. 4. Effects of *R*(+)-7-OH-DPAT on spontaneous locomotor activity in mice. Locomotor activity was measured for 10 min with a 5-min interval. *R*(+)-7-OH-DPAT (s.c.) was administered 30 min before behavioral measurements. First columns: control; second columns: *R*(+)-7-OH-DPAT 1 μg/kg; third columns: *R*(+)-7-OH-DPAT 100 μg/kg; fourth columns: *R*(+)-7-OH-DPAT 1000 μg/kg. Data are expressed as the means ± S.E.M. of 8 or 9 mice. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control.

3.2. Effects of R(+)-7-OH-DPAT on spontaneous locomotor activity

Within 5 min after the start of behavioral measurements, *R*(+)-7-OH-DPAT (1000 μg/kg) markedly decreased horizontal movement 1 ($F(3,33) = 6.46$, $P < 0.05$), horizontal movement 2 ($F(3,33) = 4.14$, $P < 0.05$) and vertical activity ($F(3,33) = 11.70$, $P < 0.01$) (Fig. 4). From 5 to 10 min after the start of measurements, *R*(+)-7-OH-DPAT (1000 μg/kg) significantly decreased vertical activity ($F(3,33) = 4.54$, $P < 0.01$). However, lower doses (1 and 100 μg/kg) of *R*(+)-7-OH-DPAT failed to influence locomotor activity (Fig. 4).

4. Discussion

Memory is divided into three phases: acquisition, consolidation and retention. In the present study, *R*(+)-7-OH-DPAT (10 and 100 μg/kg), a purported dopamine D_3 receptor agonist, impaired acquisition (pretraining administration), consolidation (post-training administration) and retention (preretention administration), strongly suggesting that stimulation of dopamine D_3 receptors results in amnesia. In other words, *R*(+)-7-OH-DPAT would induce anterograde and retrograde amnesia when administered prior to training and after training, respectively, while the compound seems to disrupt recall when administered prior to retention testing. Furthermore, a higher dose (1000 μg/kg) of *R*(+)-7-OH-DPAT administered 30 min before training and immediately after training significantly shortened step-down latency, although such a dose of *R*(+)-7-OH-DPAT was ineffective when administered 30 min before retention tests. In contrast, 30–40 min after administration of *R*(+)-7-OH-DPAT (1000 μg/kg) locomotor activity decreased significantly. *R*(+)-7-OH-DPAT (1000 μg/kg) attenuated the electroshock-induced vocalization during training (data not shown). Thus, the effects of 1000 μg/kg or higher doses of *R*(+)-7-OH-DPAT on passive avoidance response would not be associated with learning and memory.

The actual contribution of dopamine D_3 receptor agonists to memory should be determined with antagonists having high selectivity for dopamine D_3 receptors. Although (+)-AJ76, nafadotride, U99194 and S-142987 are considered to be putative dopamine D_3 receptor antagonists (Sokoloff et al., 1990; Waters et al., 1993; Millan et al., 1994; Sokoloff and Schwartz, 1995), these drugs have a considerable affinity, not only for dopamine D_3, but also for D_2 receptors. In contrast, because *R*(+)-7-OH-DPAT reportedly possesses some affinity for dopamine D_1 and D_2 receptors in addition to a much higher affinity for dopamine D_3 receptors (Svensson et al., 1994a; Millan et al., 1995), the involvement of dopamine D_3 receptors in the effects of *R*(+)-7-OH-DPAT should be rigorously clarified by using dopamine D_1- or D_2-selective receptor

antagonists. The dopamine D_1 receptor antagonist, *R*(+)-SCH23390 (2.5 and 5 μg/kg), or the dopamine D_2 receptor antagonist, *S*(−)-sulpiride (10 and 30 mg/kg), did not significantly block the *R*(+)-7-OH-DPAT (10 and 100 μg/kg)-induced amnesia. In addition, the doses of *R*(+)-SCH23390 and *S*(−)-sulpiride used were sufficient for antagonism against dopamine D_1 and D_2 receptors, respectively (Toyoshi et al., 1992). Thus, these results suggest that *R*(+)-7-OH-DPAT impairs passive avoidance learning through dopamine D_3 but not D_1 or D_2 receptors. Moreover, although it has been reported that *R*(+)-7-OH-DPAT has only slight affinity for 5-HT_{1A} and σ receptors in a receptor binding assay (Chumpradit et al., 1994; Millan et al., 1995), these receptors do not appear influential in the effects of *R*(+)-7-OH-DPAT.

In short, the present results suggest that *R*(+)-7-OH-DPAT impairs passive avoidance learning through the mediation of dopamine D_3 but not D_1 or D_2 receptors.

Acknowledgements

This research was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan.

References

Baldessarini, R.J., N.S. Kula, C.R. McGrath, V. Bakthavachalam, J.W. Kebabian and J.L. Neumeyer, 1993, Isomeric selectivity at dopamine D_3 receptors, Eur. J. Pharmacol. 239, 269.

Bracs, P.U., P. Gregory and D.M. Jackson, 1984, Passive avoidance in rats: disruption by dopamine applied to the nucleus accumbens, Psychopharmacology 83, 70.

Buresova, O. and J. Bures, 1982, Radial maze as a tool for assessing the effect of drugs on the working memory of rats, Psychopharmacology 77, 268.

Chumpradit, S., M.P. Kung, J. Vessotskie, C. Foulon, M. Mu and H.F. Kung, 1994, Iodinated 2-aminotetralins and 3-amino-1-benzopyrans: ligands for dopamine and D_3 receptors, J. Med. Chem. 37, 4245.

Daly, S.A. and J.L. Waddington, 1993, Behavioral effects of the putative D-3 dopamine receptor agonist 7-OH-DPAT in relation to other 'D-2-like' agonists, Neuropharmacology 32, 509.

Damsma, G., T. Bottema, B.H.C. Westerink, P.G. Tepper, D. Dijkstra, T.A. Pugsley, R.G. MacKenzie, T.G. Heffner and H. Wikström, 1993, Pharmacological aspects of *R*(+)-7-OH-DPAT, a putative dopamine D_3 receptor ligand, Eur. J. Pharmacol. 249, R9.

Ichihara, K., T. Nabeshima and T. Kameyama, 1988, Opposite effects induced by low and high doses of apomorphine on single-trial passive avoidance leaning in mice, Pharmacol. Biochem. Behav. 30, 107.

Kameyama, T., T. Nabeshima and Y. Noda, 1986, Cholinergic modulation of memory for step-down type passive avoidance task in mice, Res. Commun. Psychol. Psychiatry Behav. 11, 193.

Kebabian, J.W. and D.B. Calne, 1979, Multiple receptors for dopamine, Nature 277, 93.

Kesner, R.P., R.A. Bierley and P. Pebbles, 1981, Short-term memory: the role of *d*-amphetamine, Pharmacol. Biochem. Behav. 15, 673.

Levin, E.D. and R.E. Bowman, 1986, Effects of the dopamine D-2 receptor agonist, LY 171555, on radial arm maze performance in rats, Pharmacol. Biochem. Behav. 25, 1117.

Levin, E.D., S.R. McGurk, J.E. Rose and L.L. Butcher, 1989, Reversal of a mecamylamine-induced cognitive deficit with the D_2 agonist, LY171555, Pharmacol. Biochem. Behav. 33, 919.

Mattingly, B.A., 1986, The effects of apomorphine on lever press shock escape learning in rats, Pharmacol. Biochem. Behav. 25, 693.

Millan, M.J., V. Audinot, J.M. Rivet, A. Gobert, J. Vian, J.F. Prost, M. Spedding and J.L. Peglion, 1994, S14297, a novel selective ligand at cloned human dopamine D_3 receptors, blocks 7-OH-DPAT-induced hypothermia in rats, Eur. J. Pharmacol. 260, R3.

Millan, M.J., J.L. Peglion, J. Vian, J.M. Rivet, M. Brocco, A. Gobert, A. Newman-Tancredi, C. Dacquet, K. Bervoets, S. Girardon, V. Jacques, C. Chaput and V. Audinot, 1995, Functional correlates of dopamine D_3 receptor activation in the rat in vivo and their modulation by the selective antagonist, (+)-S 14297. 1. Activation of postsynaptic D_3 receptors mediates hypothermia, whereas blockade of D_2 receptors elicits prolactin secretion and catalepsy, J. Pharmacol. Exp. Ther. 275, 885.

Sokoloff, P. and J.-C. Schwartz, 1995, Novel dopamine receptors half a decade later, Trends Pharmacol. Sci. 16, 270.

Sokoloff, P., B. Giros, M.P. Martres, M.L. Bouthenet and J.C. Schwartz, 1990, Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics, Nature 347, 146.

Svensson, K., A. Carlsson, R.M. Huff, T. Kling-Petersen and N. Waters, 1994a, Behavioral and neurochemical data suggest functional differences between dopamine D_2 and D_3 receptors, Eur. J. Pharmacol. 263, 235.

Svensson, K., A. Carlsson and N. Waters, 1994b, Locomotor inhibition by the D_3 ligand *R*(+)-7-OH-DPAT is independent of changes in dopamine release, J. Neural Transm. 95, 71.

Toyoshi, T., M. Ukai and T. Kameyama, 1992, Combination of a δ opioid receptor agonist but not a μ opioid receptor agonist with the D_1-selective dopamine receptor agonist SKF38393 markedly potentiates different behaviors in mice, Eur. J. Pharmacol. 213, 25.

Ukai, M., T. Kobayashi and T. Kameyama, 1994, Effects of several amnesic drugs on the habituation of exploratory activity in mice as indexed by horizontal and vertical activities, Gen. Pharmacol. 25, 179.

Van de Buuse, M., 1993, Effects of 7-hydroxy-*N*,*N*-di-*n*-propylaminotetralin on behaviour and blood pressure of spontaneously hypertensive rats, Eur. J. Pharmacol. 243, 169.

Waters, N., K. Svensson, S.R. Haadsma-Svensson, M.W. Smith and A. Carlsson, 1993, The dopamine D_3-receptor: a postsynaptic receptor inhibitory on rat locomotor activity, J. Neural Transm. 94, 11.