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Ovariectomy ameliorates dextromethorphan - induced memory impairment in young female rats

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Abstract

We have previously found that dextromethorphan (DM), over-the-counter cough suppressant, impairs memory retention in water maze task, when it is repeatedly administrated to adolescent female rats at high doses. In this study we examined first if ovariectomy ameliorates the DM-induced memory impairment in female rats, and then whether or not the DM effect is revived by estrogen replacement in ovariectomized female rats. Female rat pups received bilateral ovariectomy or sham operation on postnatal day (PND) 21, and then intraperitoneal DM (40 mg/kg) daily during PND 28–37. Rats were subjected to the Morris water maze task from PND 38, approximately 24 h after the last DM injection. In probe trial, goal quadrant dwell time was significantly reduced by DM in the sham operated group, however, the reduction by DM did not occur in the ovariectomy group. When 17β-estradiol was supplied to ovariectomized females during DM treatment, the goal quadrant dwell time was significantly decreased, compared to the vehicle control group. Furthermore, a major effect of estrogen replacement was found in the escape latency during the last 3 days of initial learning trials. These results suggest that ovariectomy may ameliorate the adverse effect of DM treatment on memory retention in young female rats, and that estrogen replacement may revive it, *i.e.* estrogen may take a major role in DM-induced memory impairment in female rats.

Keywords: NMDA receptor • estrogen • learning and memory

Introduction

Dextromethorphan (DM), a non-competitive antagonist of the N-methyl-D-aspartate (NMDA)-type of excitatory amino acid receptors, has long been used as an over-the-counter cough suppressant at low doses (30-60 mg/day, *p.o.*) [1]. Recently, DM has attracted clinical interest for its neuroprotective [2], anticonvulsant [3] and analgesic effects [4, 5]. Clinical trials are also currently investigating for the intervention of neurodegenerative diseases and other disorders [6, 7]. Chronic administration of DM at higher doses than antitussive doses is usually recommended for these clinical indications.

However, episodic and sporadic abuse of DM at high doses (300 mg/day or more) has been reported in several countries mostly among adolescents and young adults [8, 9, 10, 11]. Abuse liability of DM at adolescence has been supported by our previous

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reports showing that acute DM, which was administrated to adolescent rats at high doses, increased gene expression of tyrosine hydroxylase, the rate limiting enzyme of catecholamine biosynthesis, in the midbrain dopaminergic neurons [12] and activated the neurons, referred by c-fos expression, in the reward pathway [13].

Repetitive DM at high doses may induce phencyclidine-like side effects, such as memory and psychotomimetic disturbances, because phencyclidine, so as DM, is a non-competitive antagonist of NMDA receptor [14] and the psychological symptoms of DM abuse are similar to phencyclidine-induced psychotomimetic symptoms [15]. It is well known that treatment with NMDA receptor antagonists impairs spatial learning tasks [16–20]. It has been reported that chronic neonatal NMDA receptor antagonists, such as phencyclidine or MK-801, impairs spatial learning both in adolescent and adult rats [21, 22]. Acute DM on each training day of water maze task impaired the ability for learning in adult rats [23]. We previously found that repetitive DM at adolescence induces behavioral sensitization [24, Jahng *et al.*, submitted] and impairs water maze learning, however, only in female rats, but not in male [25]. This suggests that female rats at adolescence may have higher vulnerability than male rats to NMDA receptor antagonists.

It was reported that estrogen impairs reference memory in Morris water maze learning, the hippocampal based learning task [26]. Estrogen has been reported to be involved in neural plasticity of the pyramidal cells in the hippocampus [27, 28], and the estrogen effects on the hippocampal neurons are believed to be mediated by NMDA receptors [29–31]. In this study, we examined first if ovariectomy ameliorates DM-induced impairment in water maze learning in female rats, and then whether or not the DM effect is revived by estrogen replacement in ovariectomized female rats.

Materials and Methods

Animals

Sprague-Dawley rats were supplied from the Division of Laboratory Animal Medicine, Yonsei University College of Medicine, and cared in a specific-pathogen-free barrier area with constant control of temperature (22 ±1°), humidity (55%), and a 12/12 h light/dark cycle (lights-on at 07:00AM). Rats had free access to standard laboratory food (Purina Rodent Chow, Purina Co., Seoul, Korea) and membrane filtered purified water *ad libitum*. Animals were cared according to The Guideline for Animal Experiments, 2000, edited by The Korean Academy of Medical Sciences, which is consistent with NIH Guideline for the Care and Use of Laboratory Animals, revised 1996. All animal experiments were approved by the Committee for the Care and Use of Laboratory Animals at Yonsei University.

For behavioral study, nulliparous female and proven breeder male were used for breeding in the laboratory of the animal facility, and the offspring reared in a controlled manner to minimize and standardize unwanted environmental stimulation from *in utero* life. Twelve hours after confirming delivery, pups were culled to 6 males and 6 females in a litter, and weaned on postnatal day (PND) 21. Three weanling female pups in each litter were used for the experiment and the rest were excluded. One female pup in a litter was assigned to ovariectomy and the rest two to sham operation. Bilateral ovariectomy was performed with dorsal approach under chloral hydrate (153 mg/kg) and pentobarbital (35 mg/kg) anesthesia on PND 21, as previously described [32], and the sham operation consisted of the same procedure without touching ovary. Body weight gain of ovariectomized pups did not differ from the sham-operated group during the experimental period.

Drug treatment

DM (Dextromethorphan HBr, Sigma Chemical Co., St. Louis, MO, USA) was dissolved at a concentration of 40 mg/2 ml in saline. One out of the two sham-operated female rats and the ovariectomized one from each litter received intraperitoneal injection of DM at a dose of 40 mg/kg daily between 8:00 AM and 9:00 AM during PND 28 – 37, and the rest sham-operated female rat received aseptic physiologic saline instead of DM with the same injection volume and schedule (n=6 in each group, *i.e.* 18 pups from 6 different litters were used). The dose was chosen because 40 mg/kg of DM produced the most significant behavioral effects acutely without mortality [13], and induced behavioral sensitization [24] and impairment in water maze learning [25] when it was given repeatedly to adolescent female rats.

Four out of 10 rats died within 20 min after 80 mg/kg of DM in our preliminary experiment (data not shown). Mean body weights of the pups on PND 28 were not different among the groups, because rats were assigned by Latin square method according to the order of body weights within a litter.

For estrogen replacement experiment, two ovariectomized female pups in each litter were used (total 12 pups from 6 different litters). One pup from each litter received subcutaneous injection of 17β-estradiol (1μg in 200μl of sesame oil, Sigma Chemical Co., St. Louis, MO, USA) and intraperitoneal DM (40 mg/kg), and the other received 200 μl of sesame oil (Sigma Chemical Co., St. Louis, MO, USA) and 40 mg/kg of DM. The injections were made daily between 8:00 AM and 9:00 AM, and 17β-estradiol or the vehicle was given from PND 24 to 37 and the DM injection from PND 28 to 37.

Morris water maze

Rats were subjected to the Morris water maze task from PND 38, approximately 24 h after the last drug injection. A modified version of the Morris water maze task was used. A circular water maze (Panlab s.l., Barcelona, Spain) in 200 cm diameter and 40 cm height was used. The pool was divided into four quadrants of equal surface area. The starting points were called north, south, east and west, and located arbitrarily at equal distances on the pool rim. A circular platform in 15 cm diameter and 30 cm height was located in the south-east quadrant, submerged 1cm under the surface of water and 42.5 cm apart from the pool rim. The water temperature was maintained at 22 ±1°. Non-toxic, black tempera paint powder (Funstuff, Reeves and Poole Group, Toronto, Canada) was used to hide the platform. The movement of rats within the tank was recorded by a video tracking system (Smart, Panlab s.l., Barcelona, Spain). A variety of pictures were posted on the walls of the test room as cued stimuli. Time of escape latency and swim distance to reach the platform were used to assess the acquisition of the task. The motor activity of rats in this task was determined by swimming speed (path length / escape latency).

To assess the spatial learning ability, the rats were subjected to initial training as the first phase of the experiment. The rats were randomly placed in one of the four starting points, approximately 5 cm apart from and facing the edge of the pool, and then given 60 sec to find the escape platform. If they found the platform within 60 sec, they were allowed to stay at the platform for 15 sec. Rats that did not find the platform within 60 sec were guided to the platform by the experimenter and allowed to stay there for 15 sec. The rats were removed from the maze, toweled dry, and placed under a heat lamp (60 Watt, 20 cm above) for 5 min before the next trial. There were four trials on each training day and the starting quadrant was changed after each trial. The order of starting points changed for each training day. Initial training was performed for 6 days in ovariectomy experiment, or for 9 days in estrogen replacement experiment, respectively.

A probe test was completed with the first trial on the next day after the initial training. The escape platform was removed from the tank. Then the rats were placed in the opposite side of where the platform used to be located, and allowed to swim in the tank for 60 sec. The animal performances, *i.e.* swimming time and distance in each quadrant, swim speed, and annulus crossings, over 60 sec were recorded by a video tracking system (Smart, Panlab s.l., Barcelona, Spain) to measure the spatial learning ability without the influence of chance encounters with the platform. The probe test was performed to demonstrate the strength of the place bias indicative of spatial learning in each animal.

Statistical analysis

Data were presented as means ± S.E.M. and statistical analyses were done with the aid of the StatView II program (version 5.01, The SAS Institute, CA, USA). Differences between means were analyzed with a one-way analysis of variance (ANOVA) and preplanned comparisons with the control performed by post-hoc Fisher's PLSD test.

Results

Rats were subjected to the initial training of water maze task from PND 38, approximately 24 h after the last injection of dextromethorphan (DM) or saline. All rats showed a progressive decline in the escape latency over the six initial training days. One-way ANOVA was performed with the average of the rat's four escape latencies per day, and no significant difference was found among the experimental groups in each training day (Fig. 1. A). Swim speeds of rats in the maze on each training

day did not differ among the experimental groups (data not shown). To evaluate memory retention, probe trial was performed on the seventh day. Goal quadrant dwell time was significantly reduced in the DM treated sham group [F (2, 13) = 4.806, $P<0.05$] compared with the saline control sham group, and the reduction was not found in the DM treated ovariectomy group (Fig.1. B). Swim speeds of rats during probe trial did not differ among the groups (sham/saline group, 32.600 ± 1.757 cm/sec; sham/DM group, 29.900 ± 1.698 cm/sec; OVX/DM group, 32.317 ± 1.134 cm/sec). These results suggest that ovariectomy may ameliorate the memory impairment by repetitive DM at adolescence in female rats.

Ovariectomized rats received 17β-estradiol or vehicle during DM treatment, and were subjected to the water maze task 24 h after the last drug injections. Both the 17β-estradiol and the vehicle groups showed a progressive decline in the escape latency over the first seven training days, without significant differences between the experimental groups (Fig. 2. A). However, one-way ANOVA revealed a major effect of estradiol replacement in the escape latency on the 8th [F (1, 10) = 28.003, $P<0.001$ *vs.* vehicle] and 9th [F (1, 10) = 14.163, $P<0.01$ *vs.* vehicle] day of initial learning trials. In the probe test, which was performed on the 10th day, goal quadrant dwell time was significantly reduced in the 17β-estradiol group [F (1, 10) = 5.211, $P<0.05$] compared with the vehicle control (Fig. 2. B). These results suggest that estrogen replacement might have revived the adverse effect of repetitive DM on memory retention in ovariectomized female rats, *i.e.* estrogen may take a major role in DM-induced memory impairment in young female rats.

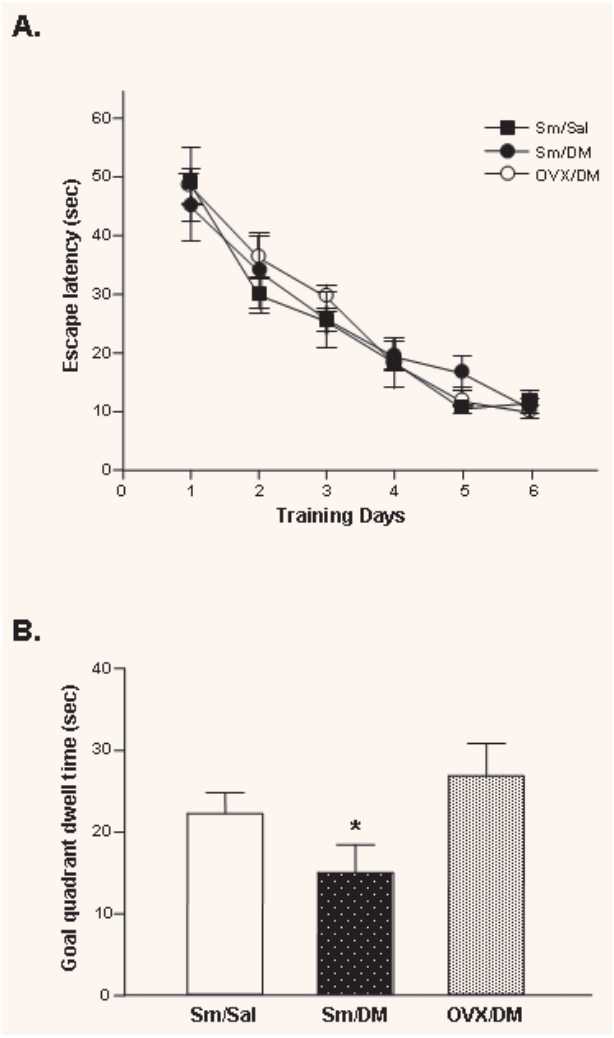

Fig. 1 Behavioral scores of ovariectomized or sham-operated female rats in the Morris water maze task. A; Escape latencies during initial training period, B; Goal quadrant dwell times of probe trial. Rats received ovariectomy on PND 21, and dextromethorphan (40 mg/kg) was intraperitoneally injected daily between 8:00AM and 9:00AM during PND 28–37. Rats were subjected to water maze task 24 h after the last injection (PND 38). One-way ANOVA analysis of escape latencies during initial training period revealed no effect of drug or ovariectomy in each training day (A). In the probe trial performed on the seventh day, goal quadrant dwell time was significantly reduced in the DM treated sham group (Sm/DM) compared with the saline control sham group (Sm/Sal), and the reduction was not found in the DM treated ovariectomy group (OVX/DM) (B). *$P<0.05$ *vs.* Sm/Sal, n= 5–6 each group, Data were presented as means ± S.E.M.

Discussion

We demonstrated that female rats treated with dextromethorphan (DM) repeatedly during adolescent period successfully completed the initial learning of the Morris water maze task, regardless of ovariectomy, in a similar pattern with the non-treated controls. This result is in accordance with our previous report [25], suggesting that repetitive DM at adolescence of female rats may not influence the acquisition of water maze learning. However, it was

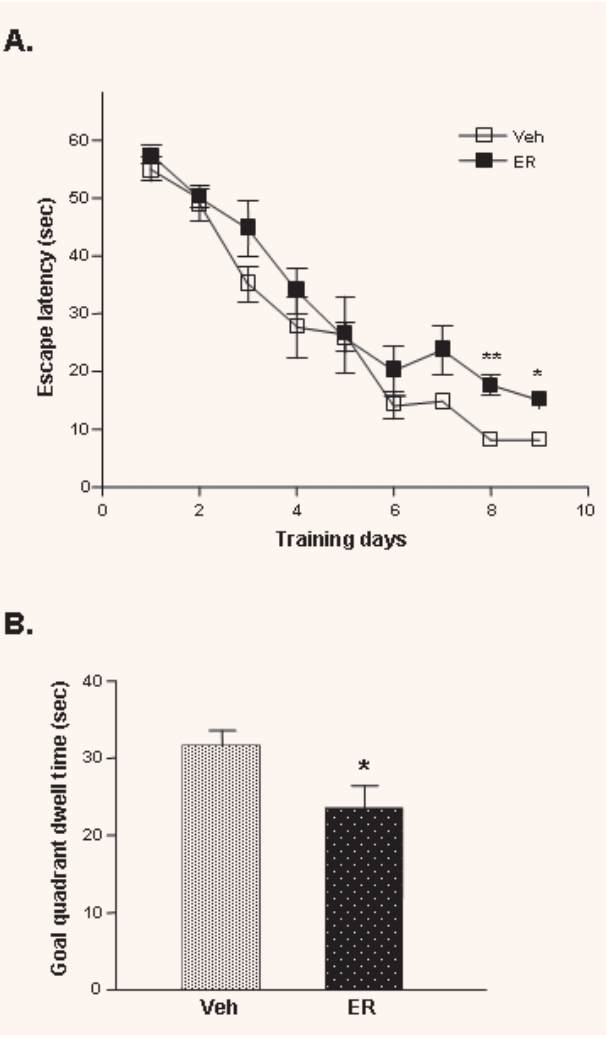

Fig. 2 Behavioral scores of ovariectomized female rats received 17β-estradiol or vehicle during DM treatment. Ovariectomized rats received 17β-estradiol (1 μg in 200 μl of sesame oil) or vehicle (200 μl of sesame oil) daily from PND 24 to 37, and intraperitoneal DM (40 mg/kg) from PND 28 to 37. Rats were subjected to the water maze task 24 h after the last drug injections. A; One-way ANOVA revealed a major effect of estradiol replacement in the escape latency on the 8th [$F(1, 10) = 28.003$, $^{**}P<0.001$ *vs.* vehicle] and 9th [$F(1, 10) = 14.163$, $^{*}P<0.01$ *vs.* vehicle], *i.e.* the estradiol replaced group (ER) took longer time to reach the hidden platform compared with vehicle control (Veh). B; In the probe test, which was performed on the 10th day, goal quadrant dwell time was significantly reduced in the 17β-estradiol group [$F(1, 10) = 5.211$, $^{*}P<0.05$] compared with vehicle control. n=6 each group, Data were presented as means ± S.E.M.

reported that DM at 40 mg/kg of dose, the same dose used in this study, impairs the acquisition of the Morris water maze task during initial training period in adult rats, when it is injected shortly, *i.e.* 15 min, before the initial training [23]. The initial training performance of rodents in the maze is generally dependent upon two factors: habituation of perimeter preference and learning the location of the escape platform. Previous report has revealed slower perimeter habituation in NMDA receptor antagonist-treated rats [33]. The impairment of water maze acquisition by acute DM could be due to impaired sensory and motor processes [34]. Indeed, Bane *et al.* [23] mentioned an alteration in motor activity induced by DM, *i.e.* DM treated adult rats appeared slow to move towards the center of the maze. Another non-competitive antagonist of NMDA receptor, MK-801, impaired the acquisition of water maze learning in rats, when it was injected 45 min before the behavioral tests, and these rats showed hyperactivity with increased swim speed, which inhibited staying on the escape platform [35]. We previously reported that ataxia and stereotyped behavior is induced in adolescent rats shortly after DM injection in a dose dependent manner [13, 12]. In the present study, swim speeds of rats in the maze did not differ among the experimental groups, supporting no effect of chronic DM on motor system. Furthermore, in our studies including the present one, rats were subjected to the initial training of water maze task 24 h after the last drug injection. Therefore, we believe that DM does not affect the acquisition of water maze learning task in adolescent rats, when a possible involvement of altered motor activity in the learning task is excluded in the experimental paradigm.

In this study, goal quadrant dwell time in the probe trial of water maze task was significantly reduced by DM in young female rats without ovariectomy, as we previously reported [25]. The purpose of probe trial was to evaluate memory retention, *i.e.* to examine whether the rats soundly remember the spot where the escape platform used to be located without the influence of chance encounters with the platform. Thus our result suggests that repetitive DM at adolescence may impair the retention of spatial memory in female rat. This result concurs with previous report that repeated treatment of a non-competitive antagonist of NMDA receptor phencyclidine induces long-term

impairment in water maze performance [22]. In this study, the DM-induced memory impairment did not occur in ovariectomized females, suggesting that ovary function is required for the adverse effect of repetitive DM. Indeed the impairment in memory retention by repetitive DM revived with estradiol replacement to the ovariectomized females. Furthermore, the estradiol replaced group showed a partial impairment in the acquisition of water maze learning as well, during the initial learning trials. This result suggests that estrogen may take a major role in the adverse effect of repetitive DM during adolescent period in female rats.

It has been suggested that spatial learning deficits induced by NMDA receptor antagonists are due to the blockade of hippocampal NMDA receptors [36]. It was reported that chronic postnatal phencyclidine upregulates NMDA receptor density in the hippocampus and the frontal cortex of adult rats, concomitantly with deficits in spatial learning task [22]. This suggests that repeated treatment with NMDA receptor antagonists may cause a permanent alteration of NMDA receptor populations in the brain regions, and this alteration may correlate with impairment of spatial learning task in rodents. It was reported that NMDA receptor activation is required for synapse formation in the hippocampus [37], suggesting a permanent increase in NMDA receptor activity could disrupt synapse formation in the hippocampus, in turn impairs spatial cognitive memory task. We previously found that repeated treatment of DM during adolescent period of rats markedly increased NMDAR1, functional subunit of NMDA receptor complex, in the brain regions, such as the hippocampus and the hypothalamus [24], and that this increase was more obvious in female rats than males, and furthermore, not observed in ovariectomized female rats (manuscript in preparation). Estrogen increases Ca^{2+} influx through NMDA receptors [38] and both the slop and the amplitude of NMDA receptor-mediated long-term potentiation [39]. It has been reported that estradiol treatment increases the number of NMDA binding sites and NMDAR1 subunits [30, 31] and NMDA receptor-mediated synaptic input [31] in the hippocampus, and impairs reference memory in Morris water maze learning, the hippocampal based learning task [26]. Taken all together, it is suggested that memory impairment by repetitive DM may be related with a permanent increase in NMDA receptor activity in the hippocampus, and estrogen may take a role in the adverse effect of repetitive DM, perhaps, via mediating, or enhancing, NMDA receptor activity in the hippocampus.

Interestingly, DM effect was not detected in the initial learning trials of intact or ovariectomized rats; however, it was disclosed by estradiol replacement. It has been reported that estrogen replacement at high doses may impair acquisition of spatial memory [40, 41]. Exogenous estrogen at high doses impaired working memory performance in the radial arm maze [40] and memory acquisition in the water maze task [41] in ovariectomized rats. It was suggested that impaired acquisition in the water maze learning by exogenous estrogen may be due to disturbances in estrogen-induced changes of the hippocampal spine density [41]. This may explain the impaired performance of initial learning trials by estradiol replacement in this study, although further studies are required to determine whether or not exogenous estrogen interacted with DM to impair the acquisition of water maze learning.

In conclusion, repetitive DM at adolescence impairs spatial memory task in young female rats, and estrogen appears to take a role in this impairment. We suggest that this adverse, perhaps sex-specific, effect of repetitive DM should be considered when the drug is clinically treated for neurodegenerative diseases and other disorders [6, 7], since chronic administration of DM at higher doses than antitussive doses is usually recommended for those clinical indications. Further studies to define molecular mechanism of repetitive DM-induced long-term adverse effects are currently under our considerations.

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References

1. **Bem JL, Peck R.** Dextromethorphan. An overview of safety issues. *Drug Safety*. 1992; 7: 190–9.

2. **Steinberg GK, Kunis D, DeLaPaz R, Poljak A.** Neuroprotection following focal cerebral ischaemia with the NMDA antagonist dextromethorphan has a favourable dose response profile. *Neurol Res.* 1993; 15: 174–80.
3. **Sagratella S.** NMDA antagonists: antiepileptic-neuroprotective drugs with diversified neuropharmacological profiles. *Pharmacol Res.* 1995; 32: 1-13.
4. **Price DD, Mao J, Lu J, Caruso FS, Frenk H, Mayer DJ.** Effects of the combined oral administration of NSAIDs and dextromethorphan on behavioral symptoms indicative of arthritic pain in rats. *Pain*. 1996; 68: 119-27.
5. **Weinbroum AA, Rudick V, Paret G, Ben-Abraham R.** The role of dextromethorphan in pain control. *Can J Anaesth.* 2000; 47: 585-96.
6. **Chase TN, Oh JD, Konitsiotis S.** Antiparkinsonian and antidyskinetic activity of drugs targeting central glutamatergic mechanisms. *J Neurol*. 2000; 247: Suppl 2: II36-42.
7. **Fisher K, Coderre TJ, Hagen NA.** Targeting the N-methyl-D-aspartate receptor for chronic pain management. Preclinical animal studies, recent clinical experience and future research directions. *J Pain Symptom Manage*. 2000; 20: 358-73.
8. **Murray S, Brewerton T.** Abuse of over-the-counter dextromethorphan by teenagers. *South Med J.* 1993; 86: 1151-3.
9. **Wolfe TR, Caravati EM.** Massive dextromethorphan ingestion and abuse, *Am J Emerg Med.* 1995; 13: 174-6.
10. **Darboe MN, Keenan GR Jr, Richards TK.** The abuse of dextromethorphan-based cough syrup: a pilot study of the community of Waynesboro, Pennsylvania. *Adolescence.* 1996; 31: 633-44.
11. **Noonan WC, Miller WR, Feeney DM.** Dextromethorphan abuse among youth. *Arch Fam Med.* 2000; 9: 791-2.
12. **Zhang TY, Jahng JW, Kim D.** Dextromethorphan increases tyrosine hydroxylase mRNA in the mesencephalon of adolescent rats. *Neurosci Lett.* 2001; 309: 85-8.
13. **Jahng JW, Zhang TY, Lee S, Kim D.** Effects of dextromethorphan on the nocturnal behavior and brain c-Fos expressions in adolescent rats. *Eur J Pharmacol.* 2001; 431: 47-52.
14. **Dingledine R, Borges K, Bowie D, Traynelis SF.** The glutamate receptor ion channels. *Pharamcol Rev.* 1999; 51: 7-61.
15. **Price LH, Lebel J.** Dextromethorphan-induced psychosis. *Am J Psychiat.* 2000; 157: 304.
16. **Morris RGM, Andersen E, Lynch GS, Baudry M.** Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist AP5. *Nature*. 1986; 319: 774-776.
17. **Morris RGM.** Synaptic plasticity and learning. Selective impairment of learning in rats and blockade of long-term potentiation in vivo by the N-methyl-D-aspartate receptor antagonist APV. *J Neurosci*. 1989; 9: 3040-3057.
18. **Shapiro ML, Caramanos Z.** NMDA antagonist MK-801 impairs acquisition but not performance of spatial working memory and reference memory. *Psychobiology*. 1990; 18: 231-243.
19. **Davis S, Butcher SP, Morris GRM.** The NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) impairs learning and LTP in vivo at intracerebral concentrations comparable to those that block LTP in vitro. *J Neurosci*. 1992; 12: 21-34.
20. **McDonald RJ, Hong NS, Craig LA, Holahan MR, Louis M, Muller RU.** NMDA-receptor blockade by CPP impairs post-training consolidation of a rapidly acquired spatial representation in rat hippocampus. *Eur J Neurosci*. 2005; 22: 1201-1213.
21. **Gorter JA, de Bruin JPC.** Chronic neonatal MK-801 treatment results in an impairment of spatial learning in the adult rat. *Brain Res.* 1992; 580: 12-7.
22. **Sircar R.** Postnatal phencyclidine-induced deficit in adult water maze performance is associated with N-methyl-D-aspartate receptor upregulation. *Int J Devl Neurosci.* 2003; 21: 159-67.
23. **Bane A, Rojas D, Indermaur K, Bennett T, Avery D.** Adverse effects of dextromethorphan on the spatial learning of rats in the Morris water maze. *Eur J Pharmacol.* 1996; 302: 7-12.
24. **Zhang TY, Jahng JW, Kim EJ, Chung H, Kim D.** Chronic dextromethorphan increases NMDAR1 immunoreactivity in the brain and induces sensitization of stereotyped behavior in female rats. *Soc Neurosci Abs*. 1999; 25: 2077.
25. **Cho HJ, Kim JG, Lee JY, Lee S, Jahng JW.** Repetitive dextromethorphan at adolescence affects water maze learning in female rats. *Intl J Neurosci.* 2005; In press.
26. **Galea LA, Wide JK, Paine TA, Holmes MM, Ormerod BK, Floresco SB.** High levels of estradiol disrupt conditioned place preference learning, stimulus response learning and reference memory but have limited effects on working memory. *Behav Brain Res.* 2001; 126: 115-26.
27. **Gould E, Woolley CS, Frankfurt M, McEwen BS.** Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. *J Neurosci.* 1990; 10: 1286-91.
28. **Woolley CS, McEwen BS.** Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. *J Comp Neurol.* 1993; 336: 293-306.
29. **Weiland NG.** Estradiol selectively regulates agonist binding sites on the N-methyl-D-aspartate receptor complex in the CA1 region of the hippocampus. *Endocrinology.* 1992; 131: 662-8.
30. **Gazzaley AH, Weiland NG, McEwen BS, Morrison JH.** Differential regulation of NMDAR1 mRNA and protein by estradiol in the rat hippocampus. *J Neurosci.* 1996; 16: 6830-8.
31. **Woolley CS, Weiland NG, McEwen BS, Schwartkroin PA.** Estradiol increases the sensitivity of hippocampal CA1 pyramidal cells to NMDA receptor-mediated synaptic input: correlation with dendritic spine density. *J Neurosci.* 1997; 17: 1848-59.
32. **Waynforth HB, Flecknell PA.** Experimental and surgical technique in the rat. New York; Academic Press; 1992
33. **Venable N, Kelly, PH.** Effects of NMDA antagonists on memory processes in a novel two-trial swimming test. *Behav Pharmacol.* 1991; 2: 161-9.

34. **Willets J, Balster RL, Leander JD.** The behavioral pharmacology of NMDA receptor antagonists. *Trends Pharmacol Sci.* 1990; 11: 423-8.
35. **Ylinen A, Pitkanen M, Sirvio J, Hartikainen T, Sivenius J, Koivisto E, Riekkinen PJ Jr.** The effects of NMDA receptor antagonists at anticonvulsive doses on the performance of rats in the water maze task. *Eur J Pharmacol.* 1995; 274: 159-65.
36. **Butelman ER.** A novel NMDA antagonist, MK-801, impairs performance in a hippocampal-dependent spatial learning task. *Pharmacol Biochem Behav.* 1989; 34: 13-6.
37. **McEwen B.** Estrogen actions throughout the brain. *Recent Prog Horm Res.* 2002; 57: 357-84.
38. **Pozzo-Miller LD, Inque T, Murphy DD.** Estradiol increases spine density and NMDA-dependent Ca++ transientsin spines of Ca1 pyramidal neurons from hippocampal slices. *J Neurophysiol.* 1999; 81: 1404-11.
39. **Foy MR, Xu J, Xie X, Brinton RD, Thompson RF, Beger TW.** 17b-Estradiol enhances NMDA receptor-mediated EPSPs and long-term potentiation. *J Neurophysiol.* 1999; 81: 925-9.
40. **Holmes MM, Wide JK, Galea LAM.** Low levels of estradiol facilitate, whereas high levels of estradiol impair, working memory performance on the radial arm maze. *Behav Neurosci*. 2002; 116: 928-34.
41. **Frick KM, Fernandez SM, Bennett JC, Prange-Kiel J, MacLusky NJ, Leranth C.** Behavioral training interferes with the ability of gonadal hormones to increase CA1 spine synapse density in ovariectomized female rats. *Eur J Neurosci*. 2004; 19: 3026-32.