NO 行政院國家科學委員會專題研究計畫成果報告

天麻成分 gastrodin 及 *p*-hydroxbenzyl alcohol 對於大鼠學習記憶及腦內單胺濃度之研究

Effects of gastrodin and *p*-hydroxybenzyl alcohol on memory processes and brain monoamines concentration in rats

計畫編號：NSC 86-2314-B-039-007 M13
執行期限：85 年 8 月 1 日至 86 年 7 月 31 日
主持人：謝明村　　執行機構及單位名稱：中國醫藥學院中國藥學研究所

一、中文摘要

天麻苷元可改善學習獲得障礙誘發劑 *p*-chloroamphetamine 及 apomorphine、記憶鞏固障礙誘發劑 cycloheximide 及記憶再現障礙誘發劑 apomorphine 之作用，且其作用均較正對照組 piracetam 為佳；而作用劑量曲線呈倒 U 型。

在記憶鞏固機轉方面，天麻苷元對記憶鞏固障礙誘發劑 cycloheximide 之改善作用，膽鹼接受器 scopolamine 或煙鹼接受器拮抗劑 mecamylamine 無法拮抗此作用，但可被 serotonin 釋放促進劑 *p*-chloroamphetamine 及 serotonin 前驅物 5-hydroxytryptophan 所阻斷；進一步，可被突觸後 5-HT_{1A} 接受器致效劑 8-OH-DPAT 或 5-HT_2 接受器致效劑 DOI 所阻斷，亦可被突觸後非選擇性之突觸後 5-HT_1 接受器拮抗劑(±)-pindolol 或 5-HT_2 接受器拮抗劑 ritanserin 所增強。

再者，在周邊作用機轉方面，大白鼠經靜脈注射 6-OHDA 前處理造成交感神經截斷後，天麻苷元對學習記憶障礙誘發劑 cycloheximide 之改善作用明顯低於正常動物下天麻　元之改善作用。其次，大白鼠經去除腎上腺後，可部份阻斷天麻苷元對誘發記憶鞏固障礙劑 cycloheximide 之改善作用。而在中樞作用機轉方面，天麻苷元對腦室給予 6-OHDA 或 5,7-DHT 誘發之學習記憶障礙均具改善作用。

綜合上述研究結果顯示：天麻苷元改善誘發大白鼠學習獲得障礙劑 *p*-chloroamphetamine 、誘發大白鼠學習獲得及記憶再現障礙劑 apomorphine 及誘發記憶鞏固障礙劑 cycloheximide ，較智能增進劑 piracetam 之作用強，其可能之機轉不僅與周邊交感神經有關，且可能經由中樞突觸後及突觸前之 5-HT_{1A} 接受器及 5-HT_2 接受器或間接經由 a_1 接受器，以降低中樞 serotonergic system 之活性；及經由中樞突觸前之 D_1 及 D_2 接受器，以降低中樞 dopaminergic system 之活性。

關鍵詞：天麻苷元、學習記憶、中樞神經、週邊神經

Abstract

We attempted to investigate the effects of HBA on learning and memory processes such as acquisition, consolidation and retrieval in the passive avoidance task in rats and piracetam was used as a positive control. HBA can attenuate the impairments of learning acquisition induced by *p*-chloroamphetamine (PCA) and apomorphine (APO), the impairments of memory consolidation induced by cycloheximide (CXM) and the impairments of memory retrieval induced by APO in rats. The above results indicated that the counteractive effects of HBA are bell-shaped dose-response curve with a maximal effect at 5 mg/kg and greater than those brought through piracetam.

In the mechanism for the counteractive effects of HBA on CXM-induced memory consolidation impairment, the counteractive effect of HBA was not depressed by either SCOP or mecamylamine. The serotonin (5-HT) releaser, PCA, and precursor, 5-hydroxytryptopan significantly antagonized the counteractive effect of HBA on the CXM-induced shortening of retention latencies. Furthermore, the counteractive effect was also inhibited by the 5-HT_{1A} receptor agonist 8-OH-DPAT and the 5-HT_2 receptor agonist DOI, but potentiated by the 5-HT receptor antagonist (+)-pindolol and the 5-HT_2 receptor antagonist ritanserin.

Moreover, recent studies have revealed that cognition enhancing drugs act via the peripheral or central mechanism. The present study was further investigated to evaluate whether the counteracting effects of HBA was mediated by the peripheral or central sites of action. In the role of peripheral sites on the counteractive effects of HBA, the counteracting effects of HBA on the CXM-induced memory impairment in 6-OHDA (i.v.)-treated rats were smaller than those of HBA in normal rats. Secondly, adrenalectomy could partially block the attenuating effects of HBA on CXM-induced memory impairment. In the role of central sites on the counteractive effects of HBA, HBA attenuated the impairment of learning and memory induced by 6-OHDA or 5,7-DHT in rats.

These above results suggest that 1) The beneficial effects of HBA on the PCA-induced impairment, the CXM-induced impairment and the APO-induced impairment were greater than those of piracetam and its mechanism may be related to decreased serotonergic activity via directly 5-HT_{1A} and 5-HT_2 receptors or indirectly α-adrenergic receptors, and decreased dopaminergic activity via D_1 and D_2 receptors. 2). The attenuating effects of HBA on the various drugs-induced impairment acted through not only the peripheral sites such as sympathetic nervous systems and adrenal gland, also the decrease of serotonergic activity mainly via postsynaptic 5-HT receptors and dopaminergic activity via presynaptic or postsynaptic receptors.

Keywords: *p*-hydroxybenzyl alcohol (HBA), Learning and memory, Central nervous system, Peripheral nervous system

二、緣由與目的

台灣社會已正式邁入高齡化的社會，據85年底行政院衛生署之人口統計，老年人口約占總人口數之百分之七強。已知癡呆症（dementia）為現今老年人最常發生的疾病，其發生率據統計65歲以上之罹患率約為百分之五，而80歲以上之罹患率則超過百分之二十[1]。然癡呆症之主要臨床症狀為嚴重的記憶能力減退現象，因此驅使近代之醫藥學家的重視及致力於智能增進劑之開發。在現今已開發之智能增進劑，已於臨床運用者有 co-dergocrine、nicergoline、pentoxifylline、tacrine 等，皆無預期對阿耳滋海默氏症記憶能力減退之治療成果，對急性中風所致之記憶能力減退亦無效，在整體之症狀改善上僅發揮約50%之效果[2]。故就已開發之智能增進劑而言，其臨床運用效果不彰；且有胃腸不適及中樞之頭痛、嗜睡等之副作用，嚴重者尚可導致精神分裂[2]。因此，研發智能增進劑以供臨床應用，仍為現今醫藥學家當務之急。

天麻為蘭科（Orchidaceae）植物天麻（*Gastrodia elata* Blume）的乾燥塊莖，始載於神農本草經上品，原名赤箭[3]，藥性論記載：「治語多恍惚，多驚失志」[4]。本研究之目的即在探討天麻有效成分之作用機轉，以期自中藥開發智能增進劑。因在記憶形成階段，神經傳遞物質及蛋白質合成均扮演重要之角色：實驗步驟（1）：擬採被動迴避學習反應及各類誘發大白鼠行為操作能力障礙物質，如誘發學習獲得障礙物質 scopolamine、*p*-chloroamphetamine、apomorphine，誘發記憶鞏固障礙物質 cycloheximide，及誘發記

憶再現障礙物質 apomorphine ，並以 piracetam 為正對照組；評估並比較對大白鼠學習記憶能力之影響。其次，學習記憶過程， dopaminergic 及 serotonergic 神經系統之各接受器亞型均參與：步驟（ 2 ）即以各類藥物誘發大白鼠行為操作能力障礙後，再併用各類神經系統接受器之致效劑與拮抗劑，評估致效劑或拮抗劑對天麻活性成分改善作用之影響。進一步，智能增進劑之增強學習記憶作用，中樞神經系統或周邊神經系統如腎上腺等參與其作用機轉：步驟（ 3)在大白鼠經 adrenalectomy 、 5,7-DHT 或 6-OHDA 腦室給藥之處置後，評估天麻活性成分改善學習記憶能力作用之變化。最終，現已開發之智能增進劑，多經腎上腺以增加 aldosterone 或 epinephrine 之分泌、 glucose 之再利用，間接活化中樞神經系統而達增進學習記憶之作用。本研究期能自天麻尋找活性成分，以開發智能增進劑並進一步了解其改善學習記憶之作用機轉，將有助於中藥現代化。

三、結果與討論

在記憶形成過程，人體首要反應便是神經活性之改變，而這則包含 acetylcholine 、 dopamine 及 serotonin 等神經系統之改變；並更進一步則牽涉蛋白質之合成及基因轉錄之過程[5]。首先，本研究於訓練前投與 scopolamine ，可縮短大白鼠在明室之滯留時間，造成學習獲得障礙，證實學習獲得過程需乙醯膽鹼神經系統之參與。天麻苷及天麻苷元在一次給藥後，不論於何劑量下（ 1-50 mg/kg ）對 scopolamine 誘發之大白鼠學習獲得障礙均無改善作用；且當 scopolamine 以較低劑量（ 0.5 mg/kg ）誘發大白鼠學習獲得障礙時，天麻苷元亦無改善作用。但正對照組 piracetam 在 100 mg/kg 下即有改善作用。其次，本研究於訓練前投與 *p*-chloroamphetamine ，亦可縮短大白鼠在明室之滯留時間，造成學習獲得障礙，證實學習獲得過程需 serotonin 神經系統之參與。天麻苷元在 1-25 mg/kg 下，對 *p*-chloroamphetamine 誘發大白鼠學習獲得障礙有明顯之改善作用，其作用曲線呈倒 U 形且於 5 mg/kg 時作用最佳；而正對照組 piracetam 於 100 mg/kg 始有改善作用，其作用曲線亦呈倒 U 型。最後，本研究於訓練前投與 apomorphine ，亦可造成學習獲得障礙，證實學習獲得過程需 dopamine 神經系統之參與。天麻苷元在 1-25 mg/kg 下，

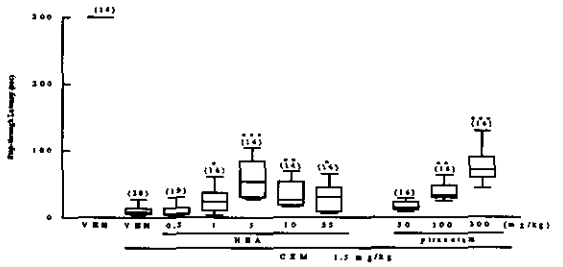

Fig 1. Effects of *p*-hydroxybenzyl alcohol (HBA) and piracetam on the CXM-induced memory consolidation impairment of passive avoidance response in rats. Each column represents the medians and the range inside 5th and 95th percentile. * P<0.05, ** P<0.01, *** P<0.001 compared with CXM group.

對 apomorphine 誘發大白鼠學習獲得障礙有明顯之改善作用，其作用曲線亦呈倒 U 型且於 5 mg/kg 之作用最佳；而正對照組 piracetam 於 100 mg/kg 具改善作用。再者，記憶鞏固過程中，本研究於訓練後投與 cycloheximide ，亦可造成記憶鞏固障礙，證實記憶鞏固過程需蛋白質、 DNA 之參與。天麻苷元在 1-25 mg/kg 下，對 cycloheximide 誘發大白鼠記憶鞏固障礙有明顯之改善作用，其作用曲線呈倒 U 形且於 5 mg/kg 作用最佳，而正對照組 piracetam 於 100 mg/kg 有改善作用。而在記憶再現過程，本研究於測定期前給予 apomorphine ，亦可縮短大白鼠在明室之滯留時間而誘發記憶再現障礙，證實記憶再現過程需 dopamine 神經系統之參與。天麻苷元在 0.5-25 mg/kg ，對 apomorphine 引發大白鼠記憶再現障礙有明顯之改善作用，其作用曲線呈倒 U 型且於 1 mg/kg 之作用最佳；而正對照組 piracetam 則於 300 mg/kg 始有改善作用。

Nabeshima 等之研究指出， cycloheximide 誘發記憶鞏固障礙之作用，可能係經由 5-HT_2 接受器增加 serotonergic

system 之活性，並因而降低 cholinergic system 活性所致[6]；在神經生化之研究，曾指出腦部 septum 區之 serotonergic neurons 支配 hippocampus 區 cholinergic neurons 之作用[7]；當 serotonergic neurons 活化時，hippocampus 區 cholinergic neurons 之 acetylcholine 釋出明顯減少[8]。而在大白鼠學習行為之研究，亦指出 cholinergic 拮抗劑可阻斷 serotonergic 拮抗劑之作用及增強 serotonergic 致效劑之作用[9]。5 mg/kg 天麻苷元於訓練前給予對 cycloheximide 誘發大白鼠記憶鞏固障礙之改善作用，並不被低於誘發障礙劑量之膽鹼接受器 scopolamine 或煙鹼接受器 mecamylamine 所阻斷；但此作用可被低於誘發障礙劑量之 serotonin 釋放促進劑 *p*-chloroamphetamine 及 serotonin 前驅物 5-hydroxytryptophan 所阻斷；更進一步，此作用可被低於誘發障礙劑量之突觸後 5-HT_2 接受器致效劑 DOI 所阻斷或被低於改善障礙劑量之突觸後 5-HT_2 接受器拮抗劑 ritanserin 所增強。雖然 Nabeshima 等之研究亦指出 cycloheximide 誘發之記憶鞏固障礙與 5-HT_{1A} 接受器無關[10]，但在近期之研究中，亦明確指出 5-HT_{1A} 接受器在記憶鞏固過程上，亦扮演重要之角色；5-HT_{1A} 接受器之致效劑 8-OH-DPAT 可誘發學習記憶障礙[11]。天麻苷元之改善作用亦可被低於誘發障礙劑量之突觸後 5-HT_{1A} 接受器致效劑 8-OH-DPAT 所阻斷或被低於改善障礙劑量之非選擇性突觸後 5-HT_1 接受器拮抗劑(±)-pindolol 所增強。由以上之

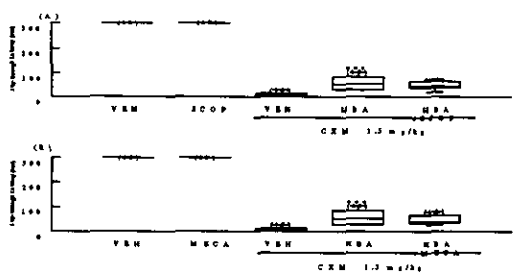

Fig 2. Effects of scopolamine (SCOP) and mecamylamine (MECA) on *p*-hydroxybenzyl alcohol (HBA)-induced recovery from CXM-induced memory consolidation impairment of passive avoidance response in rats. SCOP (A) and MECA (B). Each column represents the median and the range inside 5th and 95th percentile. *** P<0.001 compared with CXM group.

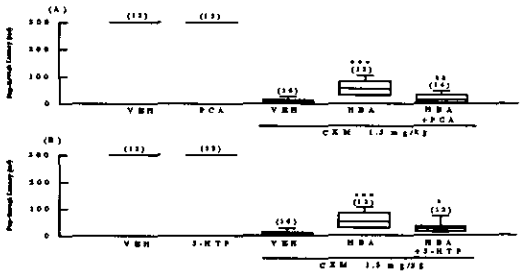

Fig 3. Effects of *p*-chloroamphetamine (PCA) and 5-hydroxytryptophan (5-HTP) on *p*-hydroxybenzyl alcohol (HBA)-induced recovery from CXM-induced memory consolidation impairment of passive avoidance response in rats. PCA (A) and 5-HTP (B). Each column represents the median and the range inside 5th and 95th percentile. *** P<0.001 compared with CXM group. a P<0.05, aa P<0.01 compared with CXM in combination with HBA group.

結果，顯示天麻苷元改善cycloheximide誘發記憶鞏固障礙之作用機轉，應不同於一般之乙醯膽鹼代謝酵素抑制劑之作用機轉，而可能係經由5-HT_{1A}及5-HT_2接受器而降低中樞serotonergic system之活性所致。

Martinez 等之研究，發現 d-amphetamine 之增進智能作用可因靜脈注射神經毒素 6-OHDA 所造成之交感神經截斷或腎上腺切除而阻斷；而 d-amphetamine 之衍生物 4-OH-amphetamine 因無法通過腦血管障壁而僅具周邊作用之性質，亦具增進智能之作用且此作用可被神經毒素 6-OHDA 所阻斷；另如 nootropics 類藥物之增進智能作用亦可因腎上腺切除而阻斷；顯示周邊之交感神經及腎上腺應參與學習記憶過程而調節中樞神經系統對外界刺激

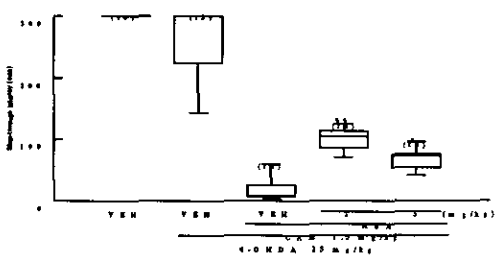

Fig 4. Effect of 6-hydroxydopamine (6-OHDA) on *p*-hydroxybenzyl alcohol (HBA)-induced recovery from cycloheximide (CXM) -induced memory consolidation impairment of passive avoidance response in rats. Each column represents the median and the range inside 5th and 95th percentile. *** P<0.001 compared with CXM group. a P<0.05 compared with HBA in combination with CXM group.

的反應。研究中發現天麻苷元在 1 及 5 mg/kg 下，均可改善經靜脈注射 6-OHDA 前處理之 cyclohexiimide 誘發學習記憶障礙，但其作用明顯低於正常動物下天麻苷元對 cyclohexiimide 誘發學習記憶障礙之改善作用；顯示天麻苷元之改善學習記憶障礙可能與周邊交感神經系統有關。其次，天麻苷元於 5 mg/kg 下，對 *p*-chloroamphetamine 及 apomorphine 誘發學習獲得障礙、cyclohexiimide 誘發記憶鞏固障礙及 apomorphine 誘發記憶再現障礙之改善作用，均可因去除腎上腺之前處理而部份阻斷，但天麻苷元於 1 mg/kg 下之改善作用則與假手術組下之作用無明顯差異。綜合上述所述及結果，天麻苷元之改善學習記憶障礙作用，不僅與周邊之交感神經系統有關，且與腎上腺有關。

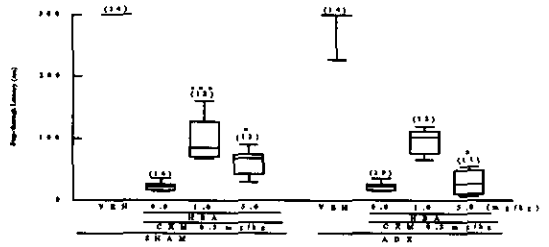

Fig 5. Effect of *p*-hydroxybenzyl alcohol (HBA) on the CXM-induced memory consolidation impairment of passive avoidance response in intact and adrenalectomized (ADX) rats. Each column represents the median and the range inside 5th and 95th percentile. * P<0.05, *** P<0.001 compared with vehicle group. a P<0.05 compared with the sham-operated group.

最後，探討天麻苷元之改善學習記憶障礙作用與中樞神經系統及突觸前或突觸後接受器之關係。6-OHDA 為中樞 dopaminergic system 之神經毒素，可造成突觸前神經節之截斷退化且可造成動物學習記憶操作能力之障礙[12-13]；腦室給予 6-OHDA（250 μg/20 μl）後 14 天，可縮短測定期大白鼠在明室之滯留時間，造成大白鼠學習記憶之障礙。天麻苷元在 1 及 5 mg/kg 對腦室給予 6-OHDA 誘發之學習記憶障礙均具改善作用，且以 1 mg/kg 較佳，但較對 PCA 誘發學習記憶障礙之改善效果為差。5,7-DHT 為中樞 serotonergic system 之神經毒素，可造成突觸前神經節之截斷

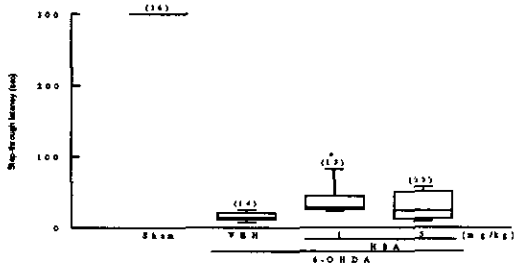

Fig 6. Effect of *p*-hydroxybenzyl alcohol (HBA) on the 6-hydroxydopamine (6-OHDA) -induced performance impairment of passive avoidance response in rats. Each column represents the median and the range inside 5th and 95th percentile. * P<0.05 compared with vehicle group.

退化且可造成動物學習記憶操作能力之障礙[14-15]；腦室給予 5,7-DHT（200 μg/5 μl）後 21 天，可縮短測定期大白鼠在明室之滯留時間，造成大白鼠學習記憶之障礙。天麻　元在 1 及 5 mg/kg 對腦室給予 5,7-DHT 誘發之學習記憶障礙均具改善作用，且以 1 mg/kg 較佳，但較對 APO 誘發學習記憶障礙之改善效果為佳。綜合上述結果顯示，天麻苷元之改善學習記憶障礙作用，主要可能係作用於中樞突觸後之 serotonergic 接受器並突觸前之 serotonergic 接受器以降低中樞 serotonergic system 之活性有關，並亦可能係作用於中樞突觸前之 dopaminergic 接受器以降低中樞 dopaminergic system 之活性有關。

綜合結果，天麻苷元在被動迴避學習之作用機轉不僅與周邊交感神經及腎上腺有關；且與阻斷中樞突觸前或突觸後之 dopaminergic、serotonergic 及 adrenergic 接受器以降低中樞 dopaminergic、serotonergic system 之活性有關。

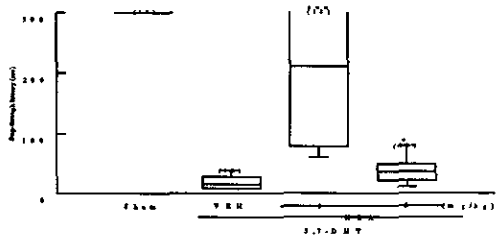

Fig 7. Effect of *p*-hydroxybenzyl alcohol (HBA) on 5,7-dihydroxytryptamine (5,7-DHT) -induced performance impairment of passive avoidance response in rats. Each column represents the median and the range inside 5th and 95th percentile. * P<0.05, *** P<0.001 compared with vehicle group.

四、計畫成果自評

本成果大致與原計畫內容及預期目標相符。天麻苷元確具改善學習記憶之能力，且其作用機轉不僅與周邊交感神經及腎上腺有關；且與阻斷中樞突觸前或突觸後之 dopaminergic 、 serotonergic 及 adrenergic 接受器以降低中樞 dopaminergic 、 serotonergic system 之活性有關。

本成果已發表於相關性學術期刊 3 篇，如下：

[1] Wu CR, Hsieh MT, Liao J: *p*-Hydroxybenzyl alcohol attenuates learning deficits in the inhibitory avoidance task: Involvement of serotonergic and dopaminergic systems. Chin J Physiol 1996;39:265-273.

[2] Hsieh MT, Wu CR, Tsai HY, Chen CF: The *p*-hydroxybenzyl alcohol-increased passive avoidance response in rats: Interactions with serotonergic and adrenergic receptors. Chin Pharma J 1996;48:411-426.

[3] Hsieh MT, Wu CR, Chen CF: Gastrodin and *p*-hydroxybenzyl alcohol facilitate memory consolidation and retrieval, but not acquisition on passive avoidance task in rats. J Ethnopharmacol 1997;56:45-54.

五、參考文獻

[1] 劉秀枝：世紀的悲哀—痴呆症（失智症）。臨床醫學 1989；23，1-5。

[2] Windisch M: Cognition-enhancing (nootropic) drugs. In: Baskys A, Remington G (eds.), Brain mechanisms and psychotropic drugs, CRC Press, United States of American, 1996, pp.239-257.

[3] 孫馮翼、孫星衍輯錄：神農本草經，卷 1，五洲出版社，台北 1985；21。

[4] 那琦、謝文全、李一宏重輯：重輯嘉祐補註神農本草，中國醫藥學院中國藥學研究所，台中 1989；92。

[5] Jodar L, Kaneto H: Synaptic plasticity: stairway to memory. Jpn J Pharmacol 1995;68:359-387.

[6] Nabeshima T, Itoh K, Kawashima K, Kameyama T: Effects of 5-HT_2 receptor antagonist on cycloheximide-induced amnesia in mice. Pharmacol Biochem Behav 1989;32:787-790.

[7] Pazos A, Palacios JM: Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. Brain Res 1985;346:205-230.

[8] Maura G, Fedele E, Raiteri M: Acetylcholine release from the hippocampal slices is modulated by 5-hydroxytryptamine. Eur J Pharmacol 1989; 165: 173-179.

[9] Matsuno K, Senda T, Matsunaga K, Mita S, Kaneto H: Similar ameliorating effects of benzomorphans and 5-HT_2 antagonists on drug-induced impairment of passive avoidance response in mice: comparison with acetylcholinersterase inhibitors. Psychopharmacology 1993;32:787-790.

[10] Nabeshima T, Itoh K, Kawashima K, Kameyama T: Effects of 5-HT_2 receptor antagonist on cycloheximide-induced amnesia in mice. Pharmacol Biochem Behav 1989;32:787-790.

[11] Medelson SD, Quartermain D, Francisco T, Shemer A: 5-HT_{1A} receptor agonists induce anterograde amnesia in mice through a postsynaptic mechanism. Eur J Pharmacol 1993;236:177-182.

[12] Pan SY: Influence of intraventricular injection of 6-hydroxydopamine on passive-avoidance response, acetylcholine, and muscarinic receptors in mouse brain. Acta Pharmacologica Sinica 1991;12:344-347.

[13] Narang N, Wamsley JK: Time dependent changes in DA uptake sites, D_1 and D_2 receptor binding and mRNA after 6-OHDA lesions of the medial forebrain bundle in the rat brain. J Chem Neuroana 1995;9:41-53.

[14] Ricaurte GA, Markowska AL, Wenk GL, Hatzidimitriou G, Wlos J, Olton DS: 3,4-methylenedioxymethamphetamine, serotonin and memory. J Pharmacol Exp Ther 1993;266:1097-1105.

[15] Patel TD, Azmitia EC, Zhou FC: Increased 5-HT_{1A} receptor immunoreactivity in the rat hippocampus following 5,7-dihydroxytryptamine lesions in the cingulum bundle and fimbria-fornix. Behav Brain Res 1996;73:319-323.