行政院國家科學委員會專題研究計畫成果報告

國科會專題研究計畫成果報告撰寫格式說明

Preparation of NSC Project Reports

計畫編號：NSC 90-2320-B-039-015
執行期限：90 年 8 月 1 日至 91 年 7 月 31 日
主持人：陳玉芳　中國醫藥學院藥理學科
共同主持人：蔡輝彥　中國醫藥學院藥理學科
計畫參與人員：許朝添　中國醫藥學院病理學科
主持人電子信箱: yfchen@mail.cmc.edu.tw

一、中文摘要

芍藥為中醫臨床上婦科常用於解痙及鎮痛之藥物，由我們先前之研究結果發現，芍藥之主成分 Paeoniflorin（芍藥□），具有鎮痛作用。然而目前相關探討 Paeoniflorin 之作用機轉之研究報告並不多見，從本研究室先前之試驗發現，Paeoniflorin 可減弱脊髓內注射高劑量嗎啡所引起之類似痙攣作用，亦可明顯減緩小鼠注射番木鱉鹼所致之痙攣及死亡，及對小鼠注射 P 物質(substance P)所引起之搔、咬等動作亦有減緩之作用。根據文獻報導，高劑量嗎啡所引起之痙攣、番木鱉鹼所致之痙攣及 P 物質所引起之搔、咬等動作被認為與中樞 NMDA 接受體之興奮有關；且在我們之試驗中發現，Paeoniflorin 可抑制鼷鼠由脊髓注射 glutamate、NMDA、AMPA 及 trans-ACPD 等藥物活化 NMDA 接受體所致之搔、咬等行為；此外，亦發現 Paeoniflorin 可抑制大鼠海馬迴腦薄片 NMDA 接受體所媒介之 field-EPSP，顯示 Paeoniflorin 對 NMDA 接受體具有抑制作用。因此本研究進一步藉由併用 Paeoniflorin 與不同 NMDA 接受體次元(subunit)反序寡去氧核□(antisense ODNs)，來探討 Paeoniflorin 對 NMDA 所致搔、咬等行為之抑制作用機轉，結果發現其作用是經由影響 NMDA 接受體次元(NR2B)而來。此結果並以免疫化學染色及 Western blot 證實；後續更以大鼠海馬迴初級培養細胞，來探討 Paeoniflorin 對海馬迴神經細胞內鈣之影響，結果發現 Paeoniflorin 明顯降低由 NMDA 所致之海馬回初級培養細胞之內鈣增加作用，其降低百分比為 83.86，且此作用呈現劑量依存性。由本研究結果可推論，芍藥□抑制 NMDA 接受體之作用可能是經由抑制鈣離子而來。

關鍵詞：芍藥□　反序寡去氧核□　免疫化學染色　海馬迴初級培養細胞　細胞內鈣離子

Abstract

Paeony roots (*Paeonia lactifloria* PALL) has been used in gynecological disorders as an antispasmodic and analgesic drug. From our previous studies, we found that paeoniflorin, the major component of paeony roots, showed analgesic effect. However, there were only few papers mentioned about the action mechanism of paeony roots. From our previous studies, we found that paeoniflorin could attentuate the seizure-like exictation induced by high dose morphine and inhibited the biting and scratching behavior induced by subatance P, which are considered to have a close relationship to NMDA receptors. From our previous studies, it also showed the inhibitory effects of paeoniflorin on the biting and scratching behavior induced by glutamate, NMDA, AMPA and trans-ACPD. Moreover, the field-EPSP mediated by NMDA in rat hippocampal brain slice is also inhibited by paeoniflorin. It revealed that paeoniflorin has inhibitory effect on NMDA receptors. In this

study, we attempt to explore the mechanism of inhibitory effect of paeoniflorin on NMDA receptors by using antisense oligodeoxynucleotides of different NMDA receptor subtypes mRNA. The inhibitory effects of paeoniflorin on NMDA receptor were via the inactivation of NR2B subunit. The effect of paeoniflorin on NR2B was also evaluated by immunohistochemistry and Western blotting of NMDA receptor proteins. Moreover, paeoniflorin inhibit the intracellular calcium of hippocampal primary culture cell culture evoked by NMDA. The inhibitory effect of paeoniflorin on NMDA-evoked calcium influx was dose-dependent. From above results, inhibitory effects of paeonilforin on NMDA receptor might via the inactivation of calcium influx.

Keywords: Paeoniflorin, Oligodeoxynucleotides, Immunohistochemistry, Primary hippocampal cell culture, Intracellular calcium

二、緣由與目的

芍藥為毛茛科多年生植物 *Paeonia lactifloria* PALL.的乾燥根，傳統中醫方劑中，常用於活血化瘀、解痙及止痛[1-2]。現代藥理學文獻指出，芍藥粗抽取物除了具鎮痛作用外[3]，對犬的冠狀動脈血管及後肢血管均有擴張作用[4]，對豚鼠、家兔之離體腸管呈現抑制作用[5]；顯示確實對不同部位平滑肌有選擇性的解痙作用。芍藥之主要成分為 paeoniflorin （芍藥□）[6-7]，對於周邊血管具有擴張及降壓作用；可抑制大鼠胃及子宮平滑肌之蠕動及張力[8-9]，並預防壓力所致潰瘍(stress ulcer)[10-11]；對於離體橫膈肌則可阻斷其 nerve-stimulated twitch responses[12-16]；可延長 hexobarbital 所誘發之睡眠時間，並具有輕微的降溫及抗痙攣作用[17-18]。此外，近年來也有文獻報告指出，芍藥及其主成分 Paeoniflorin 可改善大鼠因投與 scopolamine 所致之學習障礙的情形[19-22]，並能反轉 muscarinic M_1 接受體拮抗劑所致抑制大鼠海馬迴 LTP (long-term potentiation)之作用[23]，認為 Paeoniflorin 可作為認知強化劑(cognitive enhancer)[23,24]；此外，Paeoniflorin 可抑制由 phenyhydroquinone 所致之氧化性 DNA 斷裂(oxidative DAN cleavage)[25]，並對鈷所致海馬迴神經元損傷有保護作用[26]；至於有關 Paeoniflorin 作用機轉之探討文獻仍不多見。

有許多的文獻報告指出，痙攣、癲癇[27]、焦慮[28]、藥物之依賴性與耐受性[29]、疼痛[30]、Huntington 氏病[31]、Alzheimer 氏病[32]、Parkinson 氏病[33]等皆與 NMDA 接受體之過度活化有關，而 NMDA 接受體拮抗劑(如 MK-801 等)[34-37]亦被用於上述症狀改善之研究。由於 NMDA 接受體是中樞神經系統中興奮性突觸所釋放之神經傳遞物質—glutamate 的主要接受體之一，若能找到對 NMDA 接受體具有調節作用之化合物，是否能用於因 NMDA 接受體之過度活化所媒介之症狀或疾病改善之研究，一直是我們期望去瞭解的。另外，當由脊髓內投與高劑量嗎啡時，會引起大鼠產生 allodynia，其特徵為明顯週期性產生自發性 agitation 及 vocalization[38-40]，而由 peri-aqueductal gray (PAG)處微量注射投與高劑量嗎啡，亦引起大鼠有相同之興奮作用[41,42]，在小鼠亦觀察到類似之作用[43,44]，由脊髓內投與高劑量嗎啡時會引起小鼠產生一種 seizure-like motor syndrome，其特徵為後肢伸展(hind limb extension) 及劇烈肌陣攣(myoclonic twitches)。此外，有些臨床報告亦指出，當病人由蜘蛛膜下腔投與高劑量嗎啡時，亦會產生此種現象(即 hyperalgesic state 及 myoclonus)[45,46]。由文獻可知，脊髓內注射嗎啡所引起之 seizure-like motor behavior 並不是由 opioid-receptor 來媒介[38,39,42,44]，例如在小鼠，此作用並不被 naloxone 所拮抗， 且不會形成 tolerance[47]；在大鼠亦有類似的結果[41-45]。由最近之研究指出， strychnine 及 NMDA (N-Methyl-D-aspartate)接受體拮抗劑會阻斷這些興奮性作用，因此 NMDA 接受體被認為對此扮演著調節角色。由本研究室先前之實驗中發現， Paeoniflorin 可減輕高劑量嗎啡所引起之類似痙攣作用；且 Paeoniflorin 可明顯減緩小鼠注射

strychnine 所致之痙攣及死亡；對小鼠注射 substance P 所引起之搔、咬等動作亦有減緩之作用。而高劑量嗎啡所引起之類痙攣，strychnine 所致之痙攣以及 substance P 所引起之搔、咬等動作被認為與中樞 NMDA 接受體之性奮有關[47]；此外，Paeoniflorin 可抑制鼷鼠由脊髓注射 glutamate、NMDA、AMPA 及 trans-ACPD 等藥物活化 NMDA 接受體所致之搔、咬等行為；且我們利用大鼠海馬迴腦薄片為材料，亦發現 Paeoniflorin 可抑制 NMDA 接受體所媒介之 field-EPSP。由這些結果顯示，Paeoniflorin 對 NMDA 接受體有調節性抑制作用。因此，芍藥□拮抗 NMDA 接受體之作可能作用機轉，是本研究期望進一步加以探討的。本研究利用不同 NMDA 接受體次元(subunits)之反序寡去氧核糖核酸(antisense oligodeoxy- nucleotides)，來探討 Paeoniflorin 對小鼠脊髓內投予 NMDA 所造成之咬(biting)及抓(scratching)等行為之緩解作用是經由影響何種 NMDA 接受體次元而來；再利用此種 NMDA 接受體次元之抗體，以免疫組織染色(immunohistochemistry)及 Western blot 等方法，來證實 Paeoniflorin 對 NMDA 接受體之抑制作用。另外，由我們先前之研究發現，Paeoniflorin 抑制 veratrine 在離體大鼠主動脈[48]及離體大鼠心房[49]所致之收縮可能經由抑制細胞內鈣離子而來，故本研究進一步利用海馬迴的初代培養細胞(hippocampus primary culture cells)，來探討 Paeoniflorin 對細胞內鈣離子之影響，以瞭解 Paeoniflorin 對 NMDA 接受體之作用情形及其可能之機轉，期能進一步瞭解 Paeoniflorin 是否能用於改善因中樞 NMDA 接受體活化所造成之各種病態生理情況，俾能提供芍藥在傳統中醫用法外之另一種臨床應用參考。

三、結果與討論

一、Paeoniflorin 對 NMDA 引起鼷鼠 biting 和 scratching 行為之影響

如圖一所示，MK-801 會明顯的抑制由 NMDA 所誘發的中樞興奮行為（$p<0.001$）而單獨給予芍藥□ 48，96，240μg 亦會明顯降低 NMDA 的反應（$p<0.001$），而且呈現劑量依存性的之現象，且有比 MK-801 更低的趨勢，但並無統計學上之意義。併用 MK-801 與芍藥□之後，降低的程度更加明顯，且其降低興奮反應程度比單獨給 MK-801 更低，特別是在合併芍藥□ 96 及 240μg 時（$p<0.001$）而且亦有劑量依存性。如圖二所示，NMDA 接受器拮抗劑 AP5，可以降低 NMDA 引起之中樞興奮反應（$p<0.001$），合併芍藥□ 48，96，240μg 之後，亦有明顯之降低現象（$p<0.001$），且有比 AP5 單獨使用更降低的趨勢，但並無統計學上之差異，也有劑量依存性的趨勢存在。

二、Paeoniflorin 與 NMDA 接受體反序寡去氧核□對鼷鼠引起 biting 和 scratching 之行為影響之研究

如圖 3、4，分別代表著由椎管內投與反序寡核糖核□序列，並連續給藥 1 天、3 天、7 天的結果， 觀察於椎管內投與 NMDA 之後引起的抓與咬的行為之影響。結果發現，投與反序寡核糖核□序列給予一天後，如同之前的結果，並無差異出現，但在給予三天及七天後，出現了極明顯的差異，不管是 NR1、2A、2B、2C 等，都表現了抑制現象，其中，以 2B 的影響最明顯。

由圖 5 的結果來看，由椎管內投與反序寡核糖核□序列，並連續給藥 1、3、7 天，併用芍藥□ 48，96，240μg 之後，觀察於椎管內投與 NMDA 之後引起的抓與咬的行為之影響。結果，在處理一天後，給予芍藥□ 48，96，240μg 後，出現了劑量依存性的抑制現象，與之前我們所做的相符合，且與單獨給予 2B 比較，亦有劑量依存性的抑制作用。而處理三天後，併用芍藥□ 48，96，240μg，出現了劑量依存性的抑制現象，且與單獨給予 2B 比較，在 240μg 時，有較明顯的抑制作用。而處理七天後，併用芍藥□ 48，96，240μg，抑制的現象，已與單獨利用 2B 處理過之反應無明

顯差異了。

三、Paeoniflorin 與 NMDA 接受體次元反序寡去氧核□對鼷鼠腦部 NMDA 接受體次元之蛋白質表現之免疫化學染色研究

如圖六、七、八所示。給予 NR2B 的反序寡去氧核□處理過的鼷鼠腦縱切面中的 NMDA 接受體含量，會隨著天數增加而減少其表現量，且併用芍藥□ 48；96；240μg 之後，亦有劑量依存性的表現趨勢，且會隨時間增加而降低其表現量。

四、Paeoniflorin 與 NMDA 接受體次元反序寡去氧核□對鼷鼠腦部 NMDA 接受體次元之蛋白質表現之研究(西方墨點法)

如圖九所示，給予 NR2B 的反序寡去氧核□處理過的鼷鼠腦中蛋白質含量，會減少其表現量；且併用芍藥□ 48；96；40μg 之後，亦有劑量依存性的表現趨勢，且會隨時間增加而降低。而單獨給予芍藥□ 240μg 後第 1、3、7 天的蛋白質表現量，與控制組相比，有抑制的現象。

五、Paeoniflorin 影響 NMDA 誘發腦部海馬迴初級培養細胞鈣離子增加之研究

(一) 海馬迴初級培養細胞內鈣離子(Ca^{2+})正常的濃度為 115.69 nM，而由 45 分鐘前先經 125ìM NMDA 處理過的海馬迴初級培養細胞內鈣離子(Ca^{2+})的濃度為 554nM ，由 45 分鐘前先經 125ìM NMDA 與 10 ìM MK-801 一起處理過的海馬迴初級培養細胞內鈣離子(Ca^{2+})的濃度為 221.17 nM，由 45 分鐘前先經 125 ìM NMDA 與 10ìM Paeoniflorin 一起處理過的海馬迴初級培養細胞內鈣離子(Ca^{2+})的濃度為 89.39 nM ，由 45 分鐘前先經 125 ìM NMDA、10 ìM MK-801 與 10ìM Paeoniflorin 一起處理過的海馬迴初級培養細胞內鈣離子(Ca^{2+})的濃度為 100.59 nM。經由前述偵測海馬迴初級培養細胞內鈣離子(Ca^{2+})的濃度的方法，先將海馬迴初級培養細胞於 45mins 前以 125ìM NMDA 處理，分別加入 1、5、10、20 與 30ìM 濃度的 Paeoniflorin，則各自海馬迴初級培養細胞內鈣離子(Ca^{2+})的濃度下降百分比如下，加 1ìM 濃度的 Paeoniflorin 為(-3.865%, n=7)，加 5ìM 濃度的 Paeoniflorin 為(-13.8%, n=3)，加 10ìM 濃度的 Paeoniflorin 為(- 23.22%, n=13)，加 20ìM 濃度的 Paeoniflorin 為(- 40.13%, n=6)， 加 30ìM 濃度的 Paeoniflorin 為(- 60.10%, n=7)。由實驗結果顯示，芍藥□對 NMDA 所致之神經細胞內鈣增加之現象，呈現劑量依存性，且其降低細胞內鈣之作用比 MK801 強。

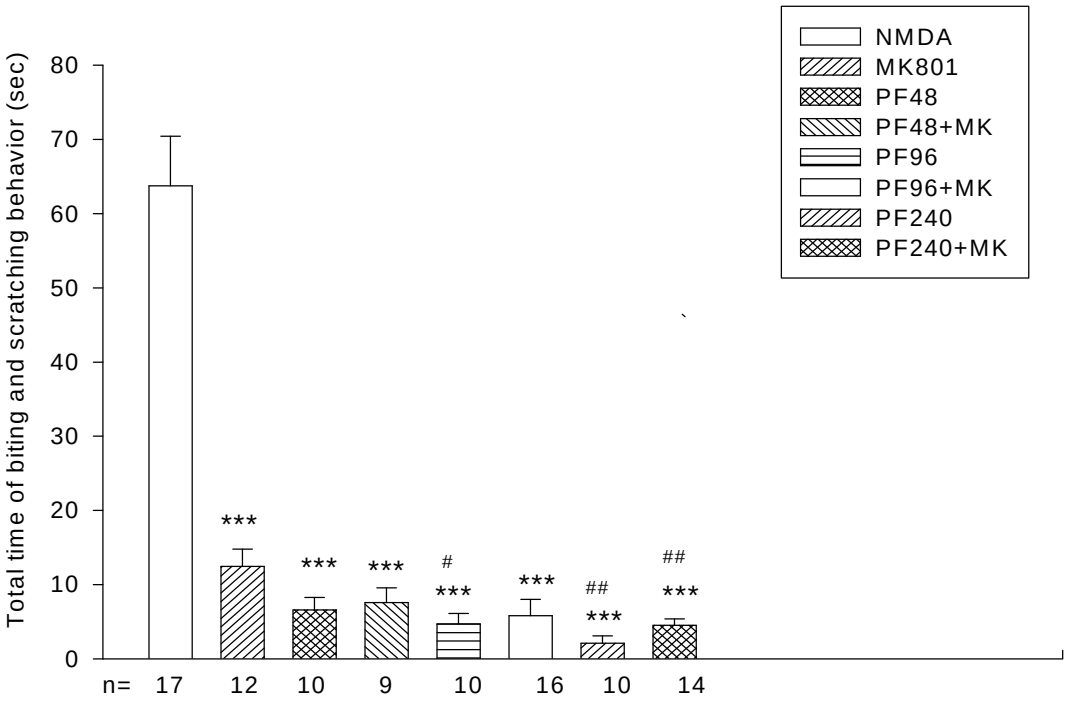


Fig 1. The effect of paeoniflorin and MK 801 on NMDA-induced biting and scratching behavior in mice. Paeoniflorin (PF 48, 96, 240 μg / 5μl) were intra-cerebroventricularly (i.c.v.) administered 15 min before intrathecal of NMDA (122 pmol / 5μl). NMDA receptor antagonist MK-801 (MK 5ng / 5μl) was administered intrathecally 5 min before NMDA injected. The time spent biting or scratching induced by NMDA during the first 120s after NMDA injected was recorded. Data were shown as mean ± S.E.

* P<0.05, ** P<0.01, *** P<0.001 compared with NMDA group.

# P<0.05, ## P<0.01, ### P<0.001 compared with MK-801-treated group.

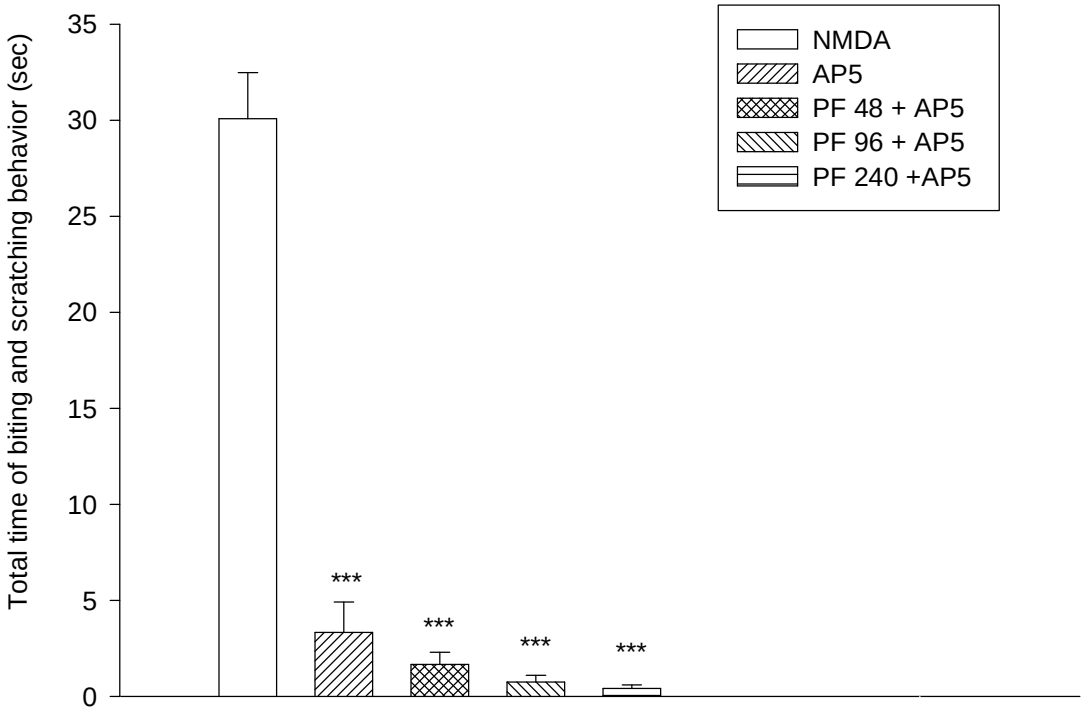


Fig 2. The effect of paeoniflorin and AP5 on NMDA-induced biting and scratching behavior in mice. Paeoniflorin (PF 48, 96, 240 μg /5μl) were intracerebroventricularly (i.c.v.) administered 15min before intrathecal injection of NMDA (122 pmol /5μl). NMDA receptor antagonist AP5 (0.1 mM /5μl) was administered intrathecally 5 min before AMPA injected. The time spent on biting or scratching induced by NMDA during the first 120s after injection NMDA was recorded. Data are shown as mean ± S.E. (n=12)

* P<0.05, ** P<0.01, *** P<0.001 compared with NMDA group.

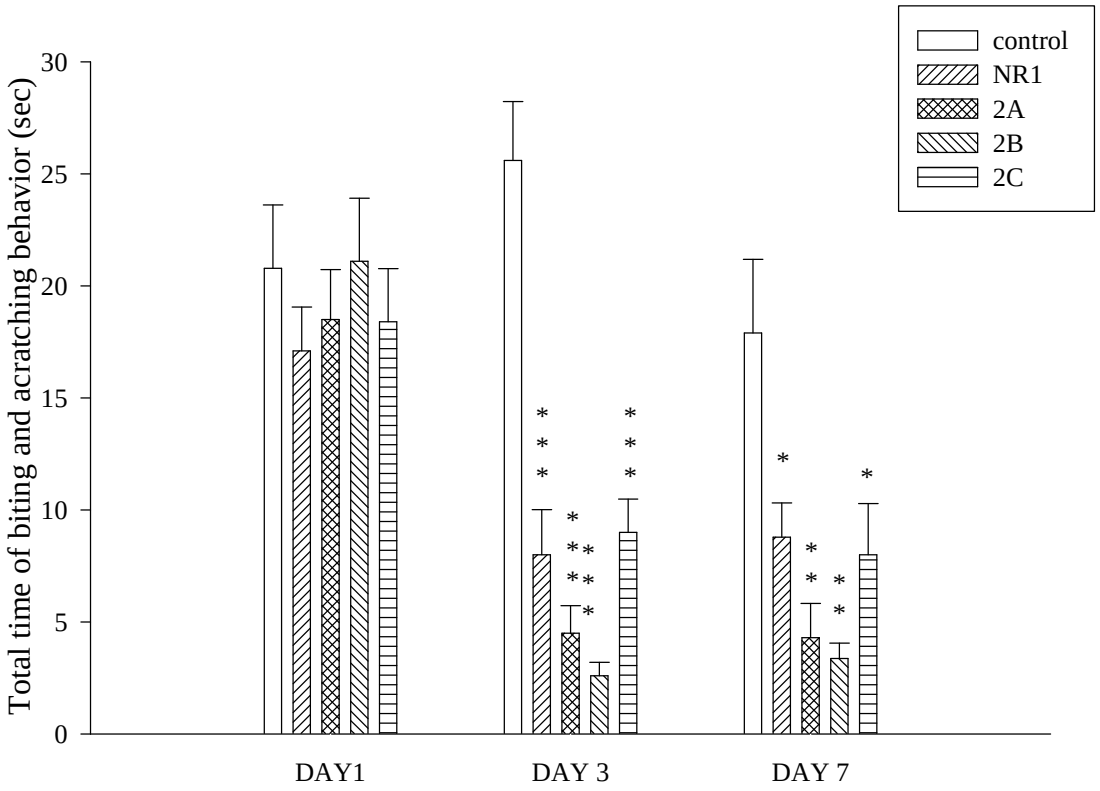


Fig 3.The time course effect of antisense oligodeoxynucleotides of NMDA receptor subunits on NMDA-induced biting and scratching behaviors in mice. Antisense oligodeoxynucleotides (15 nM / 5μl) were intracerebro-ventricularly administered 1, 3, 7 days before intrathecal of NMDA (122 pmol / 5μl). The time spent biting or scratching induced by NMDA during the first 120s after NMDA injected was recorded. Data are shown as mean ± S.E. * P<0.05, ** P<0.01, ***P<0.001 compared with NMDA group.

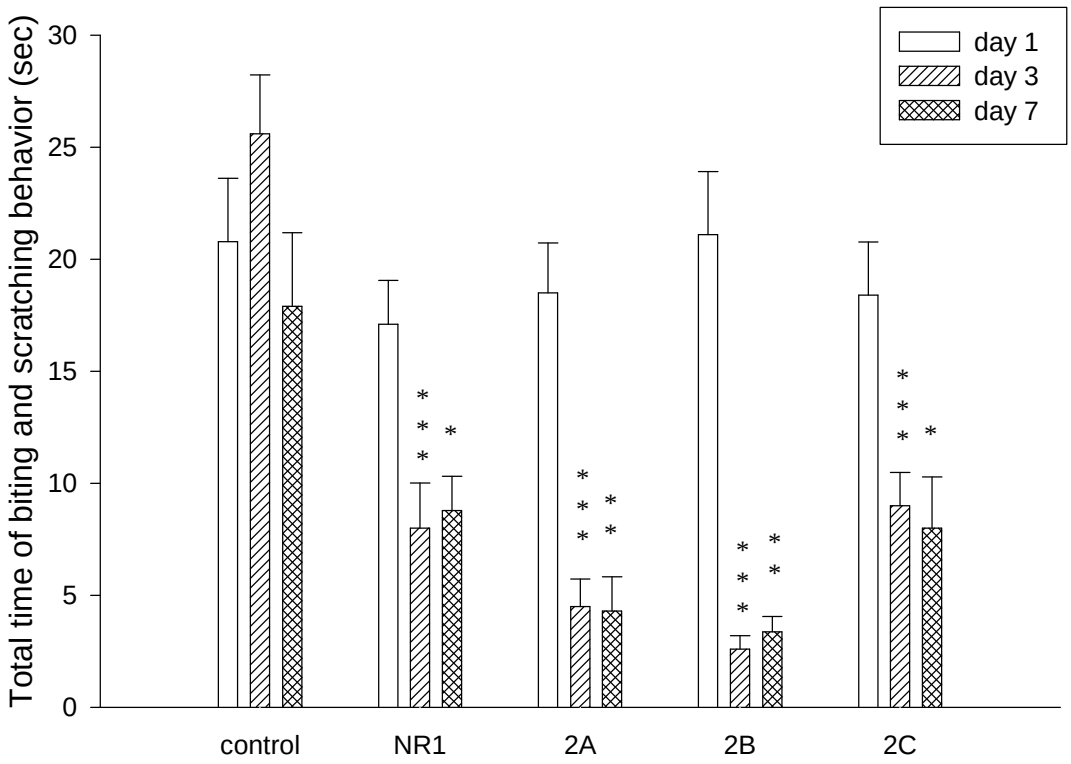


Fig 4. The time course effect of antisense oligodeoxynucleotides of NMDA receptor subunits on NMDA-induced biting and scratching behaviors in mice. Antisense oligodeoxynucleotides (15 nM / 5μl) were intracerebroventricularly administered 1, 3, 7 days before intrathecal of NMDA (122 pmol / 5μl). The time spent biting or scratching induced by NMDA during the first 120s after NMDA injected was recorded. Data are shown as mean ± S.E. * P<0.05, ** P<0.01, ***P<0.001 compared with NMDA group.

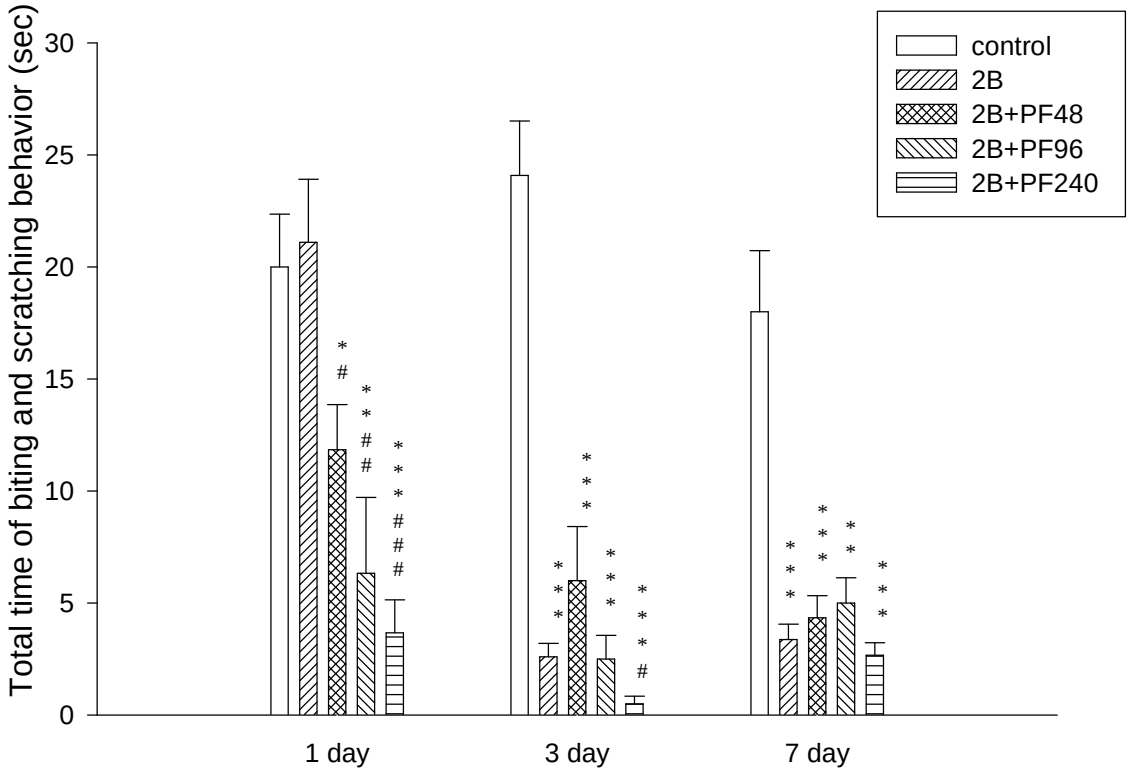


Fig 5. Effect of antisense oligodeoxynucleotide of NMDA receptor subunit (2B) on NMDA-induced biting and scratching behavior in mice. Paeoniflorin (PF 48, 96, 240 μg / 5μl) were intracerebroventricularly (i.c.v.) administered 15 min before intrathecal of NMDA. Antisense oligodeoxynucleotide (15 nM / 5μl) were intracerebroventricularly administered 1, 3, 7 days before intrathecal of NMDA (122 pmol / 5μl). The time spent on biting or scratching behavior induced by NMDA during the first 120s after NMDA injection was recorded. Data were shown as mean ± S.E. * P<0.05, ** P<0.01, *** P<0.001 compared with NMDA group. # P<0.05, ## P<0.01, ### P<0.001 compared with antisense 2B group.

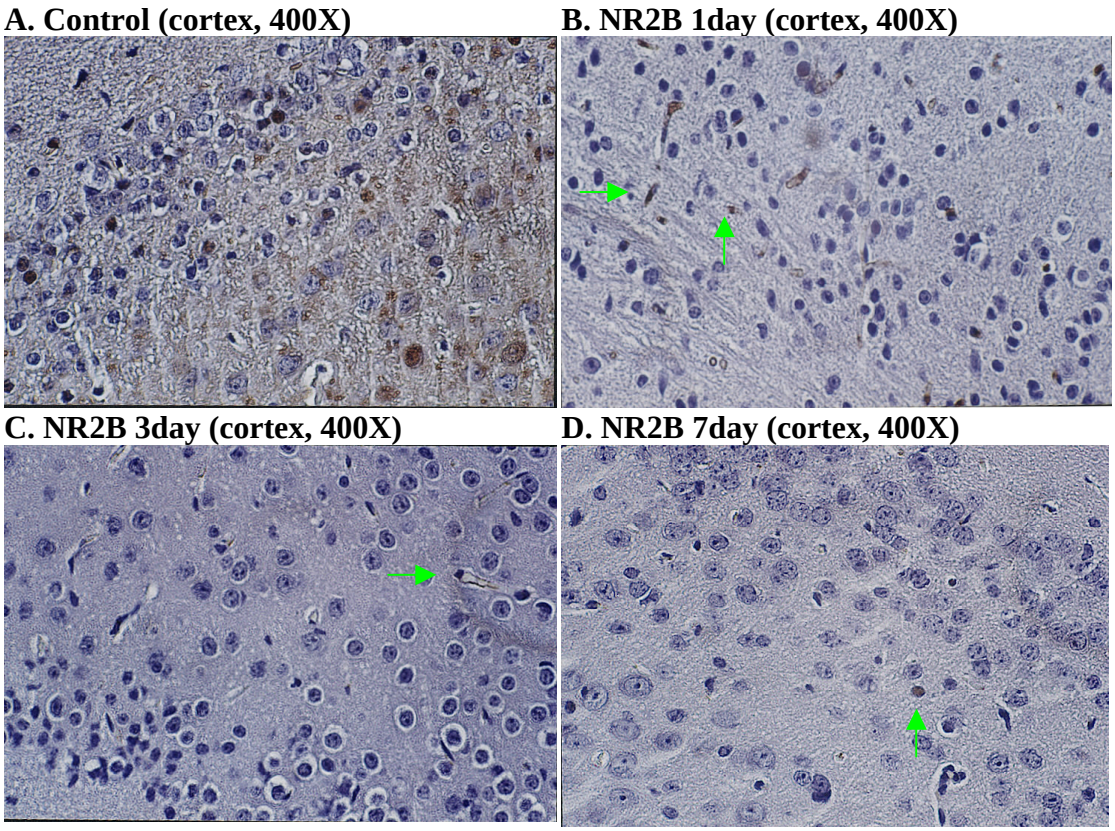


Fig 6.Immunohistochemical detection of NMDA receptor by anti-NMDA receptor NR2B antibody. Antisense oligodeoxynucleotide (ODN) of NMDA receptor subunit (NR2B) (15nM/5μl) were intrathecal admini- stered once daily for 1, 3, 7 days. (A) Normal control mice brain cortex (100X), (B) Mice treated with antisense ODNS of NMDA receptor NR2B for 1 days (400X), (C) Mice treated with antisense ODNS of NMDA receptor NR2B for 3 days (400X), (D) Mice treated with antisense ODNS of NMDA receptor NR2B for 7 days (400X).

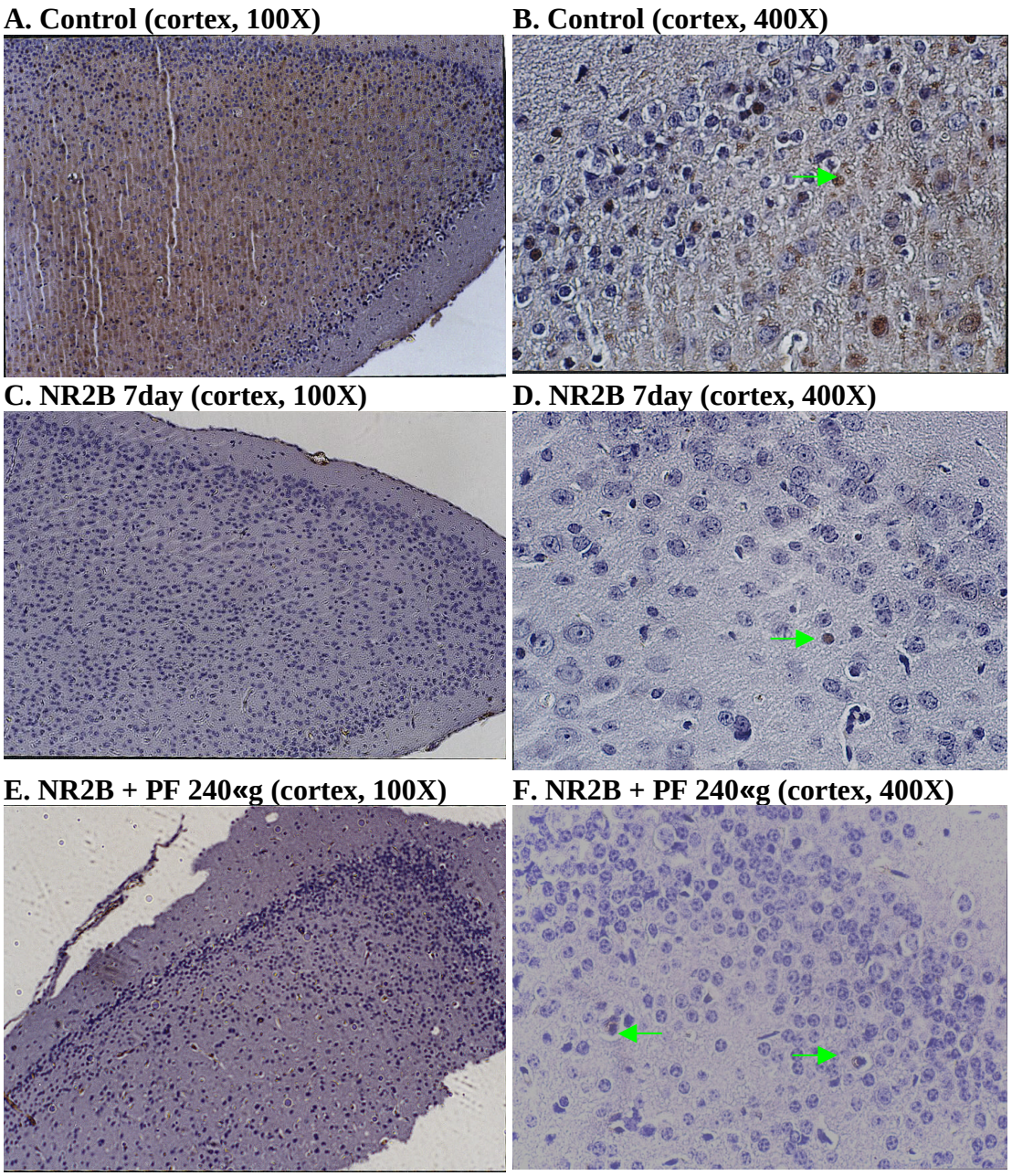
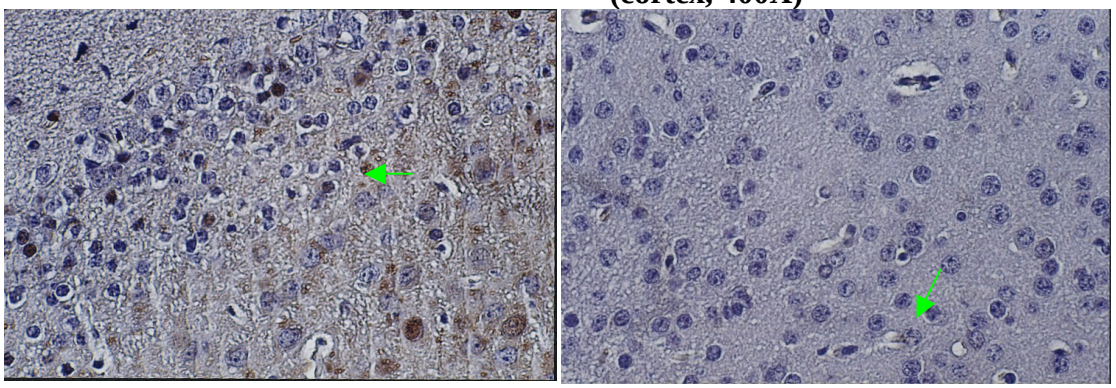


Fig 7. Immunohistochemical detection of NMDA receptor by anti-NMDA receptor NR2B antibody. Antisense oligodeoxynucleotide (ODN) of NMDA receptor subunit (NR2B) (15nM / 5μl) were intrathecal administered once daily for 7 days. Paeoniflorin (PF 240 μg / 5μl, i.c.v.) were administered 15 min before mice were sacrificed. Normal control mice brain cortex 100X(A), 400X(B). Mice treated with antisense ODNS of NMDA receptor NR2B for 7 days 100X(C), 400X (D). Mice treated with antisense for 7days and paeoniflorin was given 15 min before mice were sacrificed 100X (E), 400X (F).

A. Control (cortex, 400X)

B. NR2B 7day + PF 48 «g (cortex, 400X)



C. NR2B 7day + PF 96 «g (cortex, 400X)

D. NR2B 7day + PF 240 «g (cortex, 400X)

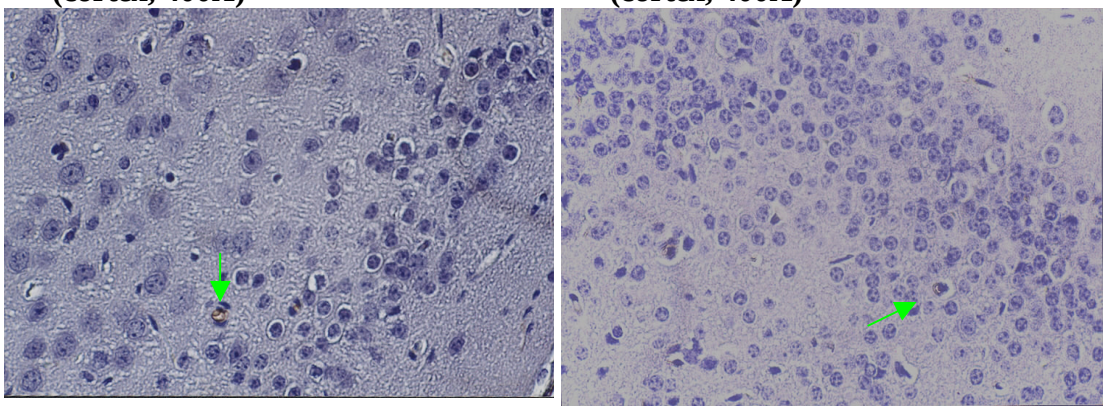


Fig 8. Effect of paeoniflorin and antisense oligodeoxynucleotide (ODN) of NMDA receptor subunit (NR2B) on the NMDA receptor by immunohistochemical detection in mice. Mice cortex was stain with anti-NMDA receptor NR2B antibody. Antisense oligodeoxy- nucleotide (ODN) of NMDA receptor subunit (NR2B) (15nM/5µl) was intrathecal administered once daily for 7 days. Paeoniflorin (PF 48, 96, 240 µg/5µl, i.c.v.) were administered 15 min before mice were sacrificed. NR2B represented mice treated with antisense ODN of NMDA receptor NR2B subunit. (A) Normal control mice (100X), (B) NR2B 7 days + PF 48µg (400X), (C) NR2B 7 day + PF 96µg (400X), (D) NR2B 7 days + PF 240µg (400X).

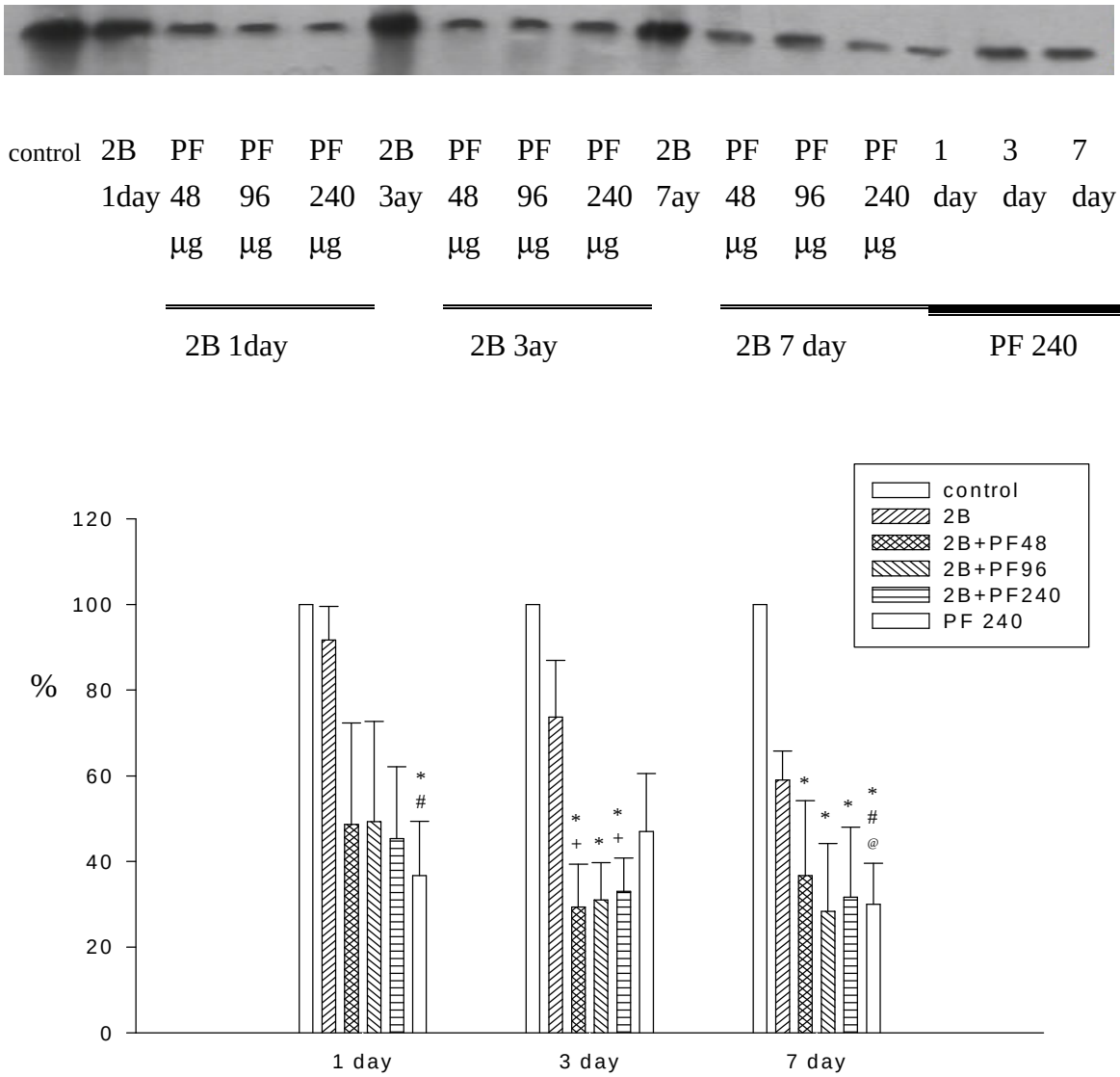
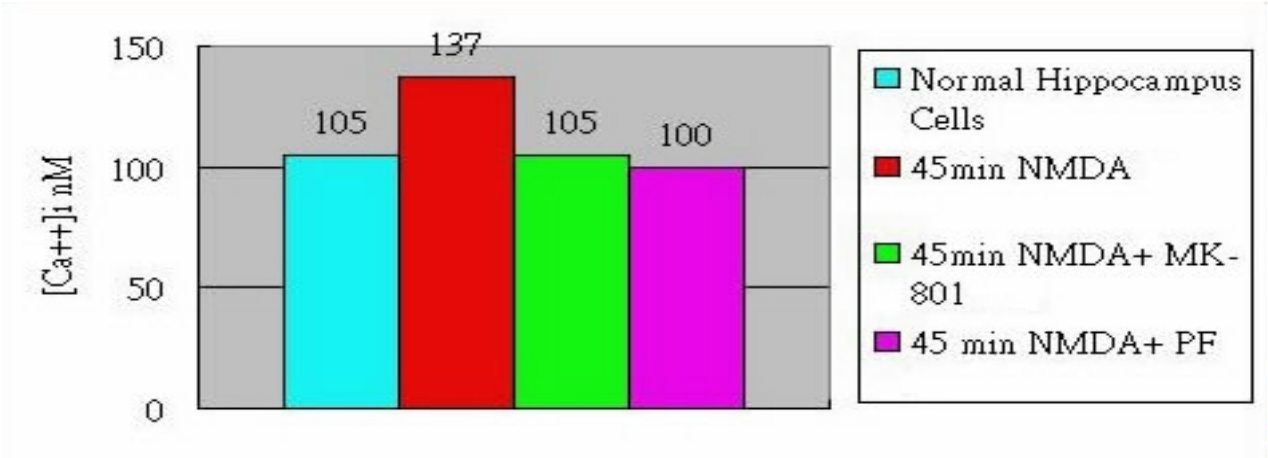
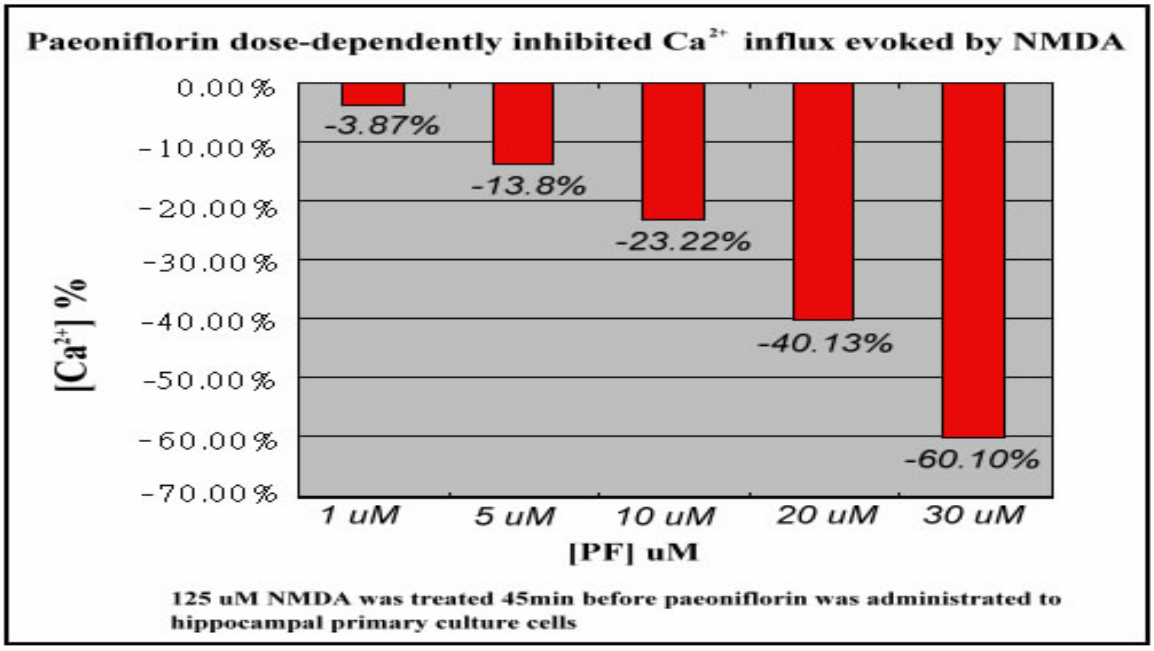


Fig 9.Western blot results of antisense oligodeoxynucleotide of NMDA receptor subunit (2B) in mice brain tissue. Paeoniflorin (PF 48, 96, 240 µg / 5µl) were intracerebroventricularly (i.c.v.) administered 15 min before sacrificed. Antisense oligodeoxynucleotide (15 nM / 5µl) were intracerebroventricularly administered 1, 3, 7 days. Paeoniflorin (PF 240 µg / 5µl) were intrathecally administered 1, 3, 7 days. Lanes were loaded with 30µg protein. *P<0.05 compared with control group # P<0.05 compared with antisense 2B 1day group, + P<0.05 compared with antisense 2B 3day group, @ P<0.05 compared with antisense 2B 7day group.

			Ca^{2+}(nM)	和 Control 比較 [(B-A) / A]	和 NMDA 比較 [(B-A) /A]
A		Control	115.7 ±37.9		
B	A	45min H Cell in 125uM NMDA	554.0 ±176.7	378.80%	
	B	45min H Cell in 125uM NMDA + 10uM MK-801	221.2 ±16.3	91.16%	-60.07%
		45min H Cell in 125uM NMDA + 10uM paeoniflorin(PF)	89.4 ±33.70	-22.74%	-83.94%
		45min H Cell in 125uM NMDA + 10uM MK-801 + 10uM paeoniflorin(PF)	100.6 ±45.1	-12.96%	-81.84%





四、參考文獻

1. 顏正華: 中藥學(上)，知音出版社， 台北，162-4，831-6，1991。
2. 李念秋: “芍藥能鎮痛、鎮痛不離經”瑣談，中國中藥雜誌，14:52-3，1989。
3. 劉冰青: 芍藥湯新用，江西中醫藥，5:31-6，1984。
4. 王欽茂:芍藥總□對心血管的藥理作用，中國藥理學通報，25:26-8，1984。
5. 崔豐年:芍藥□草湯的藥理學研究和臨床應用，中成藥，13:36-7，1991。
6. Shibata S. and Nakahara M. Studies on the constituents of Japanese and Chinese crude drugs. VIII. Paeoniflorin, A glucoside of Chinese paeony root. (1). Chem Pharm Bull 11: 372-78, 1963.
7. Shibata S., Aimi N. and Nakahara M. Studies on the constituents of Japanese and Chinese crude drugs. IX. Paeoniflorin, A glucoside of Chinese paeony root. (2) Chem Pharm Bull 11:379-81, 1963.
8. Takagi K. and Harada M. Pharmacological studies on herb paeony root. Effects of paeoniflorin on circulatory and respiratory systems and isolated organs. Yakugaku Zasshi 89:893-98, 1969.
9. Kobayashi M., Ueda C., Aoki S., Tajima K., Tanaka N. and Yamahara J. Anticholinergic action of paeony root and its active constituents. Yakugaku Zasshi 110: 964-68, 1990.
10. Takagi K. and Harada M. Pharmacological studies on herb paeony root.II Anti- inflammatory effect, inhibitory effect on gastric juice secretion, preventive effect on stress ulcer, antidiuretic effect of paeoni- florin and combined effects with licorice component FM 100. Yakugaku Zasshi 89:887-92, 1969.
11. Yamahara J., Yamada T., Kimura H., Sawada T. and Fujimura H. Biologically active principles of crude drugs. II. Anti-allergic principles in “ Shoseiryu-To” anti-inflammatory properties of paeoni- florin and its derivatives. J Pharmaco- biodynamics 5:921-29, 1982.
12. Kimura M., Kimura I., Muroi M., Nakamura T. and Shibata S. Depolarizing effects of glycyrrhizin-derivatives relating to the blend effects with paeoniflorin on mouse diaphragm muscle. Jpn J Pharmacol 41:263-65,1986.
13. Kimura M., Kimura I. and Kimura M. Decreasing effects by glycyrrhizin and paeoniflorin on intracellular Ca^{2+}-aequorin luminescence transients with or without caffeine in directly stimulated diaphragm muscle of mouse. Jpn J Pharmacol 39: 387-90, 1985.
14. Kimura M., Kimura I and Nojima H. Depolarizing neuromuscular blocking action induced by electropharmacological coupling in the combined effect of paeoniflorin and glycyrrhizin. Jpn J Pharmacol 37:395-99, 1985.
15. Kimura M., Kimura I., Takahashi K., Muroi M., Yashizaki M., Kanaoka M. and Kitagawa I. Blocking effects of blended paeoniflorin or its related compounds with glycyrrhizin on neuromuscle Junction in frog and mouse. Jpn J Pharmacol 36:275-82, 1984.
16. Dezaki K., Kimura I., Miyahara K. and Kimura M. Complementary effects of paeoniflorin and glycyrrhizin on intracellular Ca^{2+}mobilization in the nerve-stimulated skeletal muscle of mice. Jpn J Pharmacol 69:281-84, 1995.
17. Takagi K. and Harada M. Pharmacological studies on herb paeony root. I. Central effects of paeoniflorin and combined effects with licorice component FM100. Yakugaku Zasshi 89:879-86, 1969.
18. Sugishita E., Amagaya S. and Ogihara Y. Studies on the combination of Glycyrrhizae radix in Shakuyakukanzo-To. J Pharmacobio-Dynamics 7:427-35, 1984.
19. Ohta H., Ni J.W., Matsumoto K, Watanabe H., and Shimizu M. Paeony and its major constituent, paeoniflorin, improve radial maze performance impaired by scopolamine in rats. Pharmacol Bio- chem Behav 45:719-23, 1993.

20. Ohta H., Matsumoto K, Watanabe H., and Shimizu M. Involvement of beta-adrenergic systems in the antagonizing effect of paeoniflorin on the scopolamine-induced deficit in radial maze performance in rats. Jpn J Pharmacol 62:345-9, 1993.
21. Ohta H., Matsumoto K, Watanabe H., and Shimizu M. Involvement of α_1– but not α_2-adrenergic system in the antagonizing effect of paeoniflorin on the scopolamine-induced deficit in radial maze performance in rats. Jpn J Pharmacol 62:199-202, 1993.
22. Abdel-Hafez A.A., Meselhy M.R., Nakamura N., Hattori M., Watanabe H., Murakami Y., El-Gendy M.A., Mahfouz N.M. and Mohamed T.A. Effects of paeoniflorin derivatives on scopolamine-induced amnesia using a passive avoidance task in mice: structure-activity relationship. Biol Pharm Bull 21:1174-9, 1998.
23. Tabata K., Matsumoto k., Watanabe H. Paeoniflorin, a major constituent of paeony root, rverses muscarinic M_1-receptor antagonist-induced suppression of long-term potentiation in rat hippocampal slice. Jpn J Pharmacol 83:25-30, 2000.
24. Watanabe H. Candidates for cognitive enhancer extracted from medicinal plants: paeoniflorin and tetrmethylpyrazine. Behav Brain Res 83:135-41, 1997.
25. Okubo T., Nagai F., Seto T., Satoh K., Ushiyama K., Kano J. The inhibition of phenylhydroquinone-induced oxidative DNA cleavage by constituents of Moutan Cortex and Paeoniae Radix. Biol Pharm Bull 23:199-203, 2000.
26. Tsuda T., Sugaya A., Ohguchi H., Kishida. N., Nugaya E. Protective effects of paeony root extract and its components on neuron damage in the hippocampus induced by the cobalt focus epilepsy model. Exp Neurol 146:518-25, 1997.
27. Meldrum B.S. Excitotoxicity and epileptic brain damage. Epilepsy Res 10: 55-61, 1991.
28. Stephens D.N., Meldrum B.S., Weidmann R., Schneider C. and Grutzner M. (Does the excitatory amino acid receptor antagonist 2-APH exhibit anxiolytic activity? Psychopharmacology 90:166-9, 1996.
29. Trujillo K.A. and Akil H. Excitatory amino acid and drugs of abuse: A role for N-methyl-D-asparate receptors in drug tolerance, sensitization and physical dependence. Drug Alcohol Depend 38: 139-54, 1995.
30. Coderre T.J. The role of excitatory amino acid receptors and intracellular messenger in persistent nociception after tissue injury in rats. Mol Neurobiol 7: 229-46, 1993.
31. Young A.B., Greenamyre J.T., Hollingsworth Z., Albin R.L., D'Amato C., Shoulson I. and Penney J.B. NMDA receptor losses in putamen from patients with Huntington's disease. Science (Wash. D.C.) 241:981-3, 1988.
32. Francis P.T. , Sims N.R., Procter A.W. and Bowen D.M. Cortical pyramidal neurone loss may cause glutamatergic hypoactivity and cognitive impairment in Alzheimer's disease: Investigative and therapeutic perspectives. J Neurochem 60:1598-604, 1993.
33. Schmidt W.J., Bubser M. and Hauber W Behavioural pharmacology of glutamate in the basal ganglia. J Neural Transm Gen Sect 38(suppl):65-89, 1992.
34. Meldrum B. Possible therapeutic applications of antagonists of excitatory amino acid neurotransmitters. Clin Sci 68: 113-21, 1991.
35. Popik P. and Skolnick P The NMDA antagonist memantine blocks the expression and maintenance of morphine dependence. Pharmacol Biochem Behav 53: 791-7, 1996.
36. Dickenson A.H. and Aydar E. Antagonism at the glycine site on the NMDA receptor reduces spinal nociception in the rat. Neurosci Lett 121:263-6, 1991.
37. Schmidt W.J., Zadow B., Kretschmer B.D. and Hauber W. Anticataleptic potencies of glutamate-antagonists. Amino Acids 1:225-37, 1991.
38. Yaksh T.L., Harty G.J. and Onofrio B.M. High dose of spinal morphine produce a

nonopiate receptor-mediated hyperesthesia: clinical and theoretic implications. Anesthesiology 64:590-97, 1986.
39. Yaksh T.L. and Harty G.J. Pharmacology of the allodynia in rats evoked by high dose intrathecal morphine. J Pharmacol Exp Ther 244:501-7, 1988.
40. Bossut D., Frenk H. and Mayer D.J. Is substance P a primary afferent neurotransmitter for nociceptive input ? IV. 2-Amino-5-phosphonovalerate(APV) and [D-Pro^{2},D-Trp^{7,9}]- subatance P exert different effects on behaviors induced by intrathecal subatance P, strychnine and kainic acid. Brain Res 455:247-53, 1988.
41. Jacquet Y.F., Saederup E. and Squires R.F. Nonsterospecific excitatory actions of morphine may be due to $GABA_A$ receptor blockade. Eur J Pharmacol 138(2):285-8, 1987.
42. Jacquet Y.F. and Squires R.F. Excitatory amino acids: role in morphine excitation in rat peri-aqueductal gray. Behav Brain Res 31:85-8, 1988.
43. Lux F., Brase D.A. and Dewey W.L. Differential effects of subcutaneous and intrathecal morphine adminiatration on blood glcuose in mice: comparison with intracerebro ventricular administration. J Pharmacol Exp Ther 254:187-94, 1988.
44. Yoburn B.C., Lutfy K., Sierra V and Tortella F.C. Tolerance deveolps to spinal morphine analgesia but not morphine- induced convulsions. Eur J Pharmacol 176:63-7, 1990.
45. Stillman M.J., Moulin D.E. and Foley K.E. Paradoxical pain following high-dose spinal morphine. Pain Suppl 4, S389, 1987.
46. De Conno F., Caraceni A., Martini C., Spoldi E., Salvetti M. and Ventafridda V. Hyperlgesia and myo-clonus with intrathecal infusion of high-dose morphine. Pain 47:337-9, 1991.
47. Mjellem-Joly N., Lund A., Berge O., and Hole K. Potentiation of a behavioural response in mice by spinal coadministration of substance P and excitatory amino acid agonists. Neurosci Lett 133: 121-4, 1991.
48. Tsai H.Y., Lin Y.T., Chen C.F., Tsai C.H., Chen Y.F. Effects of veratrine and paeoniflorin on the isolated rat aorta. J Ethnopharmacol 66:249-55, 1999.
49. Tsai H.Y., Lin Y.T., Chen Y.F., Chen C.F. The interactions of paeoniflorin and veratrine on isolated rat atria. J Ethnopharmacol 57:169-76, 1997.

附件：封面格式

行政院國家科學委員會補助專題研究計畫成果報告

※※※※※※※※※※※※※※※※※※※※※※※※※
※　　　　　　　　　　　　　　　　　　　　　　　　※
※　　芍藥□影響 NMDA 接受體機轉之研究　　※
※　　　　　　　　　　　　　　　　　　　　　　　　※
※※※※※※※※※※※※※※※※※※※※※※※※※※

計畫類別：☑個別型計畫　　□整合型計畫

計畫編號：NSC 90－2320－B－039－015

執行期間：　90 年 8 月 1 日至　91 年 7 月 31 日

計畫主持人：陳玉芳

共同主持人：蔡輝彥

計畫參與人員：許朝添

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

執行單位：中國醫藥學院 藥理學科

中　華　民　國　91 年　10 月　28 日