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Effect of Co-administration of Morphine and Cholinergic Antagonists on Y-maze Spatial Recognition Memory Retrieval and Locomotor Activity in Mice

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Abstract: The interaction of morphine and cholinergic system was shown in previous studies. In the present study, we investigated whether morphine would interact with the cholinergic antagonists, scopolamine and atropine in a Y-maze spatial recognition memory. Pre-test treatments of morphine (5, 1.5, 0.5 mg/kg), scopolamine (1, 0.1 mg/kg), atropine (0.5, 0.1 mg/kg) were used in the experiments, relatively high or low doses were paired respectively as co-administration measures. The results showed that co-administration of morphine 0.5 mg/kg + scopolamine 0.1 mg/kg and morphine 0.5 mg/kg + atropine 0.1 mg/kg disturbed the inspective exploratory behavior (percent of arm duration) but not the inquisitive behavior (percent of arm visits) of the spatial memory retrieval, while the drugs didn't cause amnesia when single administered of the concerned low doses. Distinct interaction was found between scopolamine and morphine on increasing locomotor activity.

Key words: Retrieval; Morphine; Scopolamine; Atropine; Y-maze

吗啡和胆碱能拮抗剂联合给药对小鼠 Y 迷宫空间记忆提取及活动性的影响

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摘要：吗啡和胆碱能系统的相互作用已在多项研究中提到，本实验想查明吗啡是否能和胆碱能拮抗剂、东莨菪碱以及阿托品共同作用对小鼠的 Y 迷宫空间识别记忆提取产生影响。采用测试前腹腔给药的方法，选用 3 种剂量的吗啡(5、1.5、0.5 mg/kg)，两种剂量的东莨菪碱(1、0.1 mg/kg)，以及两种剂量的阿托品(0.5、0.1 mg/kg)，剂量由高到低相配对作为联合给药的手段。其结果表明：1) 0.5 mg/kg 低剂量吗啡与 0.1 mg/kg 低剂量的东莨菪碱，或与 0.1 mg/kg 低剂量的阿托品联合给药的小鼠，在记忆提取测试中， 空间探查行为（各臂停留时间百分比）对新异臂没有偏好，而新奇探索行为（各臂访问次数百分比）仍保持了对新异臂的偏好，而相应剂量药物单独给药的小鼠记忆提取均没有被损害；2) 吗啡能和东莨菪碱相互作用使小鼠的活动性显著增强。暗示吗啡和胆碱能拮抗剂对小鼠空间记忆提取的破坏存在一定程度的相互作用。

关键词：记忆提取；吗啡；东莨菪碱；阿托品；Y 迷宫
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Cholinergic system has been found to play a role in learning and memory (Deutsch, 1971). Cholinergic antagonists, scopolamine and atropine disrupted memory process in various tasks in animals (Meyers, 1965; Patel & Tariot, 1991; Rupniak et al, 1989; Sunderland et al, 1986), and in human (Christensen et al, 1992; Drachman & Leavitt, 1974; Ebert & Kirch, 1998; Wesnes et al, 1991). Generally, these two drugs have no qualitative difference, as to the signs of peripheral parasympathetic block and an extensive impairment of central nervous system function on human (Ketchum et al, 1973). However when combined with analgesics in

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pre-operation sedation and obstetrical amnesia, atropine failed to cause amnesia and potentiation of pentothal narcosis in doses in which scopolamine was effective (Orkin et al, 1956).

Many reports demonstrated that acute administration of opioids impairs cognitive functions in animals (Castellano & Pavone, 1985; Itoh et al, 1994; Izquierdo, 1980; Schulteis et al, 1988; Stone et al, 1991; Walker et al, 1991). Previous researches suggested that activation of opioid receptors can decrease the function of cholinergic system and cause memory deficit. Muscarinic receptor agonist oxotremorine co-administrated with naloxone significantly improved memory in an one-trial inhibitory avoidance task (Baratti et al, 1984). However the muscarinic antagonist atropine blocked the memory-enhance induced by naloxone (Baratti et al, 1984). Post-training administration of naloxone reversed memory impairment induced by scopolamine in a passive avoidance task and spontaneous alternation tests (Rush, 1986; Walker et al, 1991). Muscarinic agonists could antagonize the memory impairment induced by β-endorphine (Introini & Baratti, 1984). Thus, it has been suggested interaction between opiate and cholinergic system.

Since it has been proved that the cholinergic antagonist, scopolamine impaired retrieval of working memory in rats (Beatty et al, 1986), while morphine had an inconsistent effect on memory retrieval in mice (Kahveci et al, 2006; Shiigi & Kaneto, 1990; Shiigi et al, 1990; Zarrindast et al, 2006), and our previous data showed that morphine impaired the retrieval of spatial recognition memory in a Y-maze (Ma et al, 2007). In the present study, we used the Y-maze to test the effects of co-administration of morphine and cholinergic antagonists, scopolamine, and atropine on spatial recognition memory retrieval. According to the previous studies we hypothesized that the combined administration of morphine and scopolamine/atropine would enhance memory deficit because of the cholinergic system function may be suppressed by both morphine and the muscarinic antagonists. Meanwhile, effects of drugs on the locomotor activity can be tested in the Y-maze as well.

1 Materials and methods

1.1 Animals

Male ICR mice (24−28 g body weight, at age of 8 weeks) from breeding colonies at the Kunming Medical College were used. They were housed under standard conditions (a 12-hr light/dark cycle with light on from 07:00 to 19:00) and were reared in separate cages (8 per cage). Mice were freely feeding and familiarized with the experimenter and the testing environment for one week before the experiment started. The experiments were conducted in accordance with the Guide for Care and Use of Laboratory Animals (Publication No. 85−23, revised 1985) and were approved by the National Institutes of Health.

1.2 Drugs

Morphine hydrochloride (10 mg/mL, Shenyang Pharmaceutical Factory), scopolamine hydrobromide (0.3 mg/mL, Shanghai Harvest Pharmaceutical Co), atropine sulfate (0.5 mg/mL, Tianjin Pharmaceutical Factory), were dissolved in the saline.

1.3 Behavioral apparatus

The Y-maze was made of grey plexiglass or wood, covered with black paper, and consisted of three arms with an angle of 120° between each two arms. Each arm was 8 cm×30 cm×15 cm (width×length×height). The three identical arms were randomly designated: Start arm, in which the mouse started to explore (always open); Novel arm, which was blocked at the 1st trial, but open at the 2nd trial; and the other arm (always open).

The maze was placed in a separate room with enough light. The floor of the maze was covered with sawdust, which was mixed after each individual trial in order to eliminate olfactory stimuli. Visual cues were placed on the walls of the maze.

The Y-maze test consisted of two trials separated by an inter-trial interval (ITI) to assess spatial recognition memory. The first trial (training) was 10 min duration and allowed the mouse to explore only two arms (start arm and the other arm) of the maze, with the third arm (novel arm) blocked. After a 1 h ITI (Ma et al, 2007), the second trial (retention) was conducted, during which all three arms were accessible and novelty vs. familiarity was analyzed through comparing behavior in all three arms. For the second trial, the mouse was placed back in the maze in the same starting arm, with free access to all three arms for 5 min. By using a ceiling-mounted CCD camera, all trials were recorded on a VCR. Video recordings were later analyzed. The number of entries and time spent in each arm were to be analyzed; data were also expressed as percentage of performance in all arms during the 5-min-retention test (Akwa et al, 2001).

Because memory retention in the Y-maze test did

not last longer than a few hours, so this task can be assessed to test different drugs' effects in used animal by the interval of one week (Dellu et al, 2000).

1.4 Treatment

Drugs were intraperitoneally injected to mice 40 min after the first trial (training), 0.2 mL per mouse for each injection. The second trial (retrieval) was conducted 1 hour after 10-min-training in the Y-maze.

1.4.1 Experiment 1 There were six groups in Exp.1 (N=8 per group): 1) saline+saline (Saline); 2) scopolamine(1 mg/kg)+morphine (5 mg/kg)(Scop1+Mor5); 3) atropine (0.5 mg/kg)+morphine (5 mg/kg)(Atro0.5+Mor5); 4) scopolamine (1 mg/kg)+saline (Scop1); 5) atropine (0.5 mg/kg)+saline (Atro0.5); 6) morphine (5 mg/kg)+saline (Mor5).

The dose of morphine were chosen according to our previous study (Ma et al, 2007), which suggested morphine impaired spatial memory retrieval in mice at the dose of 5 mg/kg. The doses of scopolamine and atropine were conducted following several former researches in which the drugs effectively cause amnesia in mice (Jafari et al, 2006; Kim et al, 2006; Sakata et al, 2005).

1.4.2 Experiment 2 In experiment 1, all groups showed the impairments of the spatial recognition memory retrieval except the controls. In order to clarify the interaction of the drugs, low doses of drugs' effects were tested in experiment 2 and 3.

Four groups were conducted here (N=8 per group): 1) saline+saline (Saline); 2) scopolamine (0.1 mg/kg)+morphine (1.5 mg/kg) (Scop0.1+Mor1.5); 3) scopolamine (0.1 mg/kg) +saline (Scop0.1); 4) morphine(1.5 mg/kg)+saline (Mor1.5).

Morphine at lower dose of 0.5 mg/kg was used in order to confirm the potential interact between drugs. Another four groups were conducted (N=9,10,9,9 respectively) : 1) saline+saline (Saline); 2) scopolamine (0.1 mg/kg)+morphine (0.5 mg/kg)(Scop0.1+Mor0.5); 3) scopolamine (0.1 mg/kg)+saline (Scop0.1); 4) morphine(0.5 mg/kg)+saline (Mor0.5).

1.4.3 Experiment 3 Since morphine 1.5 mg/kg has been found enough to disturb memory retrieval in the experiment 2, so atropine 0.1 mg/kg was directly co-administrated with lower dose morphine of 0.5 mg/kg in experiment 3.

Four groups were in Exp.3 (N=8,9,9,10 respectively): 1) saline+saline (Saline); 2) atropine (0.1 mg/kg)+morphine (0.5 mg/kg)(Atro0.1+Mor0.5); 3) atropine (0.1 mg/kg)+saline (Atro0.1); 4) morphine(0.5 mg/kg)+saline (Mor0.5).

1.5 Statistical analysis

Data were expressed as: 1) percentage of duration time spent in each arm (seconds) in the 5-min-retention phase (as spatial recognition memory measure); 2) percentage of number of arm entries were used to compensate for difference of memory deficit changes between treatments; 3) the number of visits in each arm in the 5-min-retention test (as a locomotor activity index). Data were expressed as mean ± standard error of the mean (SE) and analyzed by using the SPSS statistical software package (version 10). Differences between arms within group, and differences between groups were both considered significant at P<0.05. One-way ANOVA was used to analyze the difference between the three arms. Differences between groups for number of arm visits were assessed with analysis of variance (ANOVA) with repeated measures where appropriate. Post hoc between-group comparisons were completed with Fisher's least significant difference test (LSD).

2 Results

2.1 Effects of morphine and scopolamine/atropine on retrieval of spatial recognition memory

2.1.1 Percentage of duration of arm visits in the 5 minutes retention test As shown in Fig.1a, Scop1, Atro0.5, Mor5, Scop1+Mor5, and Atro0.5+Mor5 groups all performed no difference between arms as expected. (Main arm effect, $F_{(2, 84)}$=1.236, P=0.296, while Saline group showed arm difference (LSD: novel arm vs. start arm P=0.026).

Co-administration of low doses of morphine and scopolamine/atropine showed no difference between arms with the percentage of arm duration parameter while the single-drug administered mice still kept preference to the novel arm. (Fig. 2c, main arm effect, $F_{(2, 66)}$=12.993, P<0.001, Saline group (LSD: novel arm vs. start arm P=0.02, novel arm vs. other arm P=0.04), Scop0.1 group (LSD: novel arm vs. start arm P=0.008), Mor0.5 group (LSD: novel arm vs. other arm P=0.04); Fig. 3a, main arm effect $F_{(2, 64)}$=11.051, P<0.001, Saline group (LSD: novel arm vs. start arm P=0.03, novel arm vs. other arm P=0.039), Atro0.1 group (LSD: novel arm vs. other arm P=0.013), Mor0.5 group (LSD: novel arm vs. start arm P=0.045, novel arm vs. other arm P=0.036).

2.1.2 Percentage of number of arm visits in the 5 minutes retention test As shown in Fig. 1b, merely

Saline and Mor5 groups showed difference between arms. (Main arm effect, $F_{(2, 84)}$=7.762, P=0.001, Saline group (LSD: novel arm vs. start arm P=0.006, novel arm vs. other arm P=0.036), Mor5 group (LSD: novel arm vs. start arm P=0.031).

However co-administration of the low doses of drugs didn't disrupt the memory retrieval as shown in the percentage of arm visits (Fig. 2b, main arm effect, $F_{(2, 56)}$=16.983, P<0.001, Scop0.1+Mor1.5 group (LSD: novel arm vs. start arm P=0.010, novel arm vs. other arm P=0.023); Fig. 2d, main arm effect, $F_{(2, 66)}$=31.921, P<0.001, Scop0.1+Mor0.5 group (LSD: novel arm vs. start arm P=0.013, novel arm vs. other arm P=0.004); Fig. 3b, main arm effect, $F_{(2, 64)}$=60.027，P<0.001，Atro0.1+Mor0.5 group (LSD: novel arm vs. start arm P<0.001, novel arm vs. other arm P=0.010). The low

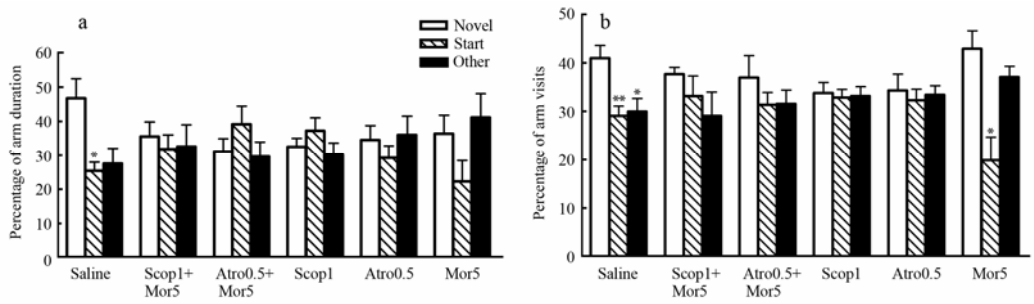

Fig. 1 Effects of pre-test co-administration of morphine and scopolamine/atropine on spatial recognition memory retrieval in mice with Y-maze

a: Only the controls showed higher percentage of arm duration of arm visits between the novel arm and the start arm within group; b: The controls showed higher percentage of the novel arm visits than the other two familiar arms. The Mor5 group showed higher percentage of the novel arm visits than the start arm.

Data were expressed as mean±SE. *P<0.05, **P<0.01 for difference between the novel arm and the start arm within group.

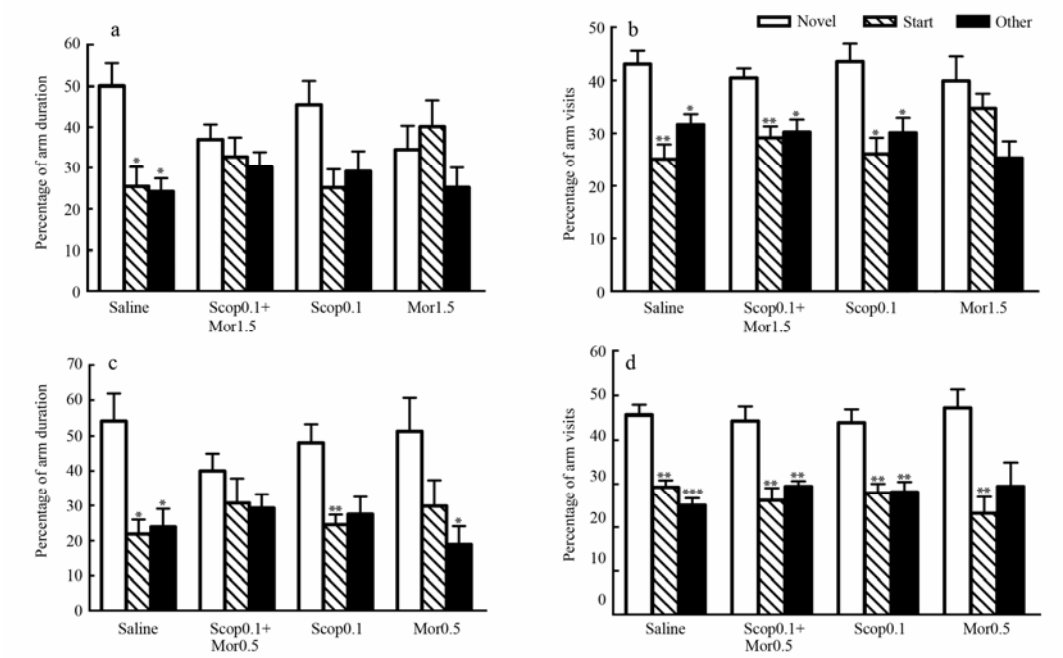

Fig. 2 Effects of pre-test low doses of morphine and scopolamine co-administration on spatial recognition memory retrieval in mice with Y-maze

a: The controls showed higher percentage of the novel arm duration than the other two familiar arms. The Scop0.1 group showed borderline higher percentage of the novel arm duration than the start arm; b: The controls, Scop0.1+Mor1.5 group and Scop0.1 group all showed higher percentage of the novel arm visits than the other two familiar arms; c: The Scop0.1+Mor0.5 group showed damaged spatial recognition memory retrieval, which was reflected by no difference was found between arms. Meanwhile other three groups all showed preference to the novel arm; d: The four groups all showed higher percentage of the novel arm visits than the other one or two familiar arms.

Data were expressed as mean±SE. *P<0.05, **P<0.01, ***P<0.001 for difference between the novel arm and the other arms within group.

doses of drugs' single-treated mice performed distinct preference to the novel arm, except Mor1.5 group (Fig. 2c, 2d, 3b, data not shown).

2.2 Effects of co-administration of morphine and scopolamine/atropine on locomotor activity and the number of arm visits in the 5 minutes retention test

There was a distinct increase of number of arm visits within the scopolamine and morphine co-administrated groups except the lowest dose of the drugs in our experiment. (Fig. 4a, Scop1+Mor5 vs. Saline P=0.035, Scop1+Mor5 vs. Atro0.5 P=0.007, Scop1+Mor5 vs. Mor5 P=0.045; Fig. 4b, Scop0.1+Mor1.5 vs. Saline P=0.022, Scop0.1+Mor1.5 vs. Scop0.1 P=0.008, Scop0.1+Mor1.5 vs. Mor1.5 P=0.008). No effect on locomotor activity was found in the morphine and atropine co-administration groups (Fig. 4a,d).

3 Discussion

According to Dellu et al (1992; 2000) the two

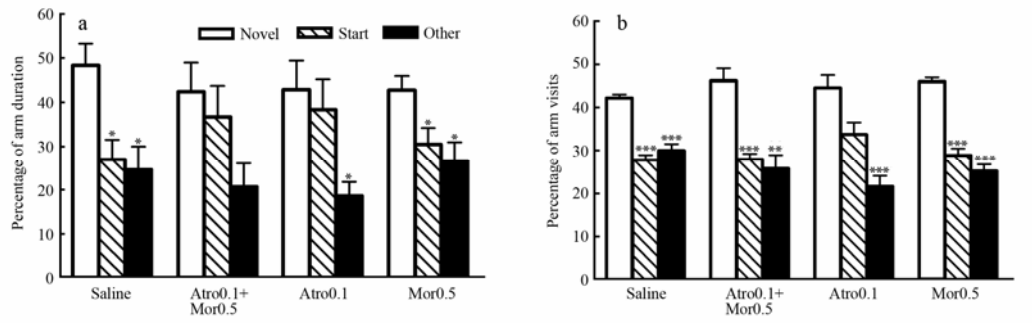

Fig. 3 Effects of pre-test low doses of morphine and atropine co-administration on spatial recognition memory retrieval in mice with Y-maze

a: The controls and Mor0.5 showed higher percentage of the novel arm duration than the other two familiar arms. The Atro0.1 group showed higher percentage of the novel arm duration than the other arm. The Atro0.1+Mor0.5 group performed damaged spatial recognition memory retrieval reflected by no difference was found between arms; b: The four groups all showed higher percentage of novel arm visits than the other one or two familiar arms.
Data were expressed as mean±SE. *P<0.05, **P<0.01, ***P<0.01 for difference between the novel arm and the other arms within group.

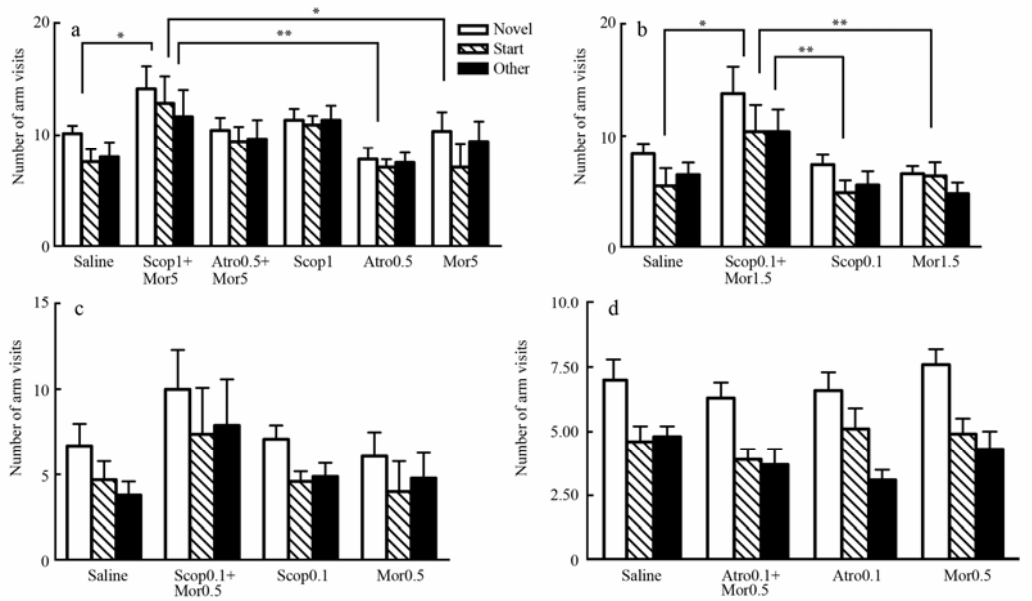

Fig. 4 Effects of co-administration of morphine and scopolamine/atropine on locomotor activity

a: The Scop1+Mor5 group showed higher number of total arm visits than the controls, Atro0.5 and Mor5 groups; b: Similar to the Scop1+Mor5 group, the Scop0.1+Mor1.5 group showed higher number of total arm visits than the other three groups; c: No difference was found between groups, while the Scop0.1+Mor0.5 group still showed the increasing trend of arm visits; d: No difference was found between groups.
Data were expressed as mean±SE. *P<0.05, **P<0.01 for difference between groups.

measures of exploratory behavior (inspective and inquisitive) should be separately analyzed. The duration time spent in arms is considered as the index of inspective exploratory behavior, while the number of arm visits is measured as inquisitive behavior. Our results indicated that morphine and cholinergic antagonists, scopolamine/atropine partly cooperated on impairing the inspective exploratory behavior of the spatial memory retrieval. Furthermore, it has been proved that morphine cooperated with scopolamine increased the locomotor activity.

In our data, the low doses of drugs' co-administered mice all performed impaired inspective exploratory behavior of spatial recognition memory, but still could distinguish the novel arm very well when analyzing the inquisitive behavior. The inconsistent performance of the two aspects of exploratory behavior is consistent with the observations from Conrad et al (1997) Also it has been found that amphetamine may affect the two measures towards opposite directions.(Dellu et al, 1992).

Our results were generally consistent to previous studies that atropine administration following morphine injection prevented memory retrieval in passive avoidance task (Jafari et al, 2006). Co-administration of scopolamine and morphine significantly impaired the performance of rats in Morris water maze(Zheng et al, 2002). Here we proved this interaction again in a Y-maze paradigm which characterized by both rewards and punishment free.

It was proved that there was an interaction between opioids and the cholinergic systems (Introini & Baratti, 1984; Rush, 1986; Walker et al, 1991). Moreover injection of morphine into the medial septum could reduce ACh release in the hippocampus (Ragozzino & Gold, 1995). Similarly, *in vivo* microdialysis has revealed that acute morphine significantly decreased the release of ACh in several brain regions (Arenas et al, 1990; Beani et al, 1982; Lapchak et al, 1989; Mulder et al, 1984, 1989; Rada et al, 1991). And decreasing the cholinergic functions generally caused impairment of the performance in rats' spontaneous alternation tests (McIntyre et al, 2002).

It has also been demonstrated that opioid agonists such as morphine and β-endorphine, possessing higher affinity for μ-opioid receptors, inhibited cholinergic activity in the hippocampus (Decker & McGaugh, 1991). Moreover, it has also been reported that μ and δ receptors locate on cholinergic terminals, which are normally under tonic inhibition by the opiate system (Heijna et al, 1990).

In our experiment morphine didn't show a regulary effect on the spatial recognition memory as doses were diminished. Taraschenko et al (2007) found that different doses of morphine produced a biphasic effect on extracellular acetylcholine levels, thus low and high dose of morphine (i.e., 5 and 20 mg/kg i.p.) significantly increased and decreased acetylcholine levels, respectively. These findings suggested that the morphine may not affect on the spatial memory directly and its influence on the cholinergic system were dose dependent.

With regard to the locomotor activity, we found a dramatic increase of locomotor activity in the mice co-administrated with scopolamine and morphine, which again proved that there was an interaction between the cholinergic and opiate systems. Former study demonstrated that morphine administration caused hyper-locomotor activity depending on dose and state. (Heidari et al, 2006; Ma et al, 2007; Stone et al, 1990) Increasing locomotor activity induced by morphine in rodents was thought to reflect dopamine release in the striatum (Murphy et al, 2001; Porras et al, 2003). Scopolamine was found to increase dopamine release depended on doses (Ichikawa et al, 2002) , and indirectly improved locomotor activity (Bauer, 1982; Chintoh et al, 2003; Joyce & Koob, 1981; Sakata et al, 2005) .Therefore we assumed that the hyper-locomotor activity, which was found in our experiment, was considered as the result from the cooperation of morphine and scopolamine to up-regulate the dopamine level. Atropine showed no effect on the locomotor activity both when administrating alone and co-administering with morphine. These results are consistent with previous studies using the conditioned place preference paradigm and passive avoidance test in rodents.(Jafari et al, 2006; Rezayof et al, 2007).

However, no significant locomotor increase was found in the co-administration of scopolamine 0.1 mg/kg+morphine 0.5 mg/kg mice, which suggested the lower limit dose.

In conclusion, morphine showed interaction with cholinergic antagonists to further impair the inspective exploratory behavior of the spatial recognition memory retrieval with Y-maze paradigm.

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