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# Effects of scopolamine and physostigmine on acquisition of morphine-treated rats in Morris water maze performance[1]

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**KEY WORDS** morphine; acquisition; scopolamine; physostigmine; Morris water maze

## ABSTRACT

**AIM**: To investigate effects of morphine on acquisition process of rats and interactions of opioid and cholinergic systems by Morris water maze performance. **METHODS**: Morris water maze was used to measure the latency of rats with drugs treatment to find the covert platform. **RESULTS**: Chronic morphine administration (10 mg/kg) impaired the acquisition process of rats in Morris water maze task. Appreciable difference was identified with morphine 10 mg/kg group compared with morphine 3 mg/kg group. Co-administration of morphine (10 mg/kg) and scopolamine (3 mg/kg) aggravated acquisition impairment induced by morphine 10 mg/kg or scopolamine alone, though scopolamine itself induced no salient changes in acquisition capabilities of rats. In addition, physostigmine (0.1 mg/kg) could appreciably attenuate morphine-induced acquisition impairment. **CONCLUSION**: Morphine 10 mg/kg evidently impaired acquisition process of rats. There was a close relationship between the acquisition capabilities of morphine-treated rats and the functions of cholinergic system.

## INTRODUCTION

Previous researches have demonstrated that there exist some common and interconnective mechanisms underlying memory and behavioral plasticity of drug abuse, including reinforcement, dependence, and drug withdrawal. The ultimate behavioral effects of addictive drugs are largely modulated by learning and memory processes[1-4]. Researches have shown that the central cholinergic system plays an important role in memory process, and is impaired by the administration of morphine. There are some interactions between central opioid and cholinergic systems through inhibitory effects of opioid-like substances on cholinergic neurons of hippocampus and prefrontal cortex *via* $\mu$ and $\delta$ receptors. Opioid-like substances inhibit the cholinergic projection from medial septum to hippocampus, which is regarded closely associated with spatial learning impairment[5-8]. It has also been reported that there is a positive correlation between the impaired performance of spontaneous alteration test and the decreased acetylcholine level in hippocampus[7]. Further, the release amount of acetylcholine drops off to 65 % – 80 % of its original level after 30 min of morphine administration[7,8]. Similar results could be found in more behavioral models, such as shuttle avoidance test, radial maze, and one-trial inhibitory avoidance performance revealing impairment of memory under treatment of morphine[8-11]. This kind of impairment can be attenuated or even reversed by naloxone, glucose, and oxotremorine *etc*[2,8,12,13]. However, to what extent and how the addictive behavioral plasticity be modulated by central cholinergic system are not very clear. In the present study, by Morris water maze, we examined the performance of morphine-treated rats after cholinergic agonists and antagonists administration.

## MATERIALS AND METHODS

**Animals** Male Wistar rats (140 – 220 g, $n = 42$, Grade Ⅰ, Certificate № 199036. Institute of Genetics, Chinese Academy of Sciences, Beijing, China) were maintained in individual steel cages (lighting 8:00 – 20:00). Water and food were given *ad libitum*.

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Experiments were carried out in 10 d successively between 13:00 – 18:00.

**Morris water maze test procedures** Morris water maze, a circular pool (diameter 98 cm, height 60 cm) was filled to a depth of 40 cm with water at room temperature of 21.0 ℃ ± 0.5 ℃. The semi-transparent cream white-colored platform was set below the surface of water (1 cm) and made unseen by milk-diluted water in the maze. Rats were placed into the water facing against the wall from a fixed position to locate the platform laid at the center of the diagonal quadrant. During experiment for 10 d, by allowing rats using intra-maze and extra-maze cues, each rat was trained one trial each day to locate the platform after 30 min of drug injection. The latency to find the platform was recorded by automatic video-tracking system of computer. Upon finding, climbing the platform, and stayed for 2 s, the rats were drawn back into cages as reinforcement. If failed in 180 s, they were helped and placed on the platform for 15 s, then sent back to cages.

**Drugs** Morphine (Qinghai Pharmaceutical Co, China), scopolamine hydrobromid (MERCK-MEDCO. USA), physostigminum (Sandoz SA, BALE, Switzerland) were used in our study. Drugs were dissolved in saline and injected ip 30 min before Morris water maze test one trial everyday.

**Groups and disposal** Rats were divided into seven groups: saline administration as control group the other six experimental groups were respectively given morphine (10 mg/kg, Mor1), morphine (3 mg/kg, Mor2), scopolamine (3 mg/kg, Sco), physostigmine (0.1 mg/kg, Phy), co-administration of morphine (10 mg/kg) and scopolamine (3 mg/kg, Sco_Mor1), co-administration of morphine (10 mg/kg) and physostigmine (0.1 mg/kg, Phy_Mor1). Considering co-administration of two injections of different drugs in the latter two groups, each rat in other groups was added with another saline injection.

**Statistics** Data were expressed as $x \pm s$, and analyzed using one-way repeated-measure ANOVA for comparisons between groups. $P < 0.05$ were considered statistically significant. The collected data of latency in 10 d were clustered, presented, and analyzed with two days as one training time.

## RESULTS

**Effects of different doses of morphine on acquisition deficit in Morris water maze performance** The latency of Mor1 administrated rats was significantly longer compared with saline control rats on training time 3, 4, and 5. On training time 5, the latency of Mor1 group significantly increased compared with Mor2 group. However, no appreciable difference was observed between Mor2 group and saline control group within the whole duration of the experiment (Fig 1).

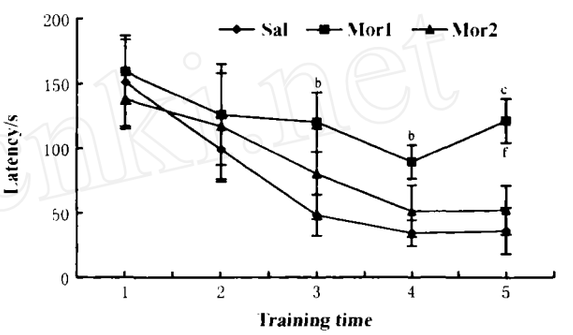


**Fig 1. Effects of different doses of morphine administration on acquisition deficit in Morris water maze performance.** $n = 5 - 6$ rats. $x \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* saline group. $^fP < 0.01$ *vs* Mor2 group.

**Effects of scopolamine on morphine-induced acquisition deficit in Morris water maze performance** The latency of Sco Mor1 group was further prolonged compared with Mor1 group and saline control group on training time 3, 4, and 5. The difference was significant on training time 4 and 5 between Sco_Mor1 and Mor1 group. The latency difference was also significant between Sco_Mor1 and Sco group on training time 3, 4, and 5. Furthermore, the latency of Mor1 group significantly increased compared with Sco group on training time 5. No significant difference was obtained between Sco group and saline control group (Fig 2).

**Effects of physostigmine on morphine-induced acquisition deficit in Morris water maze performance** On training time 5, the latency of Phy_Mor1 group significantly decreased compared with Mor1 group. However, appreciable difference was also noticed between Phy Mor1 group and saline group. No significance existed between Phy group and saline group during the whole experiment duration (Fig 3).

We set training time 5 as the ultimate testing point and found that the latency of Sco_Mor1 group increased compared with Mor1, Mor2, Sco, Phy, Phy Mor1, and saline group. Appreciable difference was also obtained with Mor1 group compared with Mor2, Sco, and

Phy_Morl group. Meanwhile, significant difference was noticed between Phy Morl group and saline group.

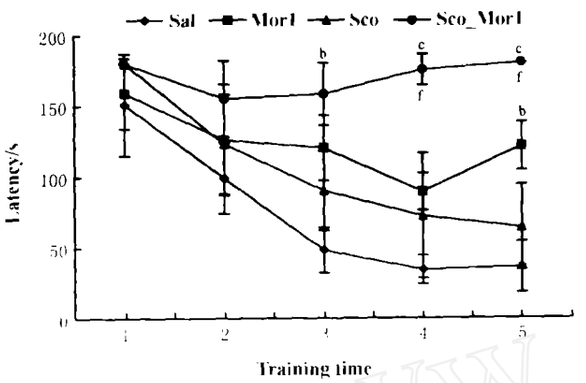


**Fig 2. Effects of scopolamine on morphine-induced acquisition deficit in Morris water maze performance. $n = 6-7$ rats. $x \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* Sco group. $^fP<0.01$ *vs* Morl group.**

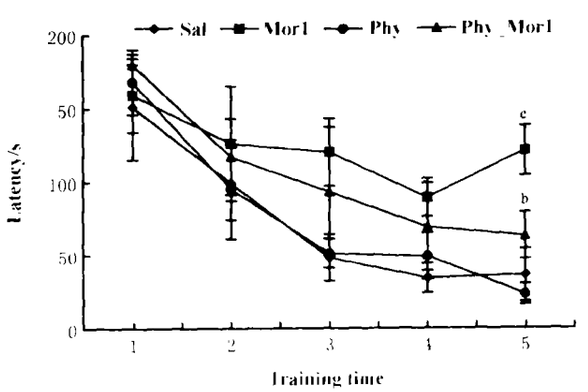


**Fig 3. Effects of physostigmine on morphine-induced acquisition deficit in Morris water maze performance. $n = 6$ rats. $x \pm s$. $^bP<0.05$ *vs* Saline group. $^cP<0.01$ *vs* Phy Morl group.**

## DISCUSSION

Morris Water Maze is believed to be able to effectively distinguish spatial memory from sensational, motivational, and retrieval processes of the brain, and often used to test the capabilities of spatial memory, which is considered closely associated with septohippocampal cholinergic activity[11,15]. It has been reported that different modalities of morphine administrations, either under single dose or escalating doses, can cause impairment for spatial memory of Morris water maze performance[12]. In our experiment, morphine had eminent dose-dependent inhibitory effects on the acquisition capabilities of rats. It has been shown that high dose of morphine treatment demonstrated significant latency increase compared with low dose of morphine group. The reason that no appreciable difference was obtained between low dose of morphine group and saline group might be, to some extent, due to the tolerance effect of morphine. Previous researches demonstrated that successive administration of morphine (5 mg/kg) produced salient tolerance in rats, leaving no significant influences on mnemonic process[16]. The same results could be found in microdialysis that the hippocampal release of acetylcholine resumed to normal in nucleus accumbens after 7 d successive morphine administration[2,16].

Though Rush and Walker reported eminent memory impairment of scopolamine 3 mg/kg in rats and mice in passive avoidance tests, our study showed no significant impairment of scopolamine 3 mg/kg to acquisition process in Morris water maze performance. This discrepancy might partially be due to different behavioral models, which activated different memory processes. Morris water maze task tests long-term memory, whereas the passive avoidance task can be worked out through activating short-term (working) memory[15]. However, co-administration of morphine and scopolamine resulted in more prolonged latency compared with morphine group and scopolamine group. This demonstrated further acquisition deficit after morphine treatment. Moreover, appreciable difference between high dose of morphine group and scopolamine group in our experiment was identified, suggesting that there was a partial separation between morphine reinforcement effect and memory impairment elicited by destruction of cholinergic activity.

Our study showed that physostigmine could effectively attenuate the memory impairment induced by morphine treatment, though the impairment could not be thoroughly reversed by physostigmine. Our result accorded with the thought that more systems were involved in morphine-induced memory impairment besides cholinergic plasticity. Since more substances besides physostigmine could change mnemonic processes, how the behavioral plasticity of drug of abuse is modulated by learning and memory details will be further studied.

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## 东莨菪碱和毒扁豆碱对吗啡处理大鼠 Morris 水迷宫空间学习能力的影响

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**关键词** 吗啡；获得；东莨菪碱；毒扁豆碱；Morris 水迷宫

**目的**：研究吗啡及胆碱能系统的药物对大鼠空间学习能力的影响．**方法**：应用 Morris 水迷宫学习程序研究吗啡处理大鼠的空间学习能力及其在东莨菪碱和毒扁豆碱作用下的变化．**结果**：高剂量吗啡（10 mg/kg）和低剂量吗啡（3 mg/kg）对大鼠学习能力的影响差异有显著性；东莨菪碱（3 mg/kg）和吗啡（10 mg/kg）联合给药，可以使吗啡对学习能力的损害进一步加重；毒扁豆碱（0.1 mg/kg）可以改善吗啡所致的学习能力损害，但不能完全逆转．**结论**：吗啡损害大鼠空间学习能力，且存在量-效关系；胆碱能系统与吗啡处理大鼠空间学习能力关系密切，有可能影响成瘾的发生及治疗．

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