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·Original Article·

Interactive effects of morphine and dopaminergic compounds on spatial working memory in rhesus monkeys

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ABSTRACT

Opiates and dopamine (DA) play key roles in learning and memory in humans and animals. Although interactions between these neurotransmitters have been found, their functional roles remain to be fully elucidated, and their dysfunction may contribute to human diseases and addiction. Here we investigated the interactions of morphine and dopaminergic neurotransmitter systems with respect to learning and memory in rhesus monkeys by using the Wisconsin General Test Apparatus (WGTA) delayed-response task. Morphine and DA agonists (SKF-38393, apomorphine and bromocriptine) or DA antagonists (SKF-83566, haloperidol and sulpiride) were co-administered to the monkeys 30 min prior to the task. We found that dose-patterned co-administration of morphine with D1 or D2 antagonists or agonists reversed the impaired spatial working memory induced by morphine or the compounds alone. For example, morphine at 0.01 mg/kg impaired spatial working memory, while morphine (0.01 mg/kg) and apomorphine (0.01 or 0.06 mg/kg) co-treatment ameliorated this effect. Our findings suggest that the interactions between morphine and dopaminergic compounds influence spatial working memory in rhesus monkeys. A better understanding of these interactive relationships may provide insights into human addiction.

Keywords: working memory; morphine; dopamine; rhesus monkey

INTRODUCTION

Working memory refers to the short-term storage and manipulation of information in memory, and is thought to be dependent upon the prefrontal cortex (PFC)[1]. The PFC mediates executive functions essential for planned and goal-directed behaviors and is modulated by several neurotransmitters, especially dopamine (DA)[2]. Impairment of executive functions, potentially by neurochemical imbalances, may cause psychiatric and neurological disorders, including mood and anxiety disorders, schizophrenia, and/or drug addiction[3].

In addition to the dopaminergic influences on executive functions, opiate agonists such as morphine have been found to induce deficits in working memory and episodic memory in humans[4-6]. An acute dose of morphine in palliative care patients has been reported to cause both anterograde and retrograde memory impairment[7]. Our previous work also shows that heroin, another opiate agonist, causes deficits in both map and landmark working memory in addicted humans[8]. Similar findings have also been reported in animal models. For example, Schulze and Paule (1991)[9] found that morphine given to rhesus monkeys 15 min prior to a battery of complex food-reinforced operant tasks,

including a delayed matching-to-sample task reflecting working memory, produced significant dose-dependent decreases in the number of reinforcers obtained.

However, a general hypothesis on drug-seeking behavior suggests that opiates like morphine stimulate the reward circuitry of the mesolimbic system coincident with learning and memory processes formulated by reinforcement conditioning and tolerance. The recent findings that morphine decreases working memory, in contrast to stimulation of the reward circuitry, only highlights the fact that the complexities of learning and memory, drug-seeking behavior and the neurochemical imbalances of drug addiction remain to be fully understood. In general, morphine influences memory in animals by agonizing μ-opioid receptors, which are widely distributed throughout the mammalian brain, including the PFC[10]. Moreover, opiate agonists have been shown to increase DA release in the ventral tegmental area, which then signals the nucleus accumbens, PFC and other areas associated with the mesolimbic system[11-13].

DA receptors, like opioid receptors, are widely dispersed within the mammalian brain and have also been reported to influence learning and memory in humans and animals. Generally, there are two subgroups of DA receptors: the D1-like and D2-like families. The D1-like family is expressed at high density in the frontal cortex, striatum, nucleus accumbens, and substantia nigra; the D2-like family is at high levels also in the striatum, nucleus accumbems, and substantia nigra, as well as in the olfactory tubercle and other cortical areas[14].

The blockade or activation of these families of receptors influences learning and memory, albeit each family influences different types of memory and memory-formation processes. Furthermore, the induction of long-term potentiation and/or short-term potentiation in the PFC, phenomena often attributed to learning and memory, depends on DA release[3].

All told, the close proximity of the opiate and dopaminergic neurotransmitter systems and their related roles in learning and memory, especially in the PFC, suggest that interactions between these two systems may exist and contribute to executive functions in animals and humans. Although similar interactions of the opiate and dopaminergic systems have been widely studied in rodents, few have been performed in primates. The rhesus monkey (*Macaca mulatta*) provides a useful model to evaluate these interactions since it has closer evolutionary ties to humans and a higher degree of intelligence and subsequent executive functions than rodents.

The current study was performed to investigate the interaction between opioid and dopaminergic neurotransmitters in learning and memory. The effects of single or co-administration of morphine and DA receptor (D1 and D2 family) agonists/antagonists were evaluated in regards to spatial working memory in the rhesus monkey. The Wisconsin General Test Apparatus (WGTA) delayed-response task, a marker task for working memory, was used to evaluate the monkeys' spatial working memory[15]. To prevent the development of opiate tolerance during the experiment, low doses of morphine and dopaminergic compounds were administered and the monkeys were allowed to recover from the drug treatments before subsequent injections.

MATERIALS AND METHODS

Animals

Four male rhesus monkeys (*Macaca mulatta*) (named KunKun, LaLa, DeDe, and XiDie) from the breeding colony at the Kunming Institute of Zoology were used. They were 8.0 ± 0.7 years and weighed 6.8 ± 0.3 kg at the onset of the experiments. After the experiments were finished, the average weight was 9.0 ± 0.8 kg. One monkey was replaced because his performance scores were too high to discriminate the effects of the study after a one-year-test, therefore the experimental group included only three monkeys.

Monkeys were individually housed under standard conditions (12-h light/dark cycle with light on from 07:00 to 19:00, humidity 60%, 21 ± 2°C). During the experimental period, monkeys were fed once per day, the normal regimen being twice daily, and were weighed once every two weeks. The experiments were conducted in accordance with the guidelines for the National Care and Use of Animals approved by the Chinese National Animal Research Authority.

Drugs

Drugs were purchased from the following suppliers: morphine hydrochloride, 10 mg/mL per ampule (Northeast Pharmaceutical Group Shenyang No.1 Pharmaceutical Co., Shenyang, China); apomorphine, sulpiride, SKF-83566, and SKF-38393 (Sigma, St. Louis, MO); bromocriptine

(Novartis Pharma Schweiz AG, Switzerland) and haloperidol (Shanghai Medical Co., Shanghai, China). Saline solutions (0.9% NaCl) of drugs were made fresh on the afternoon prior to the injection day and stored at 4°C overnight.

Apparatus

The WGTA was a wooden box (length 70 cm, width 45 cm, height 110 cm) with a small window for experimenter observation and a light (25 W) inside. A wooden gate behind the box could be lifted and lowered with a pulley by the experimenter. When the wooden gate was lifted, the monkey could see the experimenter who put one peanut into one of two wells (diameter 3.5 cm, the distance between the two wells was 8 cm) which were horizontally arranged on a wooden plate in front of the monkey. Immediately after the monkey saw the placement of the peanut, two white plastic panels (10 cm × 10 cm) were placed on the two wells to cover them and the gate was lowered to block the monkey's sight for a delay duration. After the delay, the gate was lifted and the wooden plate with the covered wells became visible to the monkey. The monkey was allowed to choose the peanut from one of the two covered wells with his hands.

Behavioral Training Procedures

Each monkey had ~1 000 training trials before the pharmacological experiments (30 trials for each workday). Each day, the peanuts were placed into the right or left well for 15 trials. The placement of the peanut was quasi-randomly arranged[16].

Five delay lengths (referred to as delays A–E) were semi-randomly distributed over the 30 trials in each session for each monkey: A = B×0 = 0 s, B = B×1 = B s, C = B×2 = 2B s, D = B×3 = 3B s, E = B×4 = 4B s. B was the delay time for each monkey according to his learning ability. For example, if monkey KunKun's delay time B = 5 s, he would be tested with the five delay lengths 0, 5, 10, 15 and 20 s (each had six trials, semi-randomly distributed over the 30 training trials).

An optimum B value was determined for each monkey as this is a necessary guideline for pharmacological tests in primates. A low B value, which indicates a short delay time, may lead to a high score in the delayed response, while a high B value may increase the difficulty of the task for the monkeys. To determine the optimal B delay length for each monkey, the value of B was increased from 0 seconds during the training process. After the monkey became familiar with the task, the B delay length was increased by 3–5 s if the monkey scored >93% correct responses (i.e. 28 correct choices out of 30 trials) in the first session. Then the delay length was further increased until the performance was stable at >93% correct responses at an optimum B-delay length for three consecutive days.

Only when monkeys reached this stable criterion was the pharmacological study started, and the effect of each drug was tested at all delay times (A–E) for each monkey. The average B-delay length for the monkeys was 11.3 ± 6.3 s at the beginning of the experiments, and 20.5 ± 6.1 s when the experiments ended.

Drug Administration and Behavioral Testing Procedures

A single-blind procedure was used during testing. The same experimenter gave the injections and performed the delayed-response experiments without knowing on which day and what compounds were given to the monkey.

On the first day of each test period, the monkeys were intra-muscularly (i.m.) injected with 0.9% saline (0.05 mL/kg body weight). After 30 min, the monkeys were tested in the delayed-response task. These responses were used as the baseline for each study and were subsequently marked as pre-treatment.

On the next (second) day, the monkeys were injected i.m. with different compounds (0.05 mL/kg body weight, according to the blind procedure) 30 min before the test. Compounds were administered either singly or in tandem with morphine. For co-administration, the two drugs were successively injected 30 min before the test. All compounds were administered according to this protocol except for bromocriptine because it is insoluble in water. One hour before the test, bromocriptine was placed into a piece of banana for oral administration, and for morphine co-administration the morphine was injected i.m. 30 min later. After 30 min, the monkey underwent 30 trials (five delay lengths, semi-randomly distributed), and the performance was indicated as: total correct responses/30 ×100%.

Follow-up tests using the delayed-response task were usually conducted on the next day to determine if the monkey's performance had recovered to its normal level. The monkeys were not given any injection before these measurements. These measurements were marked as post-treatment.

Typically, the monkey was injected with saline on Monday followed by the drugs on the next day. Then he was allowed to recover for a few days which meant he returned to a 93% correct response rate in the task before the next drug treatment. For some drugs with low doses and no effects, the next round of testing was conducted on Thursday following recovery.

During the testing period, the drug type or drug combination was randomly administered by changing the compounds, combinations and doses from week to week. This protocol helps to protect the animals from developing opiate dependence or tolerance. For most doses of the drugs, the experiments were repeated 2–5 times with random administration of each dose during the testing period.

Statistical Analysis

Data are expressed as the mean percentage of correct responses on each day (mean ± SEM). Differences between the drug treatments and pre-treatment were assessed with ANOVA, while a two-way ANOVA with repeated measures was used to analyze the difference between the co-treatment and the single treatment. Differences between treatments were considered significant when $P \leq 0.05$.

RESULTS

Effects of Single Morphine Treatment on Spatial Working Memory in Rhesus Monkeys

The monkeys showed relatively stable baselines in WGTA delayed response task before treatment with various drugs in different experimental paradigms (mean percentage of correct choice, 95.0%–96.7%).

Morphine at doses of 0.01, 0.1 and 0.2 mg/kg impaired spatial working memory in the delayed-response task, as reflected by a decrease in the percentage of correct choices made 30 min after the morphine injection when compared to the percentages scored on the day before the drug treatment (Table 1). However, lower doses of morphine (0.001 and 0.005 mg/kg) did not have a significant effect on working memory.

Morphine at high doses (0.1 and 0.2 mg/kg) changed two of the monkeys' (DeDe and LaLa) behaviors by exciting their mood and decreasing their movements (sedative behavior). Therefore, we chose two low doses (0.01 and 0.001 mg/kg) to test the effects of co-treatment on spatial working memory (Fig. 1A). Morphine at 0.01 mg/kg reduced working memory but had little effect on behavior, while the lower dose (0.001 mg/kg) had no effect on working memory or behavior.

Effects of Co-administration of Morphine and DA Receptor Agonists on Spatial Working Memory in Rhesus Monkeys

D1 Agonist SKF-38393

Treatment with the D1 agonist SKF-38393 alone did not affect the monkeys' spatial working memory (Table 1, Fig. 1A).

All three monkeys became somewhat excited after administration of high doses (0.02 and 0.04 mg/kg) of SKF-38393, as reflected by an increase in locomotor activity. However, they did not display any other serious changes in behavior. Nonetheless, the dose was not increased beyond 0.04 mg/kg and the SKF-38393 doses of 0.02 and 0.04 mg/kg were used in the morphine + SKF38393 co-treatment experiments.

SKF-38393 (0.02 and 0.04 mg/kg) + morphine (0.01 mg/kg) had no effect on memory, but ameliorated the impaired memory induced by morphine (0.01 mg/kg) alone. A slight increase in motor activity was observed after the co-treatment.

D1/D2 Agonist Apomorphine

Apomorphine, at the dose range used, did not have any effect on spatial working memory (Table 1, Fig. 1B), or on gross behaviors. Therefore, the medium (0.01 mg/kg) and the high doses (0.06 mg/kg) were continued in the morphine + apomorphine co-administration experiment.

Apomorphine + morphine had no effect on spatial working memory, but improved the spatial working memory deficits induced by 0.01 mg/kg morphine alone (Fig. 1B).

D2 Agonist Bromocriptine

Bromocriptine treatment decreased the working memory (main effect of drug between pre-treatment and treatment, $F(1,4) = 206.7$, $P < 0.001$; within effect of drug, $F(4,16) = 9.94$, $P < 0.001$; interaction drug × treatment, $F(4,16) = 6.36$, $P = 0.003$).

A single dose of bromocriptine (1 mg/kg) impaired spatial working memory 1 h after oral administration (Table 1, Fig. 1C). The monkeys made a lower percentage of correct choices compared to their pre- and post-treatment scores.

Table 1. Effect of morphine and dopaminergic compounds on WGTA spatial working memory in rhesus monkeys

Drugs (mg/kg)	*Versus* pre-treatment (*P* value)	*Versus* post-treatment (*P* value)	*Versus* drug treatment alone (*P* value)	*Versus* morphine treatment alone (*P* value)	Behavior (movement)
Morphine 0.001	-	-	-	-	-
Morphine 0.005	-	-	-	-	-
Morphine 0.01	↓ (0.007)	↓ (0.006)	-	-	-
Morphine 0.1	↓ (0.042)	↓ (0.026)	-	-	↓
Morphine 0.2	↓ (<0.001)	↓ (<0.001)	-	-	↓
DA agonists					
D1 agonist					
SKF-38393 0.001	-	-	-	-	-
SKF-38393 0.01	-	-	-	-	-
SKF-38393 0.02	-	-	-	-	↑
SKF-38393 0.04	-	-	-	-	↑
Morphine 0.01 + SKF-38393 0.02	-	-	-	↑ (0.019)	↑
Morphine 0.01 + SKF-38393 0.04	-	-	-	↑ (0.019)	↑
D1/D2 agonist					
Apomorphine (Apo) 0.005	-	-	-	-	-
Apo 0.01	-	-	-	-	-
Apo 0.04	-	-	-	-	-
Apo 0.06	-	-	-	-	-
Morphine 0.001 + Apo 0.01	-	-	-	-	-
Morphine 0.001 + Apo 0.06	-	-	-	-	-
Morphine 0.01 + Apo 0.01	-	-	-	↑ (0.019)	-
Morphine 0.01 + Apo 0.06	-	-	-	↑ (0.022)	-
D2 agonist					
Bromocriptine (Bro) 1	↓ (0.001)	↓ (0.001)	-	-	
Morphine 0.001 + Bro 1	↓ (0.016)	-	-	-	
Morphine 0.01 + Bro 1	-	-	↑ (0.038)	↑ (0.019)	
DA antagonist					
D1 antagonist					
SKF-83566 0.001	-	-	-	-	-
SKF-83566 0.01	-	-	-	-	-
SKF-83566 0.02	↓ (<0.001)	↓ (<0.001)		-	- -
SKF-83566 0.04	-	-	-	-	-
SKF-83566 0.06	-	-	-	-	↑
Morphine 0.001 + SKF-83566 0.01	-	-	-	-	-
Morphine 0.001 + SKF-83566 0.02	-	-	-	-	-
Morphine 0.001 + SKF-83566 0.06	-	-	-	-	↑

(To be continued)

(Continued)

Morphine 0.01 + SKF83566 0.01	↓ (0.003)	↓ (0.003)	-	↑ (0.058)	-
Morphine 0.01 + SKF 83566 0.02	↓ (0.05)	↓ (0.05)	↑ (0.008)	↑ (0.048)	-
Morphine 0.01 + SKF83566 0.06	↓ (0.001)	↓ (<0.001)	↓ (0.019)	-	↑
DA 2 antagonist					
Haloperidol (Hal) 0.005	-	-	-	-	-
Hal 0.01	↓ (0.053)	↓ (0.053)	-	-	↓
Morphine 0.001 + Hal 0.01	-	-	-	-	-
Morphine 0.01 + Hal 0.005	-	-	-	-	-
Morphine 0.01 + Hal 0.01	-	-	↑ (0.047)	-	-
DA2 antagonist					
Sulpiride (Sul) 0.001	↓ (0.003)	↓ (0.003)	-	-	-
Sul 0.01	↓ (<0.001)	↓ (<0.001)	-	-	-
Sul 0.1	↓ (0.014)	↓ (0.014)	-	-	-
Morphine 0.001 + Sul 0.01	-	-	↑ (<0.001)	-	-
Morphine 0.01 + Sul 0.001	-	-	- ↑ (0.057)	↑ (0.02)	-
Morphine 0.01 + Sul 0.01	↓ (0.003)	↓ (0.003)	-	↑ (0.057)	-

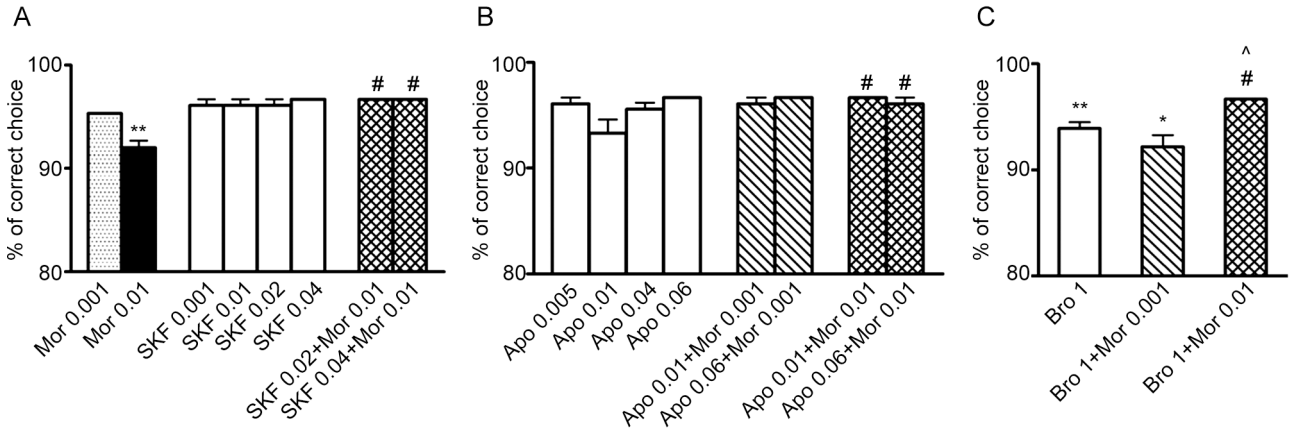

Fig. 1. Effects of single DA agonist treatment and co-administration of morphine (Mor) and the DA agonists SKF-38393 (A), apomorphine (B, Apo), and bromocriptine (C, Bro) on spatial working memory in rhesus monkeys. $^{**}P$ <0.01, $^{*}P$ <0.05 *versus* before drug treatment. $^{\#}P$ <0.05 *versus* morphine treatment alone. $^{\wedge}P$ <0.05 *versus* bromocriptine treatment alone.

Besides, co-treatment with bromocriptine (1 mg/kg) and morphine (0.001 mg/kg) impaired working memory when compared with the performance on the day before treatment, while bromocriptine (1 mg/kg) + morphine (0.01 mg/kg) restored the impairment produced by a single treatment with bromocriptine or morphine at the same doses.

Bromocriptine treatment alone and the co-treatments had similar effects on the monkeys' behaviors in that they slightly reduced their interest in finding food, but had no effect on their behavior.

Effects of Co-administration of Morphine and DA Receptor Antagonists on Spatial Working Memory in Rhesus Monkeys

D1 Antagonist SKF-83566

SKF83566 treatment decreased the working memory (main effect of drug between pre-treatment and treatment, $F(2,6) = 57.4$, $P < 0.001$; within effect of drug, $F(12,72) = 8.84$, $P < 0.001$; interaction drug × treatment, $F(24,72) = 4.76$, $P < 0.001$).

The D1 receptor antagonist SKF-83566 impaired spatial working memory in the rhesus monkey at the middle dose only (0.02 mg/kg) (Table 1, Fig. 2A).

Co-injection of SKF-83566 (0.01, 0.02, and 0.06 mg/kg) and morphine had no effect on the working memory at morphine dose of 0.001 mg/kg, but impaired it at 0.01 mg/kg, compared with the pre- and post-treatment controls. However, SKF-83566 (0.02 mg/kg) + morphine (0.01 mg/kg) co-treatment, and to a lesser extent SKF-83566 (0.01 mg/kg) + morphine (0.01 mg/kg), ameliorated the morphine effect on memory. In addition, SKF-83566 (0.02 mg/kg) + morphine (0.01 mg/kg) co-treatment also ameliorated the impaired working memory induced by 0.02 mg/kg SKF-83566 alone, while SKF-83566 (0.06 mg/kg) + morphine (0.01 mg/kg) displayed a decreased memory score when compared with single administration of SKF83566 (0.06 mg/kg).

Two monkeys (DeDe and LaLa) showed increased locomotor activity after a high dose of SKF-83566 (0.06 mg/kg). They moved more in the cage and became excited. Therefore, the dose of SKF-83566 was not increased beyond 0.06 mg/kg. All three monkeys became excited after co-treatment with SKF-83566 (0.06 mg/kg) and morphine but were able to finish the delayed-response task.

D2 Antagonist Haloperidol

Administration of haloperidol at 0.01, but not at 0.005 mg/kg, impaired spatial working memory. Interestingly, when haloperidol (0.01 mg/kg) was co-injected with morphine (0.001 and 0.01 mg/kg), no impairment in memory occurred, compared with the pre-treatment scores (Table 1, Fig. 2B). Haloperidol (0.01 mg/kg) + morphine (0.01 mg/kg) ameliorated the memory deficits induced by haloperidol alone.

Haloperidol at 0.01 mg/kg decreased the motor activity of two monkeys (DeDe and LaLa), as reflected by slow movements and abnormal joint flexibility. Haloperidol (0.01 mg/kg) + morphine (0.01 mg/kg) reduced the slow-moving behavior in one monkey (DeDe), however another monkey (LaLa) still displayed low locomotor activity. In addition, this co-administration induced slight motor impairment in the third monkey (KunKun).

D2 Antagonist Sulpiride

Sulpiride treatment decreased the working memory (main effect of drug between pre-treatment and treatment, $F(2,6) = 23.5$, $P = 0.001$; within effect of drug, $F(7,42) = 19.65$, $P < 0.001$; interaction drug × treatment, $F(14,42) = 5.4$, $P < 0.001$).

In detail, single treatment with sulpiride (0.001, 0.01, and 0.1 mg/kg) impaired spatial working memory without causing a change in behavior (Table 1, Fig. 2C). Sulpiride

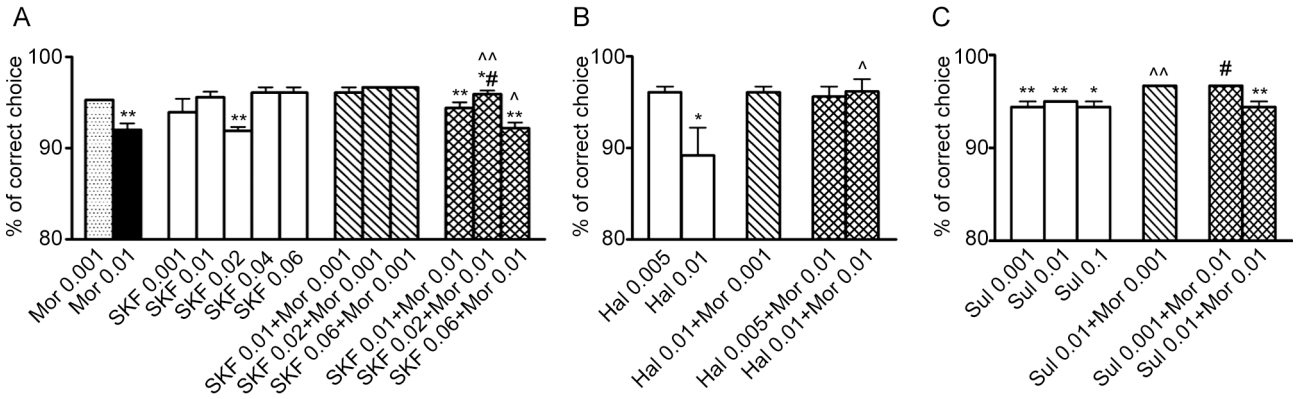

Fig. 2. Effects of single DA antagonist treatment and co-administration of morphine (Mor) and the DA antagonists SKF-83566 (A), haloperidol (B, Hal), and sulpiride (C, Sul) on spatial working memory in rhesus monkeys. **P <0.01, *P <0.05 *versus* before drug treatment. $^{\#}P$ <0.05 *versus* morphine treatment alone. ^^P <0.01, ^P <0.05 *versus* single DA antagonist treatment. Note that morphine (0.01 mg/kg) + SKF83566 (0.06 mg/kg) impaired working memory compared with SKF83566 (0.06 mg/kg) alone.

(0.01 mg/kg) + morphine (0.001 mg/kg) reversed the impairment induced by sulpiride (0.01) alone.

Co-injection of sulpiride (0.001 mg/kg) and morphine (0.01 mg/kg) reversed the impairment produced by single treatment with morphine alone. Sulpiride (0.01 mg/kg) + morphine (0.01 mg/kg) co-treatments caused a deficit in spatial working memory. No changes in behavior were found after the drug treatment.

DISCUSSION

In the current study, the spatial working memory of rhesus monkeys was tested using the WGTA delayed-response task. We found that morphine impaired the spatial working memory of the monkeys, but it depended on the dose. When the monkeys were co-treated with morphine and the DA receptor agonists SKF-38393, apomorphine and bromocriptine, the memory impairment caused by individual treatment was reversed in most instances. Similarly, when the monkeys were co-treated with the DA receptor antagonists SKF-83566, haloperidol and sulpiride, reversal of memory impairment was found, and it also depended on the drug and the dose.

The present finding that morphine caused an impairment in working memory is consistent with previous studies, including ours and those by other researchers[17,18]. The low dose of morphine (0.001 mg/kg) had no effect on working memory in any trials (5 separate injections). However, high doses of morphine caused sedation in addition to impairing working memory. This sedation may be attributed to the increases in DA levels produced by morphine administration, given that DA acts as a sedative.

DA activity in the PFC plays an important role in working memory. For example, Watanabe and Kodama found, using *in vivo* microdialysis, a significant increase in the extracellular DA levels in the dorsolateral PFC in monkeys performing a delayed-alternation task, a typical working memory paradigm[19]. Therefore changes in PFC DA levels are thought to influence working memory. The findings presented here showed that single treatment with bromocriptine, a D2 receptor agonist, impaired spatial working memory in rhesus monkeys.

On the other hand, the D1 receptor agonist SKF-38393 and apomorphine, a D1 and D2 receptor agonist, did not affect working memory in the monkeys.

Cai and Arnsten (1997)[20] found that low doses of the D1 agonists A77636 and SKF-81297 improved working memory in aging monkeys, while high doses of the same drugs impaired it. Furthermore, moderate doses of D1 agonists were shown to improve working memory by modulating the activity of the excitatory efferents of the PFC and by controlling inhibitory neuronal activity. In the current study, the D1 receptor agonist SKF-38393 did not have similar effects on the spatial working memory in the monkeys. The following are potential explanations: (1) the doses of SKF-38393 used here were too low. The monkey became impatient after injections with SKF-38393 at 0.02 mg/kg, so the doses were not increased to avoid any harmful effects to the animals; and (2) the number of correct scores by the monkeys was high before drug treatment (28–29 correct out of 30) which may have caused ceiling effects. Therefore, any memory improvement induced by treatment with the D1 agonist might not have been detectable in the current paradigm.

Apomorphine is known to stimulate autoreceptors which signal negative feedback that may inhibit the synthesis and release of DA. However, apomorphine also has a high binding-affinity for D2 receptors, which suggests that D2 receptor may play a role in the similar working memory impairment found with apomorphine and bromocriptine treatment. In the current study, a slight impairment of working memory was found in apomorphine at 0.01 mg/kg, but the difference was not significant.

In addition to the involvement of D1 receptors in working memory, D2 receptors have been found to act on the functional circuitry of working memory[21]. In the current study, the D2 receptor agonist bromocriptine impaired working memory, and so did the D2 receptor antagonists haloperidol and sulpiride, which caused dose-dependent deficits. Furthermore, the D1 receptor antagonist SKF-83566 had a similar effect, albeit only at a limited dose (0.02 mg/kg), suggesting that the blocking or modulation of D1 and D2 receptors may decrease working memory.

Interestingly, the co-administration of morphine and DA agonists restored the reduced working memory caused by morphine (0.01 mg/kg but not 0.001 mg/kg) or D2 agonist, respectively. Presumably, the morphine acts to increase DA levels in the synapse, which may further stimulate the D1 or D2 receptors, resulting in an improvement in working memory.

Evidence shows that there are interactions between the D1 receptor and the μ-opioid receptor. O'Dowd found that the μ-opioid receptor co-localizes with D1 receptors in neurons of the cortex and caudate nucleus. Further they found that the μ-opioid receptor forms a hetero-oligomer complex with the D1 receptor, and neurons expressing both receptors occur in the cortex and striatum[22]. A previous study also showed reduced μ-opioid receptor expression in striatal patches from D1 receptor-null mice[23]. This might explain why SKF-38393 (0.02 and 0.04 mg/kg) + morphine (0.01 mg/kg) ameliorated the impaired memory induced by morphine (0.01 mg/kg) administration alone.

Conversely, the co-administration of the D1 antagonist SKF-83566 (0.01 mg/kg) and morphine (0.01 mg/kg) impaired memory compared with pre-treatment. However, this co-treatment still ameliorated the memory reduction induced by a single morphine treatment, while a higher dose of SKF-83566 (0.06 mg/kg) co-aministered with morphine (0.01 mg/kg) had more serious negative effects on working memory than SKF-83566 (0.06 mg/kg) alone. No impairment was found when co-administered with morphine at a low dose (0.001 mg/kg).

Unlike the co-treatment with D1 receptor antagonist and morphine, the D2 receptor antagonists (haloperidol and sulpiride) and morphine co-administrations had an effect similar to the D2 receptor agonist and morphine co-treatment, in that they ameliorated the impaired memory produced by individual treatment with morphine or D2 antagonists. Similar reverse effects were also found in the monkeys' behavior. For example, co-treatment with morphine and haloperidol ameliorated the low locomotor activity induced by haloperidol alone. This suggested that the behavioral change might parallel the change in memory with regard to injection of D2 antagonists.

Although the monkeys' behavior paralleled their memory scores with respect to morphine and haloperidol, not all drug combinations produced similar parallels between behavior and memory. This highlights one disadvantage of using the classical WGTA method to test spatial working memory in primates under various drug paradigms; the WGTA cannot account for the possible effects of compounds on movement, attention and appetite. These types of effects could not be excluded from this study, which limited the doses of the compounds to low and safe levels to protect the monkeys from any extraneous effects of the drugs, especially since some of the higher doses began to change their behaviors.

In summary, general co-administration of morphine antagonized the effects of D1 and D2 compounds in a specific dose pattern. Morphine at 0.01 mg/kg ameliorated the effects of bromocriptine, SKF-83566, and haloperidol on working memory in the rhesus monkey. However, morphine co-treatment with high doses of SKF-83566 and sulpiride impaired working memory. Nonetheless, an overall interpretation of the relationship between morphine and the dopaminergic-like compounds investigated in this study further illustrates the importance of the tightly-regulated neurochemical balance within the PFC for proper maintenance of executive functions, like spatial working memory, in monkeys and humans.

Furthermore, the close evolutionary ties between rhesus monkeys and humans suggest that the findings here may also provide insights into how the relationships between opiate and dopaminergic neurotransmitters might induce deterioration, leading potentially to human diseases and/or addiction. The findings that morphine impairs working memory, but that this can be alleviated by the correct combination of dopaminergic agonists or antagonists may also provide support for the use of morphine and/or a combinatory therapy for humans in a clinical setting given that morphine is already commonly used in the hospital and that further combinations may be developed in the future to provide better sedative effects and/or possible therapies for human addiction or neurological disorders.

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