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Muscarinic receptors in the nucleus accumbens shell play different roles in context-induced or morphine-challenged expression of behavioral sensitization in rats

Xinhe Liu, Lin Tian, Ruisi Cui, Heng Ruan, Xinwang Li*

Beijing Key Laboratory of Learning and Cognition, Department of Psychology, Capital Normal University, Beijing, PR China

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ABSTRACT

Both drug-related cues and drug priming are the main factors that induce relapse of drug addiction. Previous research has reported that blockade of the muscarinic receptors could significantly depress addictive behavior, suggesting that the muscarinic receptors might be involved in drug use and relapse behavior. The nucleus accumbens (NAc), especially the shell of the NAc, where the muscarinic receptors are expressed, is critical for craving and relapse. This study investigated the effects of microinfusion of the muscarinic receptor antagonist scopolamine into the NAc shell on context- and morphine-induced expression of behavioral sensitization. Behavioral sensitization was established by exposure to 5 mg/kg morphine once daily for five consecutive days. Expression of behavioral sensitization was induced by saline challenge or 5 mg/kg morphine challenge. The results showed that: (a) the muscarinic receptor antagonist scopolamine (10.8 μg/rat) microinjected into the NAc shell blocked expression of conditional sensitization; (b) acetylcholinesterase inhibitor huperzine-A (0.5 and 0.1 μg/rat), but not scopolamine (10.8 μg/rat), microinjected into the NAc shell blocked morphine-induced expression of sensitization; and (c) pre-infusion of scopolamine (10.8 μg/rat) reversed the inhibitory effect of huperzine-A (0.5 μg/rat) on morphine-induced sensitization. Our findings suggest that muscarinic receptors in the NAc shell play different roles in context-induced and morphine-challenged expression of behavioral sensitization.

1. Introduction

Repeated exposure to drugs of abuse increases the rewarding and stimulant properties in animal experiments, which is known as behavioral sensitization (Castellucci and Kandel, 1976; Robinson and Berridge, 2001, 2008). Expression of behavioral sensitization can be generated not only by the drug itself but also by related cues, which is called conditional sensitization (Barr et al., 1983; Robinson et al., 1998). Thus, behavioral sensitization is not purely a consequence of neuroadaptations induced by drugs of abuse; learning and memory contribute to this process as well (Hyman, 2005; Hyman et al., 2006).

The high rate of relapse is the main obstacle in drug abuse treatment. Related conditioned cues and drug priming are the two factors that induce relapse. However, a growing body of research has indicated that they do not share the same neural mechanisms (Anagnostaras et al., 2002; Itzhak and Martin, 2000; Wei and Li, 2014).

Nucleus accumbens (NAc), including the shell of the NAc (NAcS), is mainly composed of cholinergic interneurons and medium spiny neurons which belong to gamma-aminobutyric acid system ((Calabresi et al., 2000; Meredith et al., 1992; Zahm, 1999). It receives dopaminergic projection fibers from ventral tegmental area and glutamic projection fibers from prefrontal cortex and amygdala (Yager et al., 2015). Both conditioned cues and drug challenge reinstatement have been found to facilitate changes in molecular mechanisms in the NAcS, suggesting that the NAcS is related to these two factors which induced relapse (Z. Liu et al., 2012; Lv et al., 2015).

Previous study indicated that there was a direct correlation existed between the percent of cholinergic interneurons that were activated in the NAcS and the amount of self-administered cocaine (Berlanga et al., 2003). The administration of non-selective muscarinic receptor (including M_{1-5} subtypes) antagonists in the NAcS, like scopolamine, had inhibitory effects on cocaine challenged expression of self-administration (SA) and conditioned place preference (Shinohara et al., 2014; Yee et al., 2011).

It was also known that scopolamine could inhibit different kinds of learning and memory (Klinkenberg and Blokland, 2010) and M_{1-4} receptors were related to learning and memory (Wall and Messier, 2002; Zheng et al., 2012; Poulin et al., 2010; Bubser et al., 2014). Moreover,

* Corresponding author.
E-mail address: lixw701@sina.cn (X. Li).

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muscarinic M_1 receptor antagonist biperdien has been reported to induce addiction in some clinical cases (Espi Martinez et al., 2012). These results suggest the tangle role of muscarinic receptors in drug abuse.

We proposed that scopolamine injection into the NAcS was probably related to conditional sensitization induced by morphine and the acetylcholinesterase inhibitors, such as physostigmine and donepezil, depressed opioids-induced conditioned place preference, behavioral sensitization and SA (Hikida et al., 2003; Li et al., 2010; H. Liu et al., 2012; Zhou et al., 2007) via blocking acetylcholine destruction and then activating muscarinic receptors.

This study aims to examine the role of muscarinic receptors in the NAcS in regard to context and drug challenged expression of behavioral sensitization induced by morphine. Additionally, an effort is made to investigate the relationship between muscarinic receptors and the effect of microinfusion of the acetylcholinesterase inhibitor huperzine-A in the NAcS on the expression of sensitization challenged by morphine. Scopolamine and huperzine-A were used as pharmacological tools.

2. Materials and methods

2.1. Animals

Eight-week-old male Wistar rats weighing 280 − 340 g were obtained from the Academy of Military Sciences, Beijing, P. R. China. After arriving in our laboratory, they adapted to the new environment for at least one week. Before surgery, rats were housed five per cage in an animal room with the temperature kept between 21 ℃ − 25 ℃ in a 12 h light / 12 h dark cycle (lights on from 7:00 a.m. to 7:00 p.m.). All experiments were performed form 10 a.m. to 5 p.m. during the light cycle. Over the entire experimental period, animals had free access to water and food. The treatment of rats was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996) and approved by the Institutional Animal Care and Use Committee, Capital Normal University.

2.2. Drugs

Sterile saline was prepared in our laboratory. Morphine hydrochloride (Shenyang First Pharmaceutical Factory) and chloride scopolamine (SIGMA) were dissolved in sterile saline. Huperzine-A (Jiangxi Herbal Tiangong Company) was dissolved in phosphate buffer solution (PBS, pH = 6.8); then, the pH of the solution was adjusted to 7.0 − 7.4 with the NaOH solution and diluted with saline. All drugs were freshly prepared before they were used. Three doses of scopolamine (3.6, 7.2, and 10.8 μg/rat) and two doses of huperzine-A (0.1 and 0.5 μg/rat) were chosen based on our preliminary experiments. All doses of scopolamine and huperzine-A did not significantly alter animals' locomotion (data not shown).

2.3. Implant surgery and bilateral microinfusion

Before surgery, rats were entirely anesthetized by pentobarbital sodium (50 mg/kg) and positioned in the standard stereotaxic apparatus with their head fixed. Then, rats were incised and their skull was cleaned and disinfected. Two holes were drilled by dental aiguille, and bilateral stainless steel guide cannula (35 gauge, outside diameter = 0.64 mm, inside diameter = 0.45 mm) were slowly propelled to reach the NAcS according to the rat brain atlas of Paxinos and Watson (AP = + 1.7 mm, ML = ± 0.9 mm, DV = − 6.8 mm). Three surgical screws were turned into the skull without penetrating it, and dental cement was used to fix the guide cannula and protect the wound. After surgery, rats were allowed to recover for at least one week before formal experiment.

Before microinfusion, rats were handled for 1 min to release their stress. Microinfusion was delivered by two microsyringes (RWD company, Shenzhen, 2 μl) which were connected to the bilateral injective needle with polyethylene pipes. The obstructer was removed from the guide cannula, and then, the injective needle was carefully inserted. The needle extended 0.5 mm beyond the tip of the guide cannula to reach the NAcS. Scopolamine, huperzine-A or saline was injected into the NAcS at a volume of 1 μl (0.5 μl per side) over 1 min. After the infusion, the syringe needle was kept in for another 1 min to allow the solution to totally diffuse in the target brain area. In this procedure, the rat's body was softly hold by experimenter and a cotton swab was used to tickle it's whisker to distract their attention. Then, the needle was carefully pulled out and the obstructer was inserted into the cannula.

2.4. Apparatus

Eight locomotor activity (LA) chambers (Mobile Datum Information Technology Co. Ltd., Shanghai) were made of black opaque plexiglass (40 cm × 40 cm × 50 cm, length × board × height). Each LA chamber was located in a dim, sound-proof box that was linked to the PC. A ventilating fan that provided background noise was mounted on the back wall of the chamber. An infrared sensor camera was located on the chamber's ceiling to film the rat's activity. The video was recorded on the PC and analyzed by animal locomotive activity analysis software (Institute of Psychology, Chinese Academy of Sciences, Beijing, China). Locomotion in this study was defined as the horizontal distance that rats traveled. The LA chamber was cleaned with 70% ethanol before each experiment to prevent the effect of olfactory cues.

2.5. Behavioral sensitization protocol

Before receiving the formal experimental treatment, all rats had habituated to the LA chambers for five consecutive days and were assigned to each group to match their last two days' average locomotion. On day 1, rats conducted a pretest of baseline locomotion to determine whether different motor abilities existed between groups. On days 2 – 6 (the development stage), rats were intraperitoneal injected with saline (Sal group) or morphine (5 mg/kg, Mor 1 − 4 groups in experiments 1 and 2, or Mor 1 − 3 groups in experiment 3) daily. On days 7 – 13 (the withdrawal period), rats were housed in their home cages and did not receive any treatment. On day 14 (the challenge session), rats received different treatments as described below (Table 1) according to the purpose of each experiment.

Experiment 1. Rats (n = 34) were divided into five groups and tested for the effect of scopolamine in the NAcS on context-induced expression of sensitization. Animals received microinfusion of saline (Sal and Mor1 group) or scopolamine (3.6, 7.2, and 10.8 μg/rat, Mor 2 − 4 groups) first and then were put back into the LA chamber, which was paired with morphine in the developmental stage, and received an intraperitoneal injection of saline 5 min later. Their locomotion was recorded for 1 h.

Experiment 2. Rats (n = 38) were divided into five groups and tested for the effects of scopolamine and huperzine-A on morphine-challenged expression of sensitization. Rats received each microinfusion of saline (Sal and Mor 1 group) / scopolamine (10.8 μg/rat based on experiment 1, Mor 2 group) /huperzine-A (Mor 3 and 4 groups) first, followed by a morphine (5 mg/kg) challenge 5 min later, and their locomotion was recorded for 2 h.

Experiment 3. Rats (n = 30) were divided into four groups and tested for the effects of co-microinfusion of scopolamine and huperzine-A on morphine-challenged expression of sensitization. The preferred doses of scopolamine (10.8 μg/rat) and huperzine-A (0.5 μg/rat) were chosen based on experiment 1 and experiment 2. Rats received each microinfusion of saline + saline (Sal and Mor 1 group)/saline + huperzine-A (Mor 3 group)/scopolamine + huperzine-A (Mor 4 group) first (the interval between the two microinfusions was 5 min), followed by a morphine challenge after another 5 min. Their locomotion was recorded for 2 h.