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Research Paper

The α_{2A}-adrenoceptor agonist guanfacine improves spatial learning but not fear conditioning in rats

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Abstract: It is known that stimulation of the α_{2A}-adrenoceptors (α_{2A}-ARs) by the selective α_{2A}-AR agonist guanfacine produces an important and beneficial influence on prefrontal cortical (PFC) cognitive functions such as spatial working memory and selective attention. However, it is unclear whether stimulation of the α_{2A}-ARs has a similar effect on fear conditioning that involves the amygdala and hippocampus. Here, we show that systemically administered guanfacine significantly enhances spatial learning of rats in the Lashley maze: compared with controls, the rats treated with guanfacine required significantly fewer trials and made significantly fewer errors to reach learning criterion. However, guanfacine produced no effect on acquisition of contextual and auditory fear memories. The present study suggests that beneficial effect of α_{2A}-AR stimulation is task-dependent: guanfacine improves spatial learning but not fear conditioning.

Key words: guanfacine; α_{2A}-adrenoceptors; spatial learning; fear conditioning; rats

α_{2A} 肾上腺素受体激动剂 guanfacine 增强大鼠空间学习能力而不影响条件性恐惧记忆

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摘 要：α_{2A} 肾上腺素受体选择性激动剂 guanfacine 对空间工作记忆和选择性注意等前额叶皮层认知功能有重要的、有益的影响。然而，激活 α_{2A} 受体对于依赖杏仁体和海马回路的恐惧记忆条件反射是否有影响，目前尚不清楚。本研究结果显示，全身给予 guanfacine 显著提高大鼠在 Lashley 迷宫中的空间学习能力：guanfacine 组大鼠达到学会标准所需要的训练次数和所犯错误的次数显著少于生理盐水对照组大鼠。然而，guanfacine 组大鼠场景和声音恐惧记忆的获得 / 巩固与对照组大鼠相比没有显著差异。结果提示，刺激 α_{2A} 受体产生的有益效应是任务依赖的：guanfacine 改善空间学习能力，但不影响恐惧记忆的获得 / 巩固。

关键词：guanfacine；α_{2A} 肾上腺素受体；空间学习；条件性恐惧记忆；大鼠
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The prefrontal cortex (PFC) is essential for cognitive functions such as working memory[1-4], attention regulation[5,6] and response inhibition[6,7]. Animals or humans with lesions to the PFC exhibit disorganized behaviors like poor working memory, distractibility, impulsivity and hyperactivity[6,8].

Extensive evidence suggests that norepinephrine (NE) projection from the locus coeruleus (LC) exerts, through actions at the postsynaptic α_{2A}-adrenceptors (α_{2A}-ARs),

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an important and beneficial influence on PFC cognitive functions[9-12]. For example, systemically administered clonidine, an α_2-AR agonist, becomes more potent and more effective when the presynaptic NE terminals are destroyed by 6-OHDA or NE is depleted by reserpine[9,13]. It has been reported that stimulation of prefrontal cortical α_2-ARs by the α_2-AR agonist clonidine or guanfacine enhances PFC neuronal activity that is related to working memory[14,15], while blockade of prefrontal cortical α_2-ARs inhibits working memory-related PFC neuronal activity[14,15]. Consistently, stimulation of prefrontal cortical α_2-ARs by clonidine or guanfacine enhances working memory performance in rats and monkeys, while inhibition of α_2-ARs has an opposite effect[11,12].

Guanfacine is the most selective α_{2A}-AR agonist available[16] and is effective in enhancing prefrontal cortical functions in mice, rats, monkeys and humans when administered systemically[10,17-21]. It has been reported that guanfacine is effective for clinical treatment of human psychiatric disorders that involve PFC disfunction, such as schizophrenia[22,23], attention deficit hyperactivity disorder (ADHD)[11,12,24-27] and post-traumatic stress disorder (PTSD)[28,29]. Thus, guanfacine is a potential drug for clinical application with little side-effect[11].

It is well established that acquisition and consolidation of emotional memories, such as fear responses, involve the amygdala and hippocampus[30-32]. It is reported that the PFC is also involved in formation of fear memory, especially contextual fear memory (CFM)[33]. However, it is unknown if stimulation of the α_{2A}-ARs would also produce a beneficial effect on fear conditioning. This question is clinically important because it is not expected that fear memory is enhanced when prefrontal cortical cognitive functions are improved following guanfacine treatment.

1 MATERIALS AND METHODS

1.1 *Subjects*

Sprague-Dawley rats, 6-week old, were purchased from the Animal Center of Experimental Animals, Shanghai Medical School, Fudan University, China. Rats were housed 1-2 per cage at constant temperature (23±1) ºC, with light-controlled vivarium (12 h dark: 12 h light cycles with the lights on at 08:00 am). Food and water were available *ad libitum*. All experimental protocols involving the use of animals were in compliance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (1996). All efforts were made to minimize the number of animals used and their suffering. This study was also approved by the Ethical Committee of Animal Experiments in Institute of Neurobiology, Fudan University.

1.2 *Guanfacine administration*

Guanfacine hydrochloride was purchased from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). It was dissolved in sterile saline freshly each day before administration. Rats received muscular injection of guanfacine (1 mg/kg body weight), or sterile saline (equal volume) 10 min before daily training. The 1 mg/kg body weight of guanfacine has been reported to be effective to enhance spatial working memory in mice[34].

1.3 *Lashley maze learning*

Lashley maze is a classical learning paradigm for rats. It contains sul-de-sacs (dead ends) and T-choices (Fig.1). In order to adapt to the maze, the animals were exposed to the maze and freely explored food rewards randomly placed in the maze. For formal training, a rat was placed into the

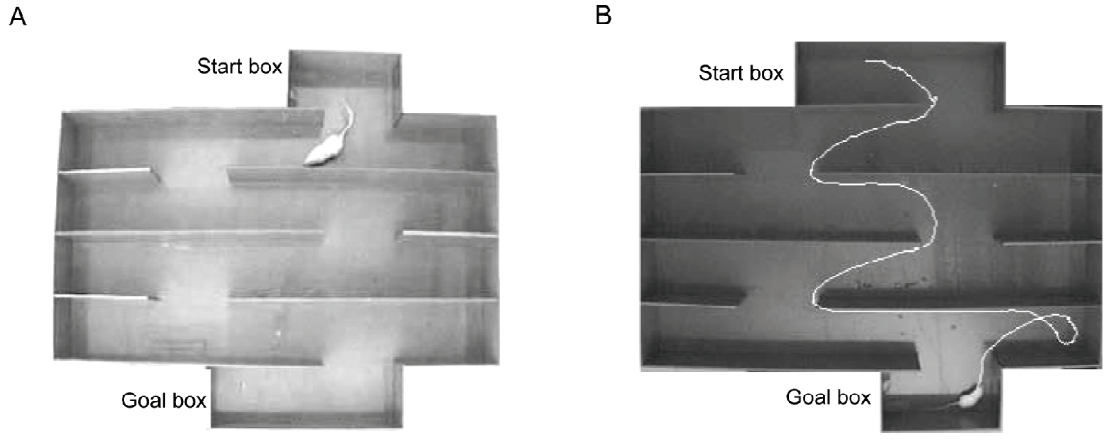

Fig. 1. Spatial learning in Lashley maze. *A*: The electronic photo and schematic drawing of Lashley maze. *B*: Running trace of a well-trained rat in the maze.

start box. The rat was required to run, within 5 min, from the start box to the goal box, where a food reward was available. If the animal failed to do so, it was removed from the maze and returned to home cage. Each animal was given two trials a day (60 min interval between two trials) for consecutive 6 d. In order to obtain the reward placed in the goal box, the rat had to avoid entering a sul-de-sac and remember correct routine to reach the goal box. Entries to a sul-de-sac or retracing were calculated as an error. Number of errors less than two in two consecutive trials was defined as learning criterion. The exploring pathway of each animal in the maze was recorded using a video camera.

1.4 *Fear conditioning*

Fear conditioning was carried out in a chamber (36 cm×23 cm×18 cm) made of transparent plexiglass (San Diego Instruments, USA). The chamber was dimly illuminated and the floor of the chamber consisted of 14 stainless steel rods (0.5 cm in diameter, spaced 1.2 cm apart), through which an electric shock to foot was delivered by a shock generator (ShockStim, San Diego Instruments, USA). The photo beam of the chamber, which was linked to a computer to make the assessment of animal's freezing response, automatically monitored the behavior of the rats, with a sampling rat of 3 times per 2 s. Freezing behavior was defined as the absence of any invisible movement except respiration[35]. The number of freezing response was summed and converted to a percentage of total observation duration. This freezing score was used as a measure for fear memory.

For conditioning, a rat was placed in chamber A and allowed to explore it for 2 min before the onset of an auditory cue, which lasted 30 s at 2 200 Hz and 96 dB (conditioned stimulus, CS). The last 1 s of the CS was paired with a foot shock (1 mA; unconditioned stimulus, US). Two CS-US pairings were given, with an interval of 30 s. The animal was allowed to stay in the chamber for an additional 30 s. Assessment of freezing response during this 30-second period was defined as "immediate test". Then, the animal was returned to home cage.

Memory retention was tested 24 h after conditioning ("24-hour test"). CFM was tested by placing the animal in chamber A, where it had been trained, for 2 min to assess freezing behavior of the animal. For the assessment of auditory fear memory (AFM), the animal was placed in a novel chamber (chamber B) for 2 min (pre-CS test) and then exposed to the CS for 2 min (CS test).

1.5 *Statistical analysis*

Data were analyzed using one-way analysis of variance (ANOVA). A probability level of less than 0.05 was accepted as statistical significance. All statistical analysis was conducted in STATISTICA (StatSoft, USA). All data in the text and figures were expressed as means±SEM.

2 RESULTS

2.1 Guanfacine improves spatial learning in Lashley maze

Guanfacine or saline was systemically administered 10 min before daily training. As shown in Fig.2, rats treated with saline needed (8.14±0.55) trials and made (66.57±4.23) errors to reach learning criterion, whereas rats treated with guanfacine needed (6.57±0.30) trials and made (52.71±4.31) errors to reach learning criterion [for trials to criterion: $F_{(1,12)}$=5.260, P=0.041, Fig.2*A*; for errors to criterion: $F_{(1,12)}$=6.529, P=0.028, Fig.2*B*]. This result indicates that stimulation of the α_{2A}-ARs by guanfacine produces a beneficial effect on spatial learning ability of rats in the Lashley

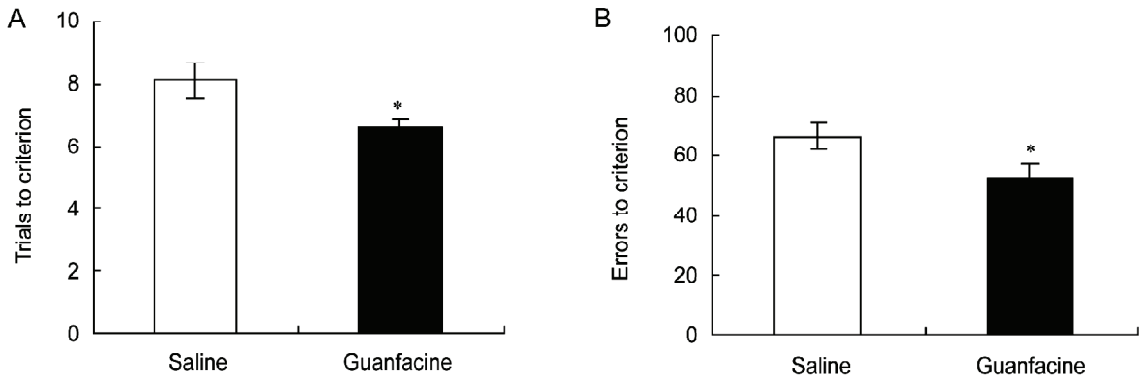

Fig. 2. Guanfacine improved spatial learning ability in Lashley maze. *A*: Trials to learning criterion in guanfacine- and saline-treated groups. *B*: Errors to learning criterion in guanfacine- and saline-treated groups. n=7. $^*P$<0.05 *vs* saline.

maze.

2.2 Guanfacine produces no effect on fear conditioning

Guanfacine or saline was systemically given 10 min before fear conditioning. As shown in Fig.3, the rats in guanfacine- and saline-treated groups exhibited comparable freezing scores for CFM during the post-conditioning 30-second period [Fig.3*A*, "immediate test": $F_{(1,11)}$=0.0117, P=0.916]. The rats in two groups demonstrated similar freezing scores when tested 24 h post-conditioning for CFM [Fig.3*A*, "24-hour test": $F_{(1,11)}$=0.336, P=0.575] or AFM [Fig.3*B*, $F_{(1,11)}$=0.229, P=0.643]. This result indicates that stimulation of the α_{2A}-ARs by guanfacine has no beneficial effect on acquisition of both CFM and AFM.

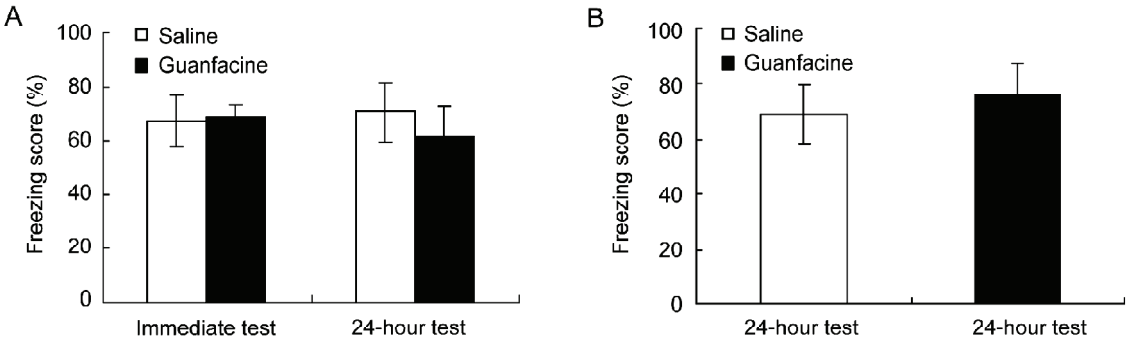

Fig. 3. Guanfacine had no effect on fear conditioning. *A*: Contextual freezing scores of the rats in guanfacine- and saline-treated groups. *B*: Auditory freezing scores of the rats in guanfacine- and saline-treated groups. n=6.

3 DISCUSSION

Lashley maze performance requires an ability of spatial memory and recognition that is dependent on the neural network between the PFC and hippocampus. It has been well documented that stimulation of the α_{2A}-ARs by guanfacine enhances spatial working memory[10,20,36], which is also dependent on the PFC-hippocampal networks[37,38]. Consistently, the present study shows that systemic treatment with guanfacine improves spatial learning in the Lashley maze, adding new evidence for the involvement of α_{2A}-ARs in behavioral performance requiring spatial information processing.

Pavlovian fear conditioning is a typical paradigm that is widely used for studying neural mechanisms underlying fear memory[30]. It has been reported that CFM is dependent on the amygdala and hippocampus, whereas AFM involves the amygdala[31,32]. The present study shows that pre-conditioning treatment with guanfacine produces no beneficial effect on the acquisition/consolidation of CFM and AFM, suggesting that the α_{2A}-ARs are not involved in fear conditioning.

It seems that α_{2A}-AR stimulation selectively improves task performance that involves the integrity of the PFC. Indeed, previous studies showed that the α_{2A}-AR agonists can improve spatial working memory in rats[17], monkeys[10] and humans[18,19], but has little effect on behavioral tasks dependent on the posterior cortices[39]. For example, learning and memory functions dependent on the medial temporal lobe or the parietal cortex are unaffected or even impaired following α_{2A}-AR stimulation[40-43].

Guanfacine has been used for treatment of psychiatric disorders like schizophrenia[22,23], ADHD[24-27] and PTSD[28,29]. Our study provides evidence that guanfacine does not enhance conditioned fear, while improves PFC-dependent cognitive functions. This is of clinical significance, as patients with these psychiatric disorders are unwilling to see an enhancement of negative emotional memory after taking guanfacine.

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