A Novel Conditioning Paradigm Enables the Dissociation Between the Formations of Context- and Cue-Dependent Memories of Drug Reward

Yossef Itzhak
Department of Psychiatry & Behavioral Sciences and Neuroscience Division
University of Miami Miller School of Medicine,
1011 NW 15th Street, Gautier Bldg Room 503 Miami, FL 33136, USA
+1-305-243-4635
yitzhak@med.miami.edu

ABSTRACT

Specific context and cues that had been associated with drug reward elicit the development of drug-associated contextual and cued memories. Re-exposure to drug-associated context and cues may cause arousal and drug-seeking behavior in humans who became addicted to drugs. This maladaptive behavior is dependent on classical Pavlovian conditioning. In animal models, the conditioned place preference paradigm has been used to investigate the motivational effects of drug reward. However, this paradigm does not allow dissociation between approach behavior to discrete contextual and cued stimuli that had been paired with drug reward. Recently, we had designed a novel conditioning paradigm that allows us to discriminate between the formations of context- and cue-dependent memories of drug reward. We used a commercially available two-compartment conditioned place preference apparatus; one compartment is painted white and the second is painted black. During the conditioning phase (4 days), mice received cocaine either in the black or in the white comportment of the cage. In our new design, we inserted a blinking light cue into the drug-paired compartment. Subsequently, we tested one group of mice for approach behavior to the context (in the original training context) in the absence of the light cue. Then, we tested a second group of mice for approach to the light cue in a ***novel context***. Mice conditioned by cocaine developed robust approach behavior for both cocaine-associated context and cue. In control experiments, mice had neither preference nor aversion to the light cue. This new straightforward paradigm allows the dissociation between context- and cue-dependent memories of drug reward. Therefore, this novel behavioral paradigm is significant for the investigation of mechanisms underlying the development of addictive behavior.

Author Keywords

Reinforcement learning, contextual and cued memory, Pavlovian conditioning, reward.

INTRODUCTION

In classical Pavlovian conditioning, pairing of an unconditioned stimulus (US) with a neutral context and cues, such as light or sound, confers conditioned stimulus (CS) properties to these entities. When a specific context is paired with reinforcing stimuli, the conditioned response is approach. Traditionally, this behavior was quantified in the conditioned place preference (CPP) paradigm. The acquisition of place conditioning requires first, an US that changes the affective state of the organism, and second, learning and memory processes [5]. The CPP paradigm has been used to investigate the motivational effects of drug and natural reward.

In a typical place conditioning apparatus, cues such as floor texture and wall color or pattern are embedded in the context, making it difficult to distinguish between cues and context. Thus the reinforcer-paired 'environment' acquires properties of a CS. Subsequent exposure to the CS elicits approach behavior to the drug-paired environment. This type of learning is also viewed as 'habit learning,' which has a major role in the development of drug addiction [3]. While the context of drug exposure may elicit drug craving, presentation of cocaine- and alcohol-related cues to cocaine and alcohol abusers elicits limbic activation, craving and physiological responses similar to the drugs' effects, suggesting the emergence of a conditioned response to drug-associated cues [2]. Given the pivotal role of cue-dependent reinforcement learning in the development of drug addiction, our goal was to modify the traditional CPP paradigm to enable us to investigate not only contextual

memories of drug reward but also discrete cued memories of drug reward. To this end, we investigated the development of approach behavior to discrete contexts and cue that were associated with cocaine administration, using a modified CPP paradigm in mice.

ANIMALS AND METHODS

Animals

Mice purchased from Jackson Laboratories (Bar Harbor, Maine, USA) were bred in our facilities at the University of Miami, Miller School of Medicine, Miami, FL, USA, as we described previously [1]. Both genotypes, wild type (WT) and neuronal nitric oxide (nNOS) knockout (KO), were generated on a mixed B6;129S genetic background. For the experiments described herein, adult (7-8 weeks old) WT and nNOS KO males were used. Animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy Press, 1996) and approved by the University of Miami Animal Care and Use Committee. Mice were conditioned by 20mg/kg cocaine, a dose we found optimal in WT and nNOS KO mice for the acquisition of cocaine conditioned place preference [1].

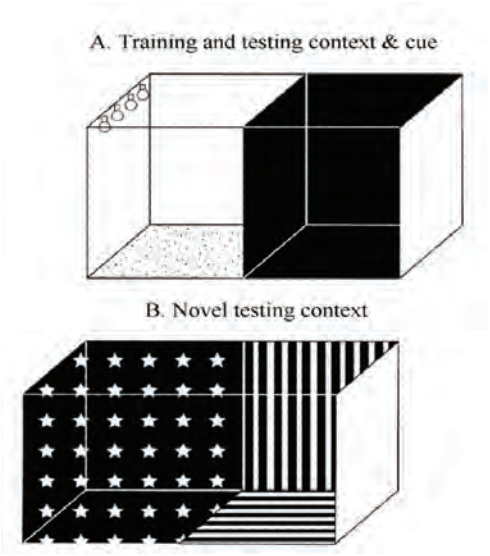

Figure 1. Schematic presentation of the training (A) and novel (B) contexts. The training context consisted of two compartments, separated by a removable guillotine door, one comprising four black walls with a smooth black floor and the other four white walls and a floor covered with sandpaper. During training four blinking lights (mini bulbs; 2.5V, each) were suspended through the ceiling perforations (not shown) of the drug-paired compartment (e.g., black or white); the light served as the drug-associated cue. Drug-associated cue preference was tested in the novel context (B). One compartment was covered with white stars (2.5cm in diameter) on a black background, and the second compartment was covered with black and white strips (2cm wide x 20cm high).

Apparatus

Custom-designed Plexiglas cages (42L x 20W x 20H cm; Opto-Max Activity Meter v2.16; Columbus Instruments, Columbus, OH, USA) were used. The training context consisted of two compartments, separated by a removable guillotine door, one comprising four black walls with a smooth black floor and the other four white walls and a floor covered with sandpaper (Figure 1A). Each compartment was covered by a transparent ceiling, perforated with an array of 16 small holes (1cm diameter, in an array of 4 rows with 4 holes each) to allow ventilation.

We introduced four blinking lights (mini bulbs; 2.5V, each) through the ceiling perforations (Figure 1A) as a cue. The compound context-cue stimulus was always paired with cocaine administration. Because experiments were carried out in an unbiased design (half of the subjects were conditioned by cocaine in the black compartment and the other half in the white compartment) the light cue was present either in the black or in the white compartment of the training apparatus. Upon completion of the training (see below), approach behavior towards cocaine-associated context and cue was tested as follows: one group was tested in the training context in the absence of the light cue and a second group was tested in a novel context (Figure 1B) in the presence of the light cue. The novel context consisted of 2 different removable wall patterns and floor covers that we designed and had laminated. One compartment was covered with white stars (2.5 cm in diameter) on a black background, and the second compartment was covered with black and white strips (2cm wide x 20cm high) (Figure 1B). Each cage was equipped with 2 horizontal sensors mounted alongside opposing lengths. The two compartments (21 x 20 x 20 cm) were each scanned by 7 infrared beams at a rate of 10Hz (2.54 cm intervals). A null zone 8 cm wide was assigned at the interface of the two compartments to ensure that only full entry into each compartment was registered as 'real' time spent in each zone.

Training

Training and testing were carried out in dimmed lighting (30Watts; a reading lamp with two 18-inch white fluorescent bulbs, 15 Watts each, faced a wall) in a test room separate from the housing room. On the first day, between 12:00-14:00h, mice were habituated (20min) to the training context (Figure 1A) in the absence of the light cue; time spent in each compartment was recorded to determine preconditioning compartment-preference/aversion. To ensure a strictly unbiased training design, mice that showed initial preconditioning preference of more than 10-12% of the total time (20min) to either compartment were discarded. For the next 4 days (days 2-5) WT and nNOS KO mice (n=32-34 per group) were trained by a morning (10:00-12:00h) saline session and an afternoon (14:00-16:00h) cocaine (20mg/kg) session, each lasting 30min. For the unbiased design, training was counterbalanced: half of the subjects were trained with drug in the black compartment and the other half in the white compartment.

Cocaine was administered immediately before the animal was placed into the appropriate compartment, and the light cue was turned on 10min later. Animals thus experienced the presence of blinking lights for the final 20min of the drug-training session. Control groups of both genotypes (n=18-20 per group) received saline instead of cocaine in the afternoon session.

Testing

All tests were carried out between 12:00-14:00h, that is during the same time period in which the pretraining habituation had been recorded. Each test was performed in a drug-free state and it lasted for only 10min to minimize extinction learning. For each genotype, half of the subjects were tested for context preference and the other half was tested for cue preference. For context preference, the mouse was placed in the training context in the absence of the light cue (Figure 1A), and time spent in each compartment was recorded for 10min. For cue preference, the light cue was introduced into the novel context (Figure 1B) and it was blinking prior to entry of the mice into the testing room. Then each mouse was brought into the room, and placed in the center of the novel context (Figure 1B). Preference for the cue was recorded for 10min. In this group, half of the mice (n=8-9) were tested with the light cue in the same side of the cage in which the cue had been present during training. To avoid influence of spatial learning of the location of the light cue, the remaining mice from this group (n=8-9) were tested with the light cue located in the part of the cage corresponding to the spatial location *opposite* to that present during training. The outcome of these two tests was remarkably similar, suggesting that animals responded to the drug-associated cue and not to the spatial location of the light; hence, the results of the two tests were combined.

Statistical Analysis

The magnitude of preference for cocaine-paired context and cocaine-paired cue across the genotypes was analyzed by two-way ANOVA: genotype x context/cue preference (Figure 2). Specific differences between groups were analyzed by post hoc Newman-Keuls test. A p value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

WT mice that had been conditioned by cocaine in a discrete context in the presence of a light cue showed significant preference for a) cocaine-paired context and b) cocaine-associated light cue (Figure 2). WT mice showed preference to the light cue that was paired with cocaine injections irrespectively of the spatial location of the light cue in the novel context. This finding suggests that following conditioning by cocaine, the light cue acquired the properties of reinforcing conditioned stimulus that elicits approach behavior. Notably, control mice that were trained only by saline showed neither preference nor aversion to the light cue.

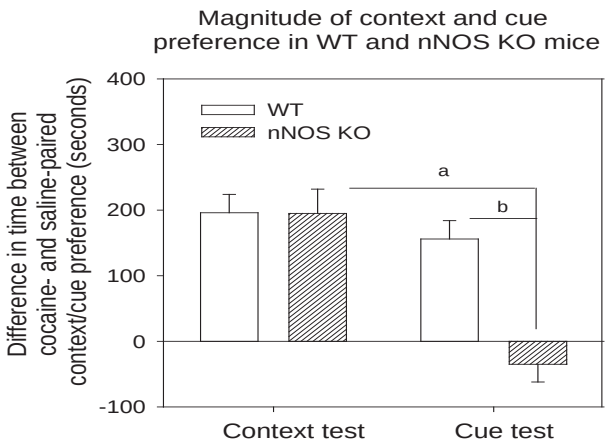

Figure 2. Comparison between WT and nNOS KO mice for context and cue preference. There was no difference between WT and nNOS KO mice in the magnitude of preference for cocaine-associated context. In nNOS KO mice, a significant difference between the preference for cocaine-associated context and cue was observed (a; $p<0.001$). A significant difference between WT and nNOS KO mice in their preference for cocaine-associated cue was also observed (b; $p<0.001$).

Hence, we have shown that a modified conditioned place preference apparatus can be used to investigate both context and cue preference following conditioning by reinforcing drug. Interestingly, nNOS KO mice developed preference for the cocaine-paired context but not for the cocaine-paired light cue (Figure 2). It is unlikely that the impairment in the acquisition of preference to cocaine-associated cue is due to visual deficits in nNOS KO mice because they acquired normal context preference. The discrimination between the two contexts is visually dependent because one compartment is black and the other is white.

Several studies suggest that contextual memory is hippocampus-dependent and cued memory is amygdala-dependent [4]. Thus, it appears that the nNOS gene is required for the formation of amygdala- more so than hippocampus-dependent memory of drug reward.

CONCLUSIONS

The present results demonstrate the utilization of a modified place-conditioning paradigm to investigate both context- and cue-dependent reinforcement learning. Unlike the traditional conditioned place preference design, this novel paradigm we developed allows dissociation between approach behavior to discrete contextual and cued stimuli that were associated with the motivational effect of drug reward. This novel paradigm will allow investigating the mechanisms underlying the development of addictive behavior.

ACKNOWLEDGEMENTS

This work was supported by award R21DA029404 from the National Institute on Drug Abuse, National Institutes of Health, USA.

REFERENCES

1. Balda, M.A., Anderson, K.L., and Itzhak, Y. Adolescent and adult responsiveness to the incentive value of cocaine reward in mice: role of neuronal nitric oxide synthase (nNOS) gene. *Neuropharmacology*, *51*, (2006), 341-349.
2. Childress, A.R., Mozley, P.D., McElgin, W., Fitzgerald, J., Reivich, M., and O'Brien, C.P. Limbic activation during cue-induced cocaine craving. *American Journal of Psychiatry*, 156 (1999), 11-18.
3. Everitt, B.J., and Robbins, T.W. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nature Neuroscience*, *8* (2005), 1481-1489.
4. Phillips, R.G., and LeDoux, J.E. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioural Neuroscience*, *106* (1992), 274–285.
5. White, N.M., and Carr, G.D. The conditioned place preference is affected by two independent reinforcement processes. *Pharmacology Biochemistry and Behavior*, *23* (1985), 37-42.