Neurobiology of Learning and Memory xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Reconsolidation and update of morphine-associated contextual memory in mice

Joao Carlos Escosteguy-Neto [a,c], Patricia Varela [a], Nelson Francisco Correa-Neto [b], Laura Segismundo Coelho [b], Emmanuel S. Onaivi [c], Jair Guilherme Santos-Junior [b,*]

[a] Laboratory of Neurobiology, Federal University of Sao Paulo, R. Pedro de Toledo, 669, 3rd floor, Sao Paulo, SP 04039-032, Brazil
[b] Department of Physiological Science, Faculty of Medical Sciences of Santa Casa of São Paulo, R. Cesário Motta Jr, 61, 12th floor, São Paulo, SP 01221-020, Brazil
[c] Department of Biology, William Paterson University, 300 Pompton Rd, Wayne, NJ 07470, USA

ARTICLE INFO

Article history:
Received 19 September 2015
Revised 19 January 2016
Accepted 20 February 2016
Available online xxxx

Keywords:
Contextual memory
Consolidation
Reconsolidation
Memory update
Morphine
Cycloheximide

ABSTRACT

Drug addiction can be viewed as a pathological memory that is constantly retrieved and reconsolidated. Since drug abuse takes place in different contexts, it could be considered that reconsolidation plays a role in memory updating. There is consistent evidence supporting the role of reconsolidation in the strength and maintenance of contextual memories induced by drugs of abuse. However, this role is not well established in memory update. The purpose of the current study was to assess the reconsolidation process over memory update. C57BL6 mice were subjected to a morphine-induced, conditioned place preference (CPP) paradigm. Based on CPP results, animals were divided into distinct experimental groups, according to the contextual characteristics of the re-exposure and a second CPP Test. Re-exposure in the original context was important for memory maintenance and re-exposure under discrete contextual changes resulted in memory updating, although original memory was maintained. Interestingly, cycloheximide, an inhibitor of protein synthesis, had different outcomes in our protocol. When the re-exposure was done under discrete contextual changes, cycloheximide treatment just after re-exposure blocked memory updating, without changes in memory maintenance. When re-exposure was done under the original context, only two subsequent cycloheximide injections (3 and 6 h) disrupted later CPP expression. Considering the temporal window of protein synthesis in consolidation and reconsolidation, these findings suggest that re-exposure, according to the contextual characteristics in our protocol, could trigger both phenomena. Furthermore, when new information is present on retrieval, reconsolidation plays a pivotal role in memory updating.

1. Introduction

One of the main characteristics of drug addiction is the emergence of a negative emotional state, reflecting a motivational withdrawal syndrome when access to the drug is prevented. This leads to craving and relapse (Koob & Volkow, 2010). Furthermore, there is evidence suggesting that learning and memory play a pivotal role in the chronic and relapsing nature of drug addiction. Since relapse is a major obstacle during withdrawal, understanding the correlation between environmental cues and drug addiction is essential for effective treatment.

Associative learning is a process whereby environmental stimuli, repeatedly paired with addictive drugs, acquire "incentive motivational value". This can evoke expectations of the drug availability and memories of the emotional aspects related to previous drug use. Conditioned responses to such stimuli activate corticostriatal-limbic structures and play a role both in maintaining ongoing drug use and causing drug craving and relapse during abstinence. The complex circuitry related to synaptic plasticity mechanisms and associative learning is characterized by structural changes in glutamatergic, gabaergic and dopaminergic synapses (Bassareo, De Luca, & Di Chiara, 2007; Di Chiara & Bassareo, 2007; Hyman, 2005; Jones & Bonci, 2005; Ungless et al., 2003), involving the Ventral Tegmental Area (VTA), Nucleus Accumbens, Prefrontal Cortex (PFC), Amygdala and Hippocampus in the animal and human brain (Berke & Hyman, 2000; Di Chiara & Bassareo, 2007; Wise, 2000).

* Corresponding author at: Department of Physiological Science, Faculty of Medical Sciences of Santa Casa of São Paulo, R. Cesário Motta Jr, 61, 12° andar, São Paulo, SP 01221 020, Brazil.
E-mail address: guilherme.stos.jr@gmail.com (J.G. Santos-Junior).

http://dx.doi.org/10.1016/j.nlm.2016.02.015
1074-7427/

Moreover, memories do not remain stable once acquired, but change dynamically over one's lifetime. Consolidated memories can return to a short-lived, labile state through memory retrieval, and trigger a re-stabilization process termed "reconsolidation" (Nader & Einarsson, 2010; Tronson & Taylor, 2007). Reconsolidation plays a pivotal role in the strengthening and updating of memory (Inda, Muravieva, & Alberini, 2011; Lee, 2008) in order to maintain its relevance after the experience of new information (Dudai, 2004; Hupbach, Gomez, Hardt, & Nadel, 2007; Lee, 2009). Thereby, inhibition of the reconsolidation process has been considered a promissory strategy for drug addiction treatment (Milton & Everitt, 2010). Previous studies showed reconsolidation as a fundamental factor in the strength of appetitive associative memories related to drugs of abuse (Fan et al., 2010; Milekic, Brown, Castellini, & Alberini, 2006; Robinson & Franklin, 2007; Valjent, Corbillé, Bertran-Gonzalez, Herve, & Girault, 2006). Curiously, there is no direct evidence concerning its role in the update of this kind of memory. Since drug exposure rarely happens under the same context, the role of memory update in drug addiction is particularly relevant. Thus, an adapted unbiased morphine Conditioned Place Preference (CPP) model was used. New contexts or discrete contextual changes were added to the previous drug-paired context after acquisition and Test 1 phases of the CPP protocol. Additionally, maintenance of morphine CPP and memory reconsolidation and update processes were verified (see Section 2.2). This information might bring new insights for memory reconsolidation based therapies and drug addiction treatment.

2. Methods

2.1. Animals

Male C57BL/6 mice ($n = 190$) from CEDEME (Center for the Development of Animal Models in Biology and Medicine of Federal University of São Paulo), were housed in standard home cages ($40 \times 34 \times 17$ cm, $n = 10$ per cage) with woodchip bedding, mouse chow pellets and tap water *ad libitum*, except during testing. The animals were 12 weeks of age (20–30 g) at the start of the experiment. The temperature (20–22 °C) and humidity (50%) controlled animal colony was maintained on a light/dark cycle (12/12 h), with lights on at 07:00 a.m. Mice were maintained in these housing conditions for at least 7 days prior to the beginning of the experiments. Principles of laboratory animal care were conducted under the protocol approved by the Animal Care and Use Ethics Committee of the University, according to the American Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research.

2.2. Experimental protocol

The experimental protocol consisted of six phases: Habituation, Preconditioning, Conditioning, Test 1, Re-exposure and Test 2 (Fig. 1).

2.2.1. Morphine-induced Conditioned Place Preference (CPP)

Morphine-induced CPP was assessed in a sound and light attenuated test room using a three-chambered CPP apparatus (adapted from McGeehan & Olive, 2003). Two larger compartments ($37 \times 15 \times 30$ cm) with distinct visual and tactile cues (one had black and white checkered walls and smooth floor, while the other one had striped walls and floor with series of 1-mm-caliber bronze bars spaced 1 cm apart) were connected by a central compartment ($7 \times 15 \times 30$ cm). The central compartment was equipped with two guillotine doors that provided access to one or both of the conditioning compartments.

In the habituation (Day 1) and pre-conditioning (Day 2) phases, all mice were placed in the central compartment with free access to both peripheral compartments for a 10-min period. On Day 2, the time spent in each compartment was measured. Mice that showed a preference for one compartment over the other (more than 60% of the time in one of the peripheral compartments) were excluded from further testing ($N = 18$).

Subsequent tests were done using an unbiased procedure (Cunningham, Ferree, & Howard, 2003). The conditioning phase was conducted two days after pre-conditioning session. For five consecutive days (Day 5–Day 9), mice were injected with morphine (20 mg/kg, s.c.) and immediately placed for 40 min in one of the peripheral compartments. A dose response curve for morphine CPP was conducted elsewhere and no significant differences from doses ranging from 10 to 20 mg/kg were reported (Ribeiro Do Couto, Aguilar, Manzanedo, Rodríguez-Arias, & Miñarro, 2003; Sala, Braida, Calcaterra, Leone, & Gori, 1992; Zhao et al., 2007). Moreover, a 20 mg/kg dose is related to an optimized morphine reward (Olson et al., 2006; Ventura, Alcaro, & Puglisi-Allegra, 2005). Since the aim of this study was focused in the post conditioning phase of the CPP paradigm (reconsolidation mechanisms), animals were not paired with saline solution in the opposite peripheral compartment (Bardo & Bevins, 2010; Milekic et al., 2006). Nonetheless, control groups were submitted to similar procedure, except that mice were injected with saline (0.9% NaCl) rather than morphine (Control A1A1 and Control A2A2 groups). These control groups were useful for detecting unlearned biases or shifts in biases that might occur due to repeated cue exposure or the passage of time. Both saline paired groups did not show any preference for either side of the apparatus, excluding a possible novelty effect related to the non-paired compartment. Two days after the conditioning phase (D11), animals were placed in the central compartment with guillotine doors open and free access to both peripheral compartments during a 10 min test (Test 1). The amount of time spent in each of the peripheral compartments was measured to define the score of CPP: the difference between the times spent in the drug-paired compartment during Test 1 and during pre-conditioning. After 7 days (D18), animals were submitted to a re-exposure procedure, as described below. The Control group was used only to determine a reliable CPP score value after the conditioning phase. Therefore, this group was not submitted to the re-exposure and Test 2 protocols.

2.2.2. Re-exposure and Test 2 protocols

In a drug-free state, the re-exposure took only one 3 min period. After 7 days from the re-exposure (D25), mice were submitted to Test 2. As in Test 1, animals were maintained for 10 min in the central compartment with free access to both peripheral compartments, allowing us to define the CPP score for Test 2 (the difference between the time spent in the drug-paired compartment during Test 2 and during pre-conditioning). Furthermore, we used an index of memory after re-exposure procedure: Cpp2:Cpp1 (%) = (Cpp2/Cpp1) * 100. Re-exposure and Test 2 protocols were performed in ten distinct iterations, according to the contextual characteristics used in the re-exposure and Test 2 (Fig. 1): i. re-exposure and Test 2 under the original context (same used in the conditioning phase) (A1A1 group); ii. re-exposure under the original context and Test 2 under discrete contextual changes (i.e. a different geometric pattern in one wall of the apparatus) (group A1A2); iii. re-exposure under original context and Test 2 in an alternative context (i.e. white walls and geometric pattern on smooth floor) (group A1B); iv. re-exposure under discrete contextual changes and Test 2 in the original context (group A2A1); v. re-exposure and Test 2 under discrete contextual changes (group A2A2); vi. re-exposure under discrete contextual changes and Test 2 in an alternative context (group A2B); vii. re-exposure in an alter-

Please cite this article in press as: Escosteguy-Neto, J. C., et al. Reconsolidation and update of morphine-associated contextual memory in mice. *Neurobiology of Learning and Memory* (2016), http://dx.doi.org/10.1016/j.nlm.2016.02.015