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Short communication

# α-Melanocyte-stimulating hormone (α-MSH) reverses impairment of memory reconsolidation induced by interleukin-1 beta (IL-1 beta) hippocampal infusions

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ABSTRACT

Interleukin-1 beta (IL-1β) significantly influences cognitive processes. Treatments which raise the level of IL-1β in the brain impair memory consolidation in contextual fear conditioning. However, the effect of IL-1β on memory reconsolidation has not yet been established. The melanocortin α-melanocyte-stimulating hormone (α-MSH) exerts potent anti-inflammatory actions by antagonizing the effect of proinflammatory cytokines. Five subtypes of melanocortin receptors (MC1R–MC5R) have been identified, of which MC3R and MC4R are predominant in the central nervous system. The present experiments show that the injection of IL-1β (5 ng/0.25 μl) in dorsal hippocampus up to 30 min after re-exposition to the context decreases freezing during the contextual fear test. Impairment of memory reconsolidation was reversed by treatment with α-MSH (0.05 μg/0.25 μl). Administration of the MC4 receptor antagonist HS014 (0.5 μg/0.25 μl) blocked the effect of α-MSH. These results suggest that IL-1β may influence memory reconsolidation and that activation of central MC4R could lead to improve cognitive performance.



## 1. Introduction

When fearful memories are formed, they are initially labile but become progressively consolidated into persistent traces via synthesis of new proteins [20]. Later retrieval of a consolidated fear memory initiates two apparently antagonistic processes: reconsolidation and extinction [17]. In the process of reconsolidation, a retrieved memory transiently returns to a labile state and requires new protein synthesis to persist further. During this labile state, the memory may be exposed to enhancement or disruption [22]. This period of instability or lability, the reconsolidation window, persists for several hours after retrieval [8]. Reconsolidation occurs in a broad range of learning paradigms [9,29] and species [14,25]. Its adaptive purpose might be to permit the integration of new information present at the time of retrieval into an updated memory representation [21,22].

One important recent advance in the biological basis of behavior is recognition of extensive communication between the central nervous system and the immune system. In this context, IL-1β was shown to produce detrimental effects on memory [12,24]. These effects are specific for consolidation of memories that depend on hippocampus, whereas hippocampal-independent memories are not altered [24]. The particularly high expression of IL-1β, IL-1 receptor 1, and proteins belonging to the IL-1 receptor family in the hippocampus may underlie the effect of IL-1β within this structure [18]. We demonstrated that the melanocortin α-MSH significantly protects against memory impairments caused by IL-1β administration [12].

α-Melanocyte-stimulating hormone (α-MSH) and other melanocortins (β-MSH, γ-MSH and ACTH) are members of a family of endogenous peptides derived from pro-opiomelanocortin (POMC). They exert their actions via five different G-protein coupled receptors (MC1/MC5), all of which couple positively via G-protein to adenyl cyclase [28]. MC4 is found in many limbic system structures, including several nuclei of the amygdala, the hippocampus and the enthorhinal cortex [1]. Melanocortins exert multiple influences on the central nervous system, including immunomodulatory effects: they can modulate the production and action of proinflammatory cytokines [2,15]. Centrally administered α-MSH is a potent antipyretic in different species [4] and can also prevent damage in brainstem ischemia and reperfusion injury

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[10]. Several different effects of central IL-1β administration are blocked by α-MSH [5,6]. Also, hippocampal infusions of alpha-MSH reverse memory consolidation deficits induced by central IL-1β administration [12].

Although the effect of IL-1β on memory consolidation has been clearly demonstrated, the effect of intrahippocampal IL-1β administration on memory reconsolidation has not yet been examined. Therefore, in the present work we studied the effect of IL-1β on the reconsolidation of a contextual fear memory. We also examined the influence of α-MSH and the role of MC4 receptors on IL-1β effects on memory reconsolidation.

## 2. Methods

### 2.1. Animals

Adult male Wistar rats weighing 270–300 g at the time of surgery were used for these studies. All animals were housed in standard laboratory plastic cages in groups of three per cage. Food and water were available ad libitum. Animals were kept on a 12 h light/dark cycle (lights on 7:00–19:00 h) with a constant room temperature of 22 ± 1 °C. Protocols used were performed according to the NIH Guide for the Care and Use of Laboratory Animals as approved by the School of Chemical Sciences, National University of Cordoba Animal Care and Use Committee. The number of animals used, as well as their suffering, was kept to the minimum necessary to accomplish the objectives of this study.

### 2.2. Surgery and drug infusion procedures

Rats were implanted bilaterally under ketamine hydrochloride (55 mg/kg) and xylazine (11 mg/kg) intraperitoneal anesthesia with 22-g guide cannulas in the dorsal CA1 region of the hippocampus on coordinates A: −3.3 mm; L: ±2.0 mm; V: −2.5 mm, as per Paxinos and Watson's atlas [23]. Cannulas were fixed to the skull with a screw and dental acrylic. After surgery, animals were gently handled daily and habituated to intrahippocampal injections throughout the recovery period. Behavioral tests commenced 7 days after surgery.

To perform local infusions into hippocampus, rats were hand-restrained and drugs or vehicle administered with infusion cannulas (30 gauge). Infusion cannulas fitted into and extended 1 mm beyond guide cannulas. Infusion cannulas were connected via polyethylene tubing (PE 10; Becton Dickinson, Sparks, MD) to 10 μl microsyringes (Hamilton, Reno, NV) mounted on a microinfusion pump (Harvard Apparatus, Holliston, MA). Each rat was injected in each infusion with 0.25 μl/side at a flow rate of 0.25 μl/min. To allow diffusion of the drug, infusion cannulas were kept in place for another minute.

### 2.3. Histological procedures

After behavioral tests, rats were killed by overdose of chloral hydrate 16%. Brains were removed and immersion-fixed in a 4% formalin solution. Frontal sections were cut in a cryostat (Leica, Nussloch, Germany), injection sites localized, and the extent of tissue damage caused by cannulation was examined under a light microscope. Injection sites were drawn on plates taken from a rat brain atlas [23]. Only animals with proper cannula placements and tissue damage not exceeding the diameter of cannulas were included in the study.

### 2.4. Drugs

The drugs used in these experiments were rrIL-1β (R&D Systems, UK), α-MSH (MW 1664) (Peninsula Laboratories Inc.) and HS024 (selective MC4-R antagonist, MW 1678) (NeoMPS, France). The rrIL-1β was dissolved in sterile 0.9% saline containing 0.1% bovine serum albumin and stored in aliquots at −70 °C. Peptides were dissolved in sterile 0.9% saline and stored in aliquots at −20 °C. The peptides are reconstituted and stored following the manufacturer's indications. Rats received bilateral infusions of sterile saline (vehicle) or drug with the following doses: IL-1β (5 ng/0.25 μl), α-MSH (0.05 μg/0.25 μl), HS014 (0.5 μg/0.25 μl).

### 2.5. Apparatus

The conditioning environment was a transparent plastic box (20 cm × 23 cm × 20 cm) with a clear lid. The floor was 10 parallel 4 mm diam. stainless steel grid bars spaced 1.5 cm apart (center to center), enclosed in a sound attenuating chamber. The grid floor was attached to a scrambled shocker to provide footshock. Illumination was provided by a 2.5 W white light bulb. The chamber was cleaned with 50% ethanol in water before and after utilization.

Experiments were always performed between 10:00 and 14:00 h by experimenters unaware of treatment condition.

### 2.6. Behavioral procedure

Each experiment consisted of three phases: conditioning, re-exposure (reactivation session), and testing sessions. An acoustically isolated room was used to run the training and other phases (re-exposure and tests) of the experiment.

*Contextual fear conditioning*: Training consisted in placing the rat in the chamber and allowing a 3 min acclimatization period (preshock period). After this period, rats received three unsignaled footshocks (0.3 mA; 2.5 s duration; 30 s intershock interval; unconditioned stimuli). Animals were left in the chamber for an additional 2 min (postshock period) and immediately after were placed in their home cages and returned to the colony room.

*Re-exposure session* (*reactivation*): Twenty-four hours after training, subjects were re-exposed to the training context without shocks for 2 min.

*Test session*: Contextual fear conditioning was assessed 24 h after re-exposition by placing the rats in the training environment for a 5 min period. Memory was assessed and expressed as percentage of time rats spent freezing. This behavior, commonly used as an index of fear in rats, was observed during this exposure period. An animal was considered to be freezing when it was crouching without body or head movements except those associated with breathing. Total time spent freezing in each period was quantified (in seconds) with a stopwatch and expressed as percentage of total time.

#### 2.6.1. Experiment 1

This experiment was performed to determine the effect of IL-1β on reconsolidation of a contextual fear memory when administered at different times (0, 15, and 30 min) after reactivation. Animals were tested in the conditioning chamber 24 h later (test). Other groups of animals were injected with vehicle at the same times.

A control experiment without reactivation was also run. Twenty-four hours after training different groups of animals were injected with IL-1β or vehicle without being re-exposed to the context (no reactivation session) and assessed for contextual fear conditioning (test session) 24 h later.

#### 2.6.2. Experiment 2

To study interaction between IL-1β and α-MSH in reconsolidation of contextual fear memory, animals randomly assigned to different groups were injected immediately following re-exposure session as follows: (a) IL-1β (5 ng); (b) IL-1β (5 ng) and α-MSH (0.05 μg); (c) IL-1β (5 ng) co-administered with HS024 (0.5 μg) and α-MSH (0.05 μg); (d) α-MSH (0.05 μg) and (e) HS024 (0.5 μg).

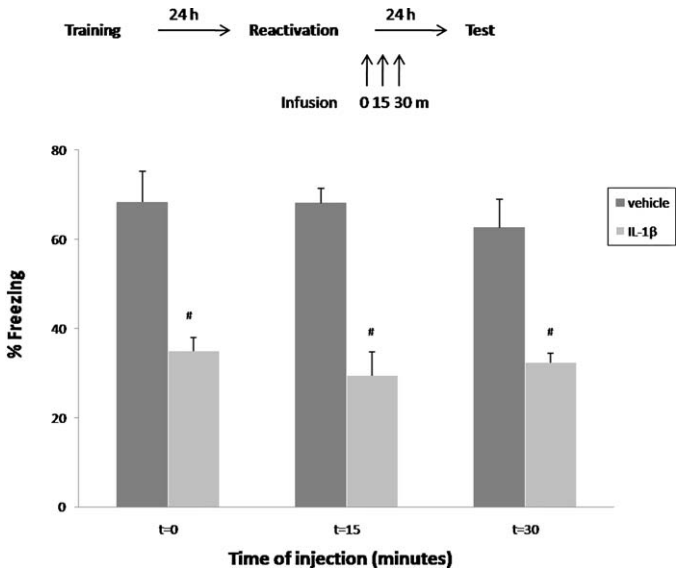


**Fig. 1.** Effect of IL-1β (5 ng/0.25 μl) administration at different time intervals after re-exposition to context. (a) Behavioral procedure used in this experiment and (b) IL-1β injected up to 30 min post-reactivation caused a profound deficit in reconsolidation of contextual fear memory. Data are the mean ± S.E.M. percentage of time rats spent freezing during 5 min exposure to training context (test). #Significantly different from vehicle group ($p < 0.001$) ANOVA followed by Newman–Keuls test. no: 4–7 per group.

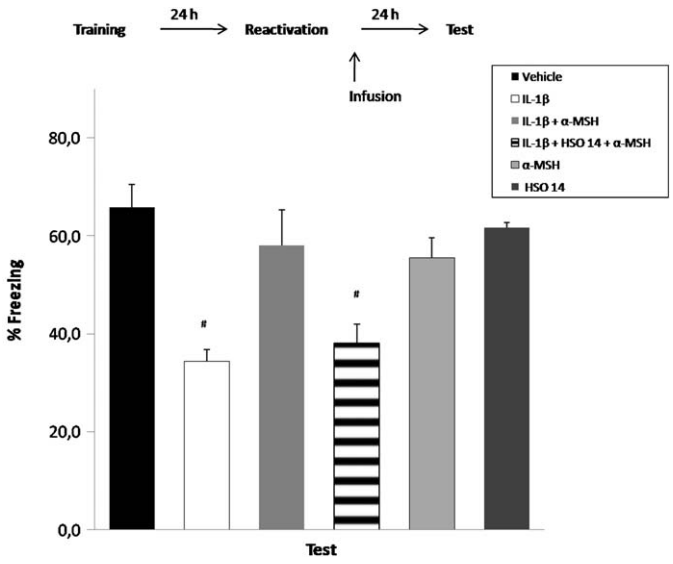


**Fig. 2.** Effect of melanocortins on IL-1β-induced impairment of contextual fear memory reconsolidation. (a) Behavioral procedure used in this experiment. (b) α-MSH (0.05 μg/0.25 μl) blocked the inhibitory effect of IL-1β on memory reconsolidation. Administration of HS014 (0.5 μg/0.25 μl), an MC4R antagonist, blocked the effect of α-MSH. α-MSH and HS014 had no effect by themselves. Data are the mean ± S.E.M. percentage of time rats spent freezing during 5 min exposure to training context. #Significantly different than vehicle group ($p < 0.001$) ANOVA followed by Newman–Keuls test. no: 3–10 per group.

Doses were determined by dose–response experiments and previous work [17]. The melanocortin was injected 10 min after IL-1β. Animals were re-exposed to the context for 5 min 24 h later and the time they spent freezing was assessed.

### 2.7. Statistics

All data shown are mean ± standard error of the mean (S.E.M.).

Data were analyzed by one-way or two-way ANOVA. If any statistically significant difference was found, post hoc analysis was performed using Student–Newman–Keuls multiple comparison test. Differences with $p \leq 0.05$ were considered statistically significant.

## 3. Results

### 3.1. Effect of intrahippocampal injection of IL-1β on reconsolidation of contextual fear memory

IL-1β impairs reconsolidation of contextual fear memory when administered immediately following re-exposition to the context. Fig. 1 shows that rats which received intrahippocampal injection of IL-1β (5 ng/0.25 μl) displayed lower levels of freezing than animals injected with saline immediately after re-exposition. ANOVA revealed a significant effect of cytokine treatment ($F(1,26) = 53.21$, $p < 0.001$). Subsequent post hoc analysis revealed that IL-1β-treated groups injected immediately, 15 or 30 min after conditioning were significantly different from saline groups. These findings clearly show that the amnesic effect of IL-1β was effective for up to 30 min.

The control experiment without reactivation ensures that IL-1β affects a process initiated by retrieval. Animals were injected with vehicle or IL-1β 24 h after training without reactivation and tested 24 h later. Both groups of animals displayed similar percentages of freezing (% of freezing: saline = 69.89 ± 6.16; IL-1β = 67.17 ± 0.17). There were no statistical differences between groups. Thus, the cytokine specifically impairs the reconsolidation process.

### 3.2. Influence of α-MSH on IL-1β-induced decreased fear contextual memory reconsolidation

Our results showed that IL-1β impairs reconsolidation of contextual memory. This effect is blocked by α-MSH (0.05 μg/0.25 μl) injection into dorsal hippocampus. Thus, results indicate that treatment of rats with α-MSH following IL-1β significantly prevented the deficit in reconsolidation of contextual representation memory. ANOVA revealed a significant effect of the treatments ($F(5,36) = 9.07$, $p < 0.001$).

α-MSH administration 10 min after IL-1β completely antagonized the effect of the cytokine and percentage of freezing was similar to that observed after saline (Fig. 2). However, co-administration of HS014 (0.5 μg/0.25 μl) and IL-1β (5 ng/0.25 μl) 10 min before administration of α-MSH (0.05 μg/0.25 μl) produced effects similar to IL-1β treatment. Thus, treatment with HS014 blocked α-MSH suppression of IL-1β-induced memory reconsolidation impairment. Treatment with HS014 or α-MSH alone had no effect on contextual fear conditioning (Fig. 2).

## 4. Discussion

Our results demonstrated once more that effects of the proinflammatory cytokine IL-1β extend beyond its role in peripheral response to infection. In the central nervous system, IL-1β and other cytokines are responsible for behavioral adaptation (sickness syndrome) that reorganizes an organism's priorities in order to fight infection and preserve energy [7]. High levels of IL-1β in brain, which occurs during sickness behavior, also impair memory consolidation [12,24].

The present findings demonstrate that IL-1β, administered after reactivation, impaired memory reconsolidation and more specifically reconsolidation of an aversive memory that depends on the hippocampus. We also demonstrated that the effect of IL-1β is specific to the reconsolidation process since IL-1β had no effect if memory was not reactivated.

In order to examine the time course of the effect of IL-1β, the cytokine or vehicle were microinjected immediately, 15 min or 30 min after re-exposition and always tested 24 h later. These experiments provide information concerning the time course of the effect produced by brain IL-1β. Our results showed that IL-1β injected into the hippocampus up to 30 min after re-exposition interfered with processes responsible for reconsolidation of memory of the contextual representation.

We also demonstrated that the melanocortin α-MSH significantly protects against impairment in memory reconsolidation caused by IL-1β administration. This clear neuroprotective effect is probably mediated by brain melanocortin MC4 receptors. Melanocortins are largely distributed in the CNS, and MC4 receptors have been found in various brain areas including hippocampus [28]. Administration of HS014, a selective MC4-R antagonist, reversed the effect of α-MSH on impairment of memory reconsolidation induced by IL-1β, suggesting that α-MSH may exert this effect by activating central MC4-R. We previously demonstrated that α-MSH, through activation of MC4R, also reversed the effect of IL-1β on memory consolidation [12]. Although the effect of α-MSH on memory has been demonstrated before [2] the intrahippocampal administration of alpha-MSH at the doses described here, have no effect on memory consolidation [12] or reconsolidation.

Several transcription factors have been implicated in memory reconsolidation [30]. There are also notable differences in patterns of transcription factor activation between consolidation and reconsolidation. Within the hippocampus, a dissociation has been reported between the transcription factor zinc finger 268 (Zif-268), selectively required for reconsolidation, and brain-derived neurotrophic factor (BDNF), selectively required for consolidation of contextual fear conditioning [16]. Transcription factors are phosphorylated by upstream kinases. Two kinases, extracellular signal-regulated kinase (ERK) and protein kinase A (PKA) have been implicated in reconsolidation process [30]. PKA is activated by cAMP and acts directly or indirectly through ERK and ribosomal protein S6 kinase (RSK) to activate transcription factors including cAMP response element-binding protein (CREB) [13] and Zif-268 [3]. IL-1β was shown to modify Zif-268 activation in human queratinocites [19]. This cytokine also reduces ERK phosphorylation induced by LPS + IFN-gamma on microglial cells [27]. On the other hand, since MCRs are G-protein coupled receptors that activate adenylate cyclase and the molecular cascade of cAMP and CREB, α-MSH was expected to induce CREB activation in hippocampus. Concordantly, α-MSH in vivo induces phosphorylation of CREB in neurons of the paraventricular nucleus in rats [26]. Also, the protective effect of α-MSH in experimental ischemic stroke was associated with Zif-268 overexpression [11].

Thus, although mechanisms underlying the effect of IL-1β on memory reconsolidation have not yet been established, it is possible that alpha-MSH could counteract IL-1β effect by modifying Zif-268 expression through CREB activation.

In conclusion, we determined the detrimental effect of IL-1β on reconsolidation of contextual memory, and also that α-MSH, through activation of MC4-R, could reverse the effect of IL-1β on reconsolidation of a fear memory.

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