Neurobiology of Learning and Memory 106 (2013) 185–192

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

# Divergent short- and long-term effects of acute stress in object recognition memory are mediated by endogenous opioid system activation

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ARTICLE INFO

*Article history:*
Received 20 July 2013
Revised 31 August 2013
Accepted 2 September 2013
Available online 11 September 2013

*Keywords:*
Endogenous opioid system
Object recognition memory
Naltrexone
Acute stress
Retroactive interference

ABSTRACT

Acute stress induces short-term object recognition memory impairment and elicits endogenous opioid system activation. The aim of this study was thus to evaluate whether opiate system activation mediates the acute stress-induced object recognition memory changes. Adult male Wistar rats were trained in an object recognition task designed to test both short- and long-term memory. Subjects were randomly assigned to receive an intraperitoneal injection of saline, 1 mg/kg naltrexone or 3 mg/kg naltrexone, four and a half hours before the sample trial. Five minutes after the injection, half the subjects were submitted to movement restraint during four hours while the other half remained in their home cages. Non-stressed subjects receiving saline (control) performed adequately during the short-term memory test, while stressed subjects receiving saline displayed impaired performance. Naltrexone prevented such deleterious effect, in spite of the fact that it had no intrinsic effect on short-term object recognition memory. Stressed subjects receiving saline and non-stressed subjects receiving naltrexone performed adequately during the long-term memory test; however, control subjects as well as stressed subjects receiving a high dose of naltrexone performed poorly. Control subjects' dissociated performance during both memory tests suggests that the short-term memory test induced a retroactive interference effect mediated through light opioid system activation; such effect was prevented either by low dose naltrexone administration or by strongly activating the opioid system through acute stress. Both short-term memory retrieval impairment and long-term memory improvement observed in stressed subjects may have been mediated through strong opioid system activation, since they were prevented by high dose naltrexone administration. Therefore, the activation of the opioid system plays a dual modulating role in object recognition memory.



## 1. Introduction

Stress is a non-specific and adaptive response to homeostasis-threatening conditions; such response involves autonomic nervous system and hypothalamus–pituitary–adrenal axis activation, resulting in glucocorticoid and epinephrine release (Pacak & Palkovits, 2001). Both acute and chronic stress affects many cognitive functions, including memory processes (McEwen, 2000). Acute stress has either impairing or improving effects on memory, depending on its intensity (Conrad, Lupien, & McEwen, 1999), the degree of emotional arousal (Okuda, Roozendaal, & McGaugh, 2004; Roozendaal, Okuda, Van der Zee, & McGaugh, 2006), its temporal relationship to specific memory stages (McEwen & Sapolsky, 1995; Roozendaal, 2002), and the degree to which the motivation to perform a particular task is aversive (Conrad, 2005).

Central and peripheral components of the endogenous opioid system become activated during stress response, giving rise to stress-induced analgesia (Akil, Madden, Patrick, & Barchas, 1976; Coventry et al., 2001; Hebb et al., 2004; Marinelli, Quirion, & Gianoulakis, 2004; Mousa, Miller, & Couri, 1983). Moreover, endogenous opiod system activation itself modifies the stress response (Bodnar, 2012; Carrasco & Van de Kar, 2003; Drolet et al., 2001;

*Abbreviations:* ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance; DI, discrimination index; FO, familiar object; N1NS, 1 mg/kg naltrexone plus no-stress; N3NS, 3 mg/kg naltrexone plus no-stress; N1S, 1 mg/kg naltrexone plus stress; N3S, 3 mg/kg naltrexone plus stress; Nal 1, 1 mg/kg naltrexone; Nal 3, 3 mg/kg naltrexone; NO, novel object; ORM, object recognition memory; ORT, object recognition task; RI, retroactive interference; SEM, standard error of the mean; SNS, saline plus no-stress; SS, saline plus stress; TFO, time spent exploring the familiar object; TNO, time spent exploring the novel object; Veh, vehicle.

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http://dx.doi.org/10.1016/j.nlm.2013.09.002

Ignar & Kuhn 1990; McLaughlin, Marton-Popovici, & Chavkin, 2003).

The central opioid system is involved in many central nervous system functions and plays a modulating role in several cognitive processes, such as learning and memory (Bodnar, 2012; Jamot, Matthes, Simonin, Kieffer, & Roder, 2003; Martínez & Rigter, 1980; McGaugh, 1983; Messing et al., 1979, 1981). Brain structures involved in memory processing (such as the hippocampus) have several opiod neurons (Morris & Johnston, 1995; Shirayama et al., 2004) and opioid receptors (mu, kappa, and delta). In fact, it has been reported that the activation of different opioid receptors in the hippocampus modifies its synaptic plasticity (Mansouri, Motamedi, & Fathollahi, 1999; Terman, Wagner, & Chavkin, 1994; Wagner, Etemad, & Thompson, 2001). Moreover, it has been found that hippocampal mu-opioid receptors play a critical role in spatial learning and memory (Meilandt, Barea-Rodriguez, Harvey, & Martinez, 2004).

Previous studies have shown that opioid antagonism regulates hypothalamus-pituitary-adrenal axis response, hippocampal activity and cognitive functions (for a pertinent review, see Bodnar, 2012; Carrasco & Van de Kar, 2003). Interestingly, opioid antagonists either improve (Gallagher, King, & Young, 1983; Zhao, Xu, & Xu, 2004) or impair (Meilandt et al., 2004) spatial memory, depending on their specificity and reversibility; the opioid system's role regarding memory processing is thus complex.

The effect of opioid receptors blocking on recognition memory has been evaluated in a few studies. When administered to monkeys trained to perform a visual recognition task the dose-effect curve of naloxone has an inverted-U shape, where low doses have little effect, intermediate doses improve recognition memory, and high doses impair performance in the task (Aigner & Mishkin, 1988). Naltrexone administration prior a recognition memory task in humans has no effects on memory retention (Chaves, Pezzin, Jardim, & Izquierdo, 1990). However, it has been reported that naltrexone administration may either improve or impair recognition memory depending on the state of physiological arousal of the subjects (Katzen-Perez, Jacobs, Lincoln, & Ellis, 2001).

Since both spatial and contextual memory depend on brain structures having high concentrations of corticosteroid and opioid receptors (Morris, 1991; Morris, Garrud, Rawlins, & O'Keefe, 1982; Roozendaal, 2002; Selden, Everitt, Jarrard, & Robbins, 1991; Simmons & Chavkin, 1996) then an interaction between stress and opioid system activity concerning memory processes is plausible. In fact, opioid receptor blockade attenuates glucocorticoid or acute restraint stress-induced deficit in contextual memory retrieval (Rashidy-Pour, Sadeghi, Taherain, Vafaei, & Fathollahi, 2004). Moreover, intrahippocampal naltrexone administration blocks the glucocorticoid-induced impairment of long-term spatial memory retrieval (Sajadi, Samaei, & Rashidy-Pour, 2007).

Object recognition memory (ORM) is a non-rewarded task which exploits the rat's natural tendency to explore novel objects more intensely than familiar ones (Ennaceur & Delacour, 1988). ORM has been considered a valid model of declarative memory in rats (Kart-Teke, De Souza Silva, & Huston, 2006; Martins de Lima, Presti-Torres, Dornelles, Bromberg, & Schroder, 2007), and depends on appropriate activity in the hippocampus (Mumby, Tremblay, Lecluse, & Lehmann, 2005), the perirhinal cortex (Winters & Bussey, 2005), and the insular cortex (Bermudez-Rattoni, Okuda, Roozendaal, & McGaugh, 2005).

Taking into account that the stress response involves opioid system activation and that such system exerts a regulatory role on memory processing, then stress-induced memory changes could be attributed to an interaction between the mechanisms mediating stress responses and the opioid system. However, previous studies have not evaluated such possible interaction during episodic memory acquisition and retrieval. This study was thus aimed at evaluating whether opiate receptor blockade modifies the acute stress-induced changes in ORM. An object recognition task allowing both short- and long-term memory evaluations in the same subject was specifically designed and applied to fulfill such aim. Evidence was found for the first time in this study suggesting that acute stress either impairs or facilitates ORM, depending on the timing of evaluation, and that such dual effect is mediated by strong opioid system activation.

## 2. Materials and methods

### 2.1. Subjects

Forty-three adult male Wistar rats, supplied by the Instituto Nacional de Salud (Bogotá, Colombia), weighting 280 ± 3 g (mean ± SEM), were used as subjects. The animals were housed in polycarbonate cages (32 × 38 × 18 cm) in groups of four with free access to water and food during the whole experiment and kept in controlled environmental conditions: 12-h light/dark cycle (lights on at 07:00 h), 20 ± 1 °C room temperature, and 50 ± 10% relative humidity. All experimental procedures were conducted during the light phase of the cycle. Local regulations and international guidelines (NIH Guide for the Use and Care of Laboratory Animals) were strictly followed throughout the experiment. All efforts were made to minimize the number of animals involved and avoid the experimental subjects' unnecessary suffering.

### 2.2. Experimental design

Rats were randomly assigned either to be submitted to restraint (stress, S) or to receive no manipulation before the training (no stress, NS). The subjects in each group were then randomly assigned to receive an intraperitoneal injection of vehicle (0.9% saline), low naltrexone dose (1 mg/kg), or high naltrexone dose (3 mg/kg). Six experimental groups were thus formed: (1) SNS (control), non-stressed rats received a saline injection 4 h and 35 min before training ($n = 8$); (2) SS, rats received a saline injection 5 min before stress induction ($n = 7$); (3) N1NS, non-stressed rats received a 1 mg/kg naltrexone injection 4 h and 35 min before training ($n = 7$); (4) N1S, rats received a 1 mg/kg naltrexone injection 5 min before stress induction ($n = 7$); (5) N3NS, non-stressed rats received a 3 mg/kg naltrexone injection 4 h and 35 min before training ($n = 7$); and (6) N3S, rats received 3 mg/kg naltrexone injection 5 min before stress induction ($n = 7$).

### 2.3. Procedures

#### 2.3.1. Acute stress induction

Acute stress was induced by movement restraint involving the use of 20 cm long, 6.5 cm diameter polycarbonate tubes designed to restrain major head and limb movements. Four and a half hours before the sample trial, subjects randomly assigned to the S condition ($n = 21$) were gently placed into the tubes, where they remained for 4 h; these subjects were allowed to get out of the restrainers 30 min before the sample trial and to move freely around their home cages to avoid non-specific motor effects due to movement restriction.

#### 2.3.2. Drugs

The non-specific and competitive opiate receptor blocker naltrexone hydrochloride doses (1 mg/kg and 3 mg/kg) were chosen on the basis of studies reporting the effectiveness of such doses to suppress opiate-mediated hedonic responses (Bienkowski, Kostowski, & Koros, 1999; Richardson, Reynolds, Cooper, & Berridge, 2005) or to prevent the stress-induced memory impairment

in a passive avoidance task (Rezvanfard, Zarrindast, & Ownegh, 2011); moreover, such reports indicate that the effect of naltrexone at 3 mg/kg is significantly larger than its effect at 1 mg/kg.

Naltrexone hydrochloride (Sigma) was dissolved in 0.9% saline and its concentration was adjusted to obtain a final 1 ml/kg injection volume. A 1 ml/kg dose of 0.9% saline was injected into subjects designated to receive vehicle. Intraperitoneal injections of saline or naltrexone were administered either 5 min before stress induction (for SS, N1S, and N3S subjects) or 4 h and 35 min before sample trial (for SNS, N1NS, and N3NS subjects), according to the experimental treatment to which each subject had been assigned.

*2.3.3. Object recognition task (ORT)*

The ORT was evaluated using a uniformly-illuminated black acrylic open field (60 × 60 × 60 cm) and different shaped (pyramids or rectangular prisms) and textured (either smooth or rough) objects which were otherwise identical regarding the material they were made from (acrylic), their size (14 cm height × 6 cm depth × 14 cm width) and color (black) and heavy enough to ensure that they could not easily be displaced by a rat. Subjects were gently handled every day for 10 min, starting four days before habituation session, to minimize manipulation-related stress. ORT consisted of four 10-min trials distributed over a three-day period (Fig. 1). Each animal was placed in the open field during the first trial (habituation trial, day 1) and allowed to become familiarized with it. During the second trial (sample trial, day 2), performed 24 h after the habituation trial, each animal was placed in the open field containing two objects identical in texture but different in shape (e.g. a smooth pyramid and a smooth rectangular prism; familiar objects, named respectively FO1 and FO2), located near two opposite corners chosen at random (12 cm away from the walls). Each animal was placed in the open field during the third trial (short-term memory test, day 2), performed one hour after the sample trial, and allowed to explore two objects: FO1 in its original location and a novel object (NO1), identical in shape but different in texture to FO1 and located in the place where FO2 had been placed during the sample trial. The fourth trial (long-term memory test, day 3), performed twenty-four hours after the sample trial, involved each animal being placed in the open field and allowed to explore two objects: FO2 in its original location and a novel object (NO2), identical in shape but different in texture to FO2 and located in the place where FO1 had been placed during the sample trial. The use of texture as novelty cue was based on a previous study in our laboratory (Moreno et al., 2010). Olfactive clues were removed after every trial by cleansing the objects and the open field with a 10% ethanol solution.

*2.3.4. Data recording and analysis*

The behavior of each subject during the experimental trials was observed and recorded by video camera linked to a video recorder and a video monitor located in an adjacent room. All behavioral measurements were made off-line by a researcher blinded to the pretraining treatments using X-Plo-Rat 3.3 software (Cardenas, Lamprea, & Morato, 2001).

The open field was virtually divided into nine squares (20 × 20 cm each) and the number of complete crossings from one square to another was counted to evaluate locomotive activity. Each trial was divided into five 2-min intervals, and the relative number of crossings per interval was used to evaluate habituation to the open field.

Object exploration was defined as sniffing, touching the object with the nose, or pointing the nose towards the object from a distance shorter than 2 cm. Accordingly, the time spent exploring each object was recorded for each sample or test trial and expressed as a percentage of exploration time (relative exploration time) to evaluate object preference. Four rats spending less than 15 s total exploration time on either sample or test trials were excluded from analysis. Discrimination index (DI) was calculated

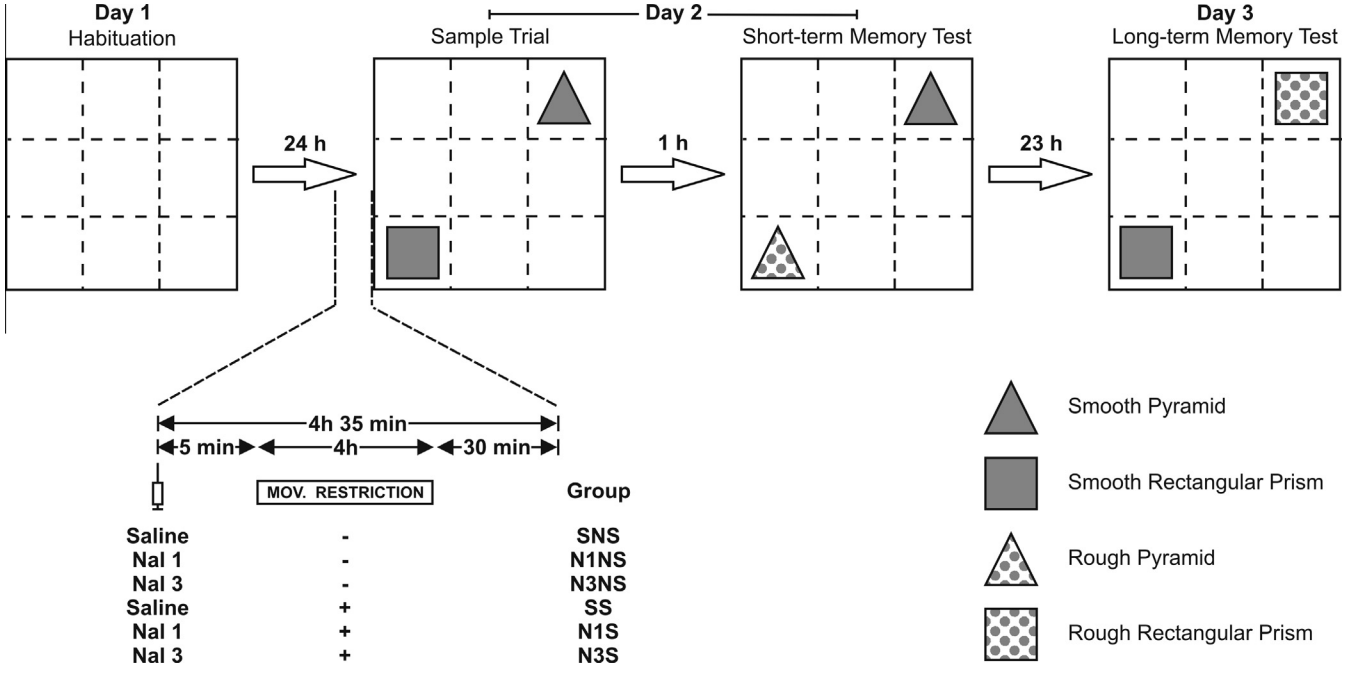


**Fig. 1.** Experimental design. Upper row, timeline for habituation, sample, and test trials regarding the object recognition task used to test both short- and long-term memory in the same individual. The shape, texture, and location of the objects presented during the sample and test trials are illustrated. The open field where object recognition took place was virtually divided (dashed lines) into nine square (20 × 20 cm each) to evaluate locomotor activity during each trial. Lower row, the timing and nature of the experimental treatments as well as the corresponding denomination of the experimental group are illustrated. Abbreviations: Mov. restriction, movement restriction; Veh, vehicle (0.9% saline); Nal 1, 1 mg/kg naltrexone; Nal 3, 3 mg/kg naltrexone); SNS, saline plus no-stress; SS, saline plus stress; N1NS, 1 mg/kg naltrexone plus no-stress; N3NS, 3 mg/kg naltrexone plus no-stress; N1S, 1 mg/kg naltrexone plus stress; N3S, 3 mg/kg naltrexone plus stress.

based on the difference in time spent exploring each object during a given trial. For the sample trial, the DI was calculated as the difference in exploration time between FO1 and FO2 (TFO1 and TFO2, respectively) divided by the total time spent exploring both objects: 100*(TFO1-TFO2)/(TFO1 + TFO2). The DI for the memory test trials was calculated as the difference in exploration time between NO and FO (TNO and TFO, respectively), divided by the total time spent exploring both objects: 100*(TNO-TFO)/(TNO + TFO). Such DIs allowed not only intragroup comparisons of the subjects' performance between the memory test trials and the sample trial, but also intergroup comparisons of the subjects' preference for the novel object in a given trial.

The behavioral measures of the subjects were grouped to perform intragroup and intergroup comparisons. Changes in each group's locomotor activity during each ORT trial were characterized using two-way repeated-measures ANOVA, followed by *post-hoc* comparisons using the Holm–Sidak method. Relative exploration time for each object in a given group was compared using either Student's *t*-test or Wilcoxon signed-rank test, according to data distribution. Differences in discrimination indexes were evaluated using two-way repeated-measures ANOVA, followed by *post-hoc* comparisons using the Holm–Sidak method, to compare object preference between groups and trials. Significance levels were set at $p < 0.05$.

## 3. Results

### 3.1. Subjects became successfully habituated to the open field in a single trial

Two-way repeated-measures ANOVA followed by Holm–Sidak *post-hoc* test indicated that the subjects' locomotor activity significantly decreased during the habituation trial (Fig. 2). In fact, there was a significant decrease in relative crossings during successive time intervals (time interval factor: $F_{(20,\ 148)} = 38.835$; $p < 0.001$; interval 1 *vs.* interval 3: $t = 8.006$, $p < 0.0001$; interval 1 *vs.* interval 4: $t = 10.237$, $p < 0.0001$; interval 1 *vs.* interval 5: $t = 11.074$, $p < 0.0001$). As expected, there were no significant differences between groups regarding locomotor activity during the habituation trial (Supplementary Table 1; group factor: $F(5,\ 148) = 0.868$, $p = 0.512$), nor significant interaction between group and time interval factors (group × time interval: $F(4,\ 148) = 1.027$, $p = 0.435$). This analysis showed that a single trial exposure to the open field was enough for the subjects to become habituated.

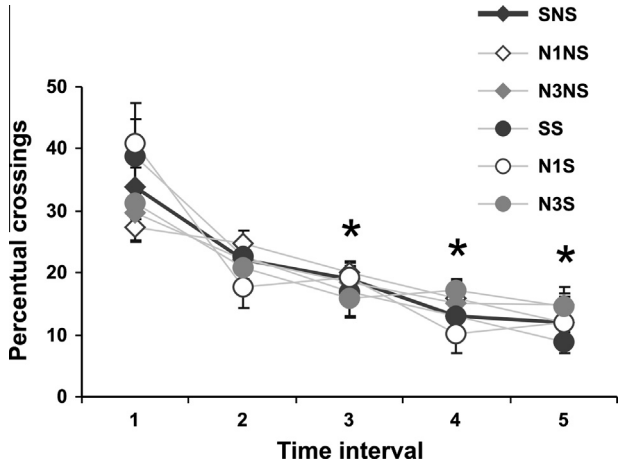


**Fig. 2.** Evolution of the percentage of crossings through virtual borders in the open field during the habituation trial. The habituation trial time span (10 min) was divided into five 2-min intervals. A progressive decline in exploratory locomotion was observed in all experimental groups, a fact indicating successful habituation to the open field. Each point and its associated whiskers represent the mean ± SEM. Abbreviations: SNS, saline plus no-stress; SS, saline plus stress; N1NS, 1 mg/kg naltrexone plus no-stress; N3NS, 3 mg/kg naltrexone plus no-stress; N1S, 1 mg/kg naltrexone plus stress; N3S, 3 mg/kg naltrexone plus stress; *, indicates a significant difference respecting the relative crossings performed during the first 2-min interval, $p < 0.05$.

### 3.2. Neither stress nor naltrexone affected overall exploratory behavior

No treatment affected the tendency to explore the objects placed in the open field. In fact, there were no statistically significant differences between the experimental groups regarding the overall time spent exploring both objects presented during either sample trial ($F_{(5,\ 37)} = 0.675$, $p = 0.646$), short-term memory test ($F_{(5,\ 37)} = 0.643$, $p = 0.669$), or long-term memory test ($F_{(5,\ 37)} = 0.741$, $p = 0.599$). Moreover, there were also no statistically significant differences between groups concerning locomotor activity (Supplementary Table 1) as evidenced by the total number of crossings during either sample trial ($F(5, 37) = 0.739$, $p = 0.599$), short-term memory test ($F(5, 37) = 0.401$, $p = 0.845$), or long-term memory test ($F(5, 37) = 0.263$, $p = 0.929$).

Subjects from each experimental group spent a comparable amount of time during the sample trial exploring each familiar object (Fig. 3 and Supplementary Fig. 1; SNS: $t_{(7)} = 0.095$, $p = 0.927$; SS: $t_{(6)} = 0.146$, $p = 0.888$; N1NS: $t_{(6)} = 0.781$, $p = 0.464$; N3NS: $t_{(6)} = 0.401$, $p = 0.703$; N1S: $t_{(6)} = 1.082$, $p = 0.321$; N3S: $t_{(6)} = -1.263$, $p = 0.253$).

### 3.3. Opiate receptor blockade reverted stress-induced short-term ORM impairment

Acute restraint stress induced before the sample trial impaired ORM during the short-term memory test (Fig. 4 A), as evidenced by the absence of significant differences in the relative exploration time of NO and FO (SS: $t_{(6)} = -0.659$, $p = 0.534$). By contrast, naltrexone administered prior to movement restriction stress prevented acute stress-induced ORM impairment during short-term memory test (Fig. 4 A), as evidenced by a significant preference for exploring the NO (N1S: $t_{(6)} = 3.638$, $p = 0.011$; N3S: $t_{(6)} = 3.533$, $p = 0.012$). Such preference for exploring the NO was also displayed by control subjects (SNS: $t_{(7)} = 2.923$, $p = 0.022$) as

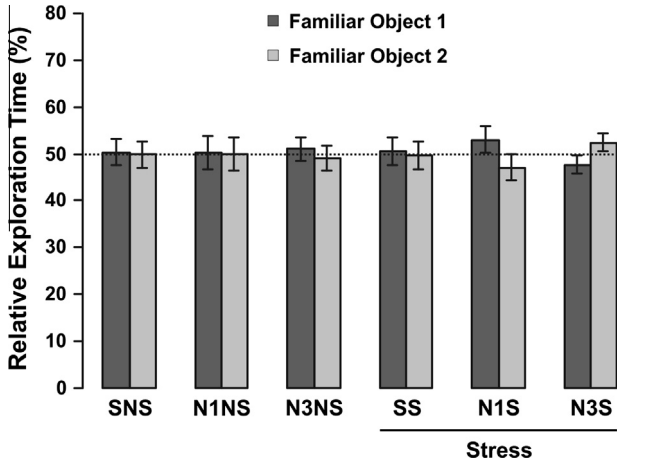


**Fig. 3.** Relative exploration time for the objects presented during the sample trial. Bar charts illustrating the time subjects from every experimental group spent exploring each object presented during the sample trial. Bars and error whiskers represent the mean + SEM. No group displayed a significant object preference. Abbreviations: SNS, saline plus no-stress; SS, saline plus stress; N1NS, 1 mg/kg naltrexone plus no-stress; N3NS, 3 mg/kg naltrexone plus no-stress; N1S, 1 mg/kg naltrexone plus stress; N3S, 3 mg/kg naltrexone plus stress.

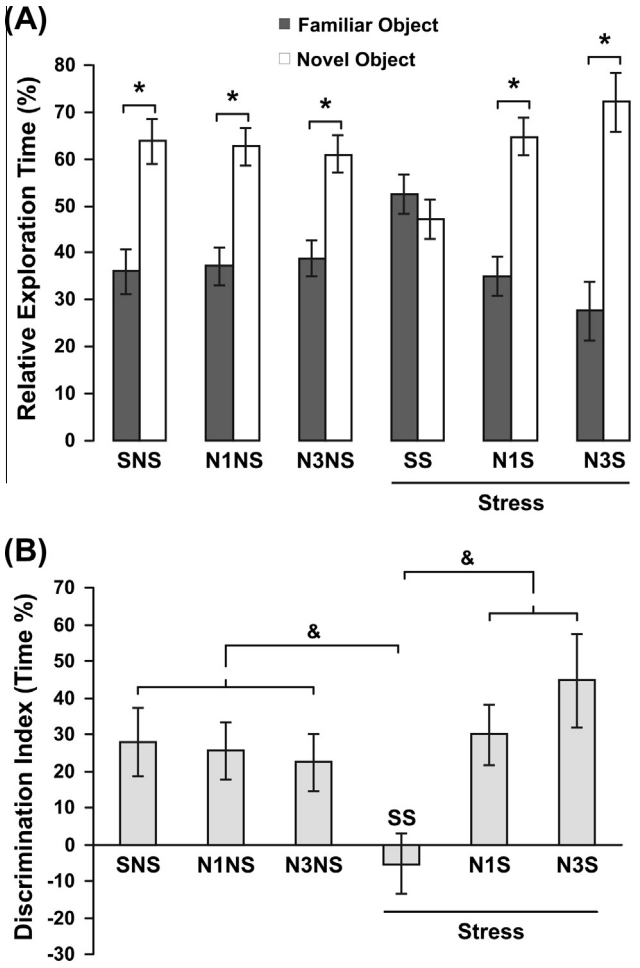


**Fig. 4.** Object exploration preference during the short-term memory test. (A) Bar charts illustrating the percentage time subjects from each experimental group spent exploring either the familiar or the novel object during the short-term memory test. (B) Bar chart illustrating the discrimination index calculated for each experimental group during the short-term memory test. Bars and error whiskers represent the mean ± SEM. Abbreviations: SNS, saline plus no-stress; SS, saline plus stress; N1NS, 1 mg/kg naltrexone plus no-stress; N3NS, 3 mg/kg naltrexone plus no-stress; N1S, 1 mg/kg naltrexone plus stress; N3S, 3 mg/kg naltrexone plus stress; *, indicates a significant difference in relative exploration time, $p < 0.05$; &, indicates a significant difference between groups in the discrimination index calculated during the short-term memory test.

well as non-stressed subjects who had been treated previously with naltrexone in either 1 or 3 mg/kg doses (N1NS: $t_{(6)} = 3.284$, $p = 0.017$; N3NS: $t_{(6)} = 2.823$, $p = 0.030$). This pattern was also found when the raw exploration time of NO and FO was compared for each group (Supplementary Fig. 2 and Supplementary Table 2).

The DI was used for making intragroup and intergroup comparisons during the sample trial and the short-term memory test. Taken overall, there were no significant difference in the DI between the experimental groups (group factor: $F_{(5,\ 85)} = 1.615$, $p = 0.18$); however, there was a significant difference between trials (trial factor: $F_{(1,\ 85)} = 48.313$, $p < 0.001$), and a significant interaction of the factors (group x trial: $F_{(5,\ 85)} = 4.498$, $p = 0.003$). Adequate ORM, as indicated by significant DI increase during the short-term memory test compared to the sample trial, was observed in control subjects (SNS: $t = 3.886$, $p < 0.001$) as well as in non-stressed subjects receiving a naltrexone injection (N1NS: $t = 2.978$, $p = 0.005$; N3NS: $t = 2.371$, $p = 0.023$) and animals receiving naltrexone before stress (N1S: $t = 2.811$, $p = 0.008$; N3S: $t = 5.792$, $p < 0.001$), but not in animals receiving vehicle injection before stress (SS: $t = 0.74$, $p = 0.464$).

There were no significant differences in DI between groups during the sample trial ($p > 0.05$). By contrast, during the short-term memory test the DI of subjects who had received a vehicle injection before stress was significantly lower than that of control subjects (SS *vs.* SNS: $t = 3.56$, $p < 0.001$) and that of animals who had received a naltrexone injection with or without stress (Fig. 4B; SS *vs.* N1NS: $t = 2.889$, $p = 0.005$; SS *vs.* N3NS: $t = 2.582$, $p = 0.012$; SS *vs.* N1S: $t = 3.293$, $p = 0.002$; SS *vs.* N3S: $t = 4.673$, $p < 0.001$). Taken together, these results suggest that acute stress-induced short-term ORM impairment was reverted by either low or high doses of naltrexone and that naltrexone by itself did not affect ORM in non-stressed subjects.

### *3.4. Acute stress improved long-term ORM; however, this effect was prevented by high dose naltrexone treatment*

Control animals were not able to retrieve ORM when tested 24 h after the sample trial (Fig. 5A), as indicated by the absence of significant differences in NO and FO exploration (SNS: $W_{(7)} = 6.000$, $p = 0.742$). However, naltrexone alone, disregarding the dose used, changed the pattern observed in control subjects showing significant preference for exploring the novel object (Fig. 5A; N1NS: $t_{(6)} = 3.099$, $p = 0.021$; N3NS: $t_{(6)} = 2.645$, $p = 0.038$).

Contrasting with the finding during the short-term memory test, acute restraint stress improved ORM when tested 24 h after the sample trial, as evidenced by a significant preference to explore the NO (SS: $t_{(6)} = 2.081$, $p = 0.046$). Similarly, animals receiving a low dose of naltrexone before acute stress preferentially explored the NO (N1S: $t_{(6)} = 5.327$, $p = 0.002$). However, a high naltrexone dose administered before movement restriction reverted the improving effect of acute stress on long-term ORM, as evidenced by the absence of significant differences in NO and FO exploration time (N3S: $t_{(6)} = 1.061$, $p = 0.329$). This pattern was also found when the raw exploration time of NO and FO was compared for each group (Supplementary Fig. 3 and Supplementary Table 2).

Taken overall, there were no significant difference in the DI between the experimental groups (group factor: $F_{(5,\ 85)} = 1.173$, $p = 0.341$); however, there was a significant difference between trials (trial factor: $F_{(1,\ 85)} = 21.669$, $p < 0.001$), and significant factor interaction (group × trial: $F_{(5,\ 85)} = 4.456$, $p = 0.045$). Adequate ORM, as indicated by significantly increased DI in the long-term memory test compared to the sample trial, was observed in subjects who received saline and were submitted to acute stress before the sample trial (SS: $t = 2.421$, $p = 0.018$), as well as in subjects who received naltrexone but no stress before the sample trial (N1NS: $t = 2.206$, $p = 0.034$; N3NS: $t = 2.855$, $p = 0.007$) and in subjects who received a low naltrexone dose and were subjected to acute stress before the sample trial (N1S: $t = 3.366$, $p = 0.002$). The performance of subjects of the remaining groups during the long-term memory test was no significantly different from that during the sample trial (SNS: $t = 0.436$, $p = 0.665$; N3S: $t = 0.0279$, $p = 0.978$), indicating impaired long-term ORM.

There were no significant differences in DI between groups during the sample trial ($p > 0.05$). Control subjects as well as subjects who received a high naltrexone dose and were subjected to acute stress before the sample trial displayed a significantly lower DI than that for the remaining groups (Fig. 5B; SNS *vs.* N1NS; $t = 2.031$, $p = 0.046$; SNS *vs.* N3NS: $t = 2.558$, $p = 0.013$; SNS *vs.* SS: $t = 2.198$, $p = 0.031$; SNS *vs.* N1S: $t = 2.726$, $p = 0.008$; N3S *vs.* N3NS: $t = 2.361$, $p = 0.021$; N3S *vs.* SS: $t = 2.013$, $p = 0.048$; N3S *vs.* N1S: $t = 2.524$, $p = 0.014$). Taken together, such results suggest that acute stress improved long-term ORM and that this effect was not changed by naltrexone in low doses; however, such ORM-improving effect of stress was reverted when high naltrexone doses were administered.

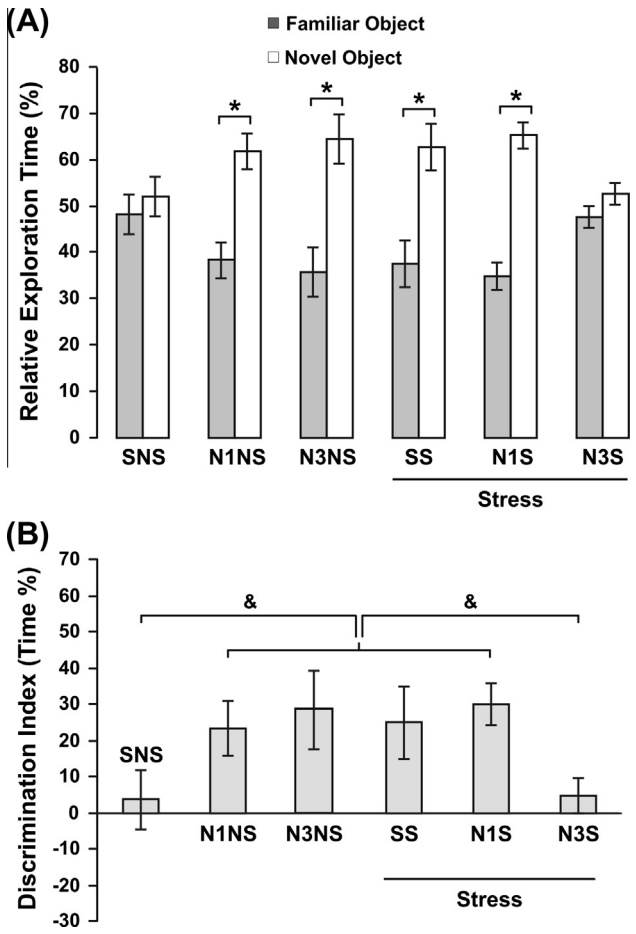


**Fig. 5.** Object exploration preference during the long-term memory test. (A) Bar charts illustrating the percentage time subjects from each experimental group spent exploring either the familiar or the novel object during the long-term memory test. (B) Bar chart illustrating the discrimination index calculated for each experimental group during the long-term memory test. Bars and error whiskers represent the mean ± SEM. Abbreviations: SNS, saline plus no-stress; SS, saline plus stress; N1NS, 1 mg/kg naltrexone plus no-stress; N3NS, 3 mg/kg naltrexone plus no-stress; N1S, 1 mg/kg naltrexone plus stress; N3S, 3 mg/kg naltrexone plus stress; *, indicates a significant difference in relative exploration time, $p < 0.05$; &, indicates a significant difference between groups in the discrimination index calculated during the long-term memory test.

## 4. Discussion

In this work, it was found that acute restraint stress induced before the sample trial impaired subjects' performance on the short-term memory test. Although naltrexone administered before the sample trial to non-stressed subjects did not change short-term ORM, acute stress-induced short-term memory impairment was prevented when naltrexone, regardless of the dose used, was applied just before the stress. Control subjects displayed adequate short-term ORM; however, when such subjects were tested 24 h after the sample trial (long-term ORM) they did not display preference for exploring the NO. By contrast, subjects submitted to acute stress alone, subjects who received naltrexone but were not submitted to stress, and subjects who received a low naltrexone dose before being stressed, performed correctly in the ORT when tested 24 h after the sample trial (long-term ORM). However, high naltrexone doses prevented the effect of stress on long-term ORM when administered jointly.

Control subjects (SNS) displayed adequate short-term, but inadequate long-term ORM. Since short- and long-term ORM were evaluated in the same subjects, it may have been possible that experience during the short-term memory test had had negative effects on the persistence of the information acquired during the sample trial; an effect known as retroactive interference (RI) (Bartko, Cowell, Winters, Bussey, & Saksida, 2010; Lupien & McEwen, 1997). In fact, non-stressed subjects who were not submitted to an interposed short-term memory test displayed adequate ORM when tested 24 h after the sample trial (Martins de Lima et al., 2007; Vargas-López, Torres-Berrio, González-Martínez, Múnera, & Lamprea, 2013). Since memory is more susceptible to RI when the items presented during the interfering experience are perceptually similar to those presented during the training (Bartko et al., 2010), our experimental design was prone to RI.

The fact that stressed subjects (SS) displayed adequate long-term ORM in spite of having previously displayed short-term memory impairment suggests that acute stress transiently impairs memory retrieval but not acquisition nor consolidation. Substantial evidence has indicated that acute stress induces transient retrieval impairment of short-term ORM (Baker & Kim, 2002; Li, Fan, Wang, & Tang, 2012; Morrow, Roth, & Elsworth, 2000; Okuda et al., 2004), contextual memory (Celerier, Pierard, Rachbauer, Sarrieau, & Beracochea, 2004), and spatial memory (de Quervain, Roozendaal, & McGaugh, 1998). Acute stress-induced impairment of ORM retrieval may have suppressed the RI effect of the short-term memory trial, in turn, allowing adequate ORM consolidation.

Subjects treated only with naltrexone, irrespectively of the dose (N1NS and N3NS), performed adequately during both short- and long-term memory trials. Adequate performance during the short-term memory test, which was no different than that of control subjects, indicates that naltrexone had no effect on ORM acquisition or retrieval. On the other hand, appropriate performance during the long-term memory test indicates that the RI effect induced by short-term memory test was reverted by naltrexone. It is thus possible that the interfering experience impaired ORM consolidation through transient and low level activation of the opioid system, since it was blocked even when low naltrexone doses were used. In fact, it has been described that opioid system activation makes a memory trace from recent learning more vulnerable to RI and that opiate antagonist administration blocks such effect (Pinheiro & Wright, 1991).

Regardless of the dose (N1S and N3S), naltrexone reverted the detrimental effect of acute stress on short-term ORM retrieval, which suggests that acute stress-induced impairment of short-term memory was mediated by activation of the endogenous opioid system. Rashidy et al., (2004) reported that naloxone blocks acute stress- or dexametasone-induced retrieval impairment of a passive avoidance task. Moreover, transgenic mice lacking the gene for dynorphin (the endogenous agonist of kappa opioid receptors) do not display stress-induced ORM impairment (Carey, Lyons, Shay, Dunton, & McLaughlin, 2009); suggesting that the memory impairing effect of stress could be mediated by dynorphin interacting with kappa opioid receptor. The memory impairing effect of stress may be due to enhanced endogenous opioid activity specifically located in the hippocampus. In fact, immobilization stress induces a significant increase in endogenous opioid activity in the hippocampus (Chen et al., 2004; Shirayama et al., 2004); in addition, intrahippocampal naltrexone injection blocks glucocorticoid-induced impairment of spatial memory retrieval (Sajadi et al., 2007). Conversely, intrahippocampal opioid agonists administration impairs spatial memory (Sandin et al., 1998; Daumas et al., 2007).

Appropriate long-term ORM performance displayed by acutely-stressed subjects was found when a low naltrexone dose was injected before stress (N1S), but not when a high naltrexone dose was used (N3S). The acute stress blocking of the RI induced by the short-term memory test became abolished only when a high naltrexone dose was used. Acute stress induces increased activity

in the opioid system; therefore, it is plausible that acute stress-induced RI blocking was due to enhanced activation of the opioid system. Since such effect was prevented only with a high dose of naltrexone, opioid system activation induced by acute stress must have been intense. Opioid system activation would be inversely proportional to a recently-acquired memory trace's susceptibility to RI: low level activation (for example after the short-term memory test) may enable RI; conversely, high level activation (for example after acute stress) may prevent RI occurrence. Since naltrexone administered alone did not affect short- and long-term ORT performance, our results suggest that opioid system basal activity does not affect ORM acquisition, consolidation, and/or retrieval. However, this system's enhanced activity, induced whether by memory reactivation or by acute stress, plays a key modulating role having dual effects over ORM.

## Acknowledgements

This experiment was financed by a grant from the Universidad Nacional de Colombia's Dirección de Investigaciones de la Sede Bogotá (DIB-6634). We would like to thank Mr. Jason Garry for his help in editing the manuscript.

## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.nlm.2013.09.002.

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