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# Spironolactone and low-dose dexamethasone enhance extinction of contextual fear conditioning

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ABSTRACT

Glucocorticoids play a role in memory formation, and they may contribute to memory changes in stress-related mental disorders, such as posttraumatic stress disorder. Cortisol may act through mineralocorticoid (MR) or glucocorticoid (GR) receptors, and the objective of the present study was to evaluate the effects of the MR antagonist spironolactone, the GR antagonist mifepristone, the MR agonist fludrocortisone, and the GR agonist dexamethasone on the extinction of contextually conditioned fear in rats. Propranolol was used as a positive control. As expected, propranolol administered before the test session increased memory extinction. Pre-test administration of spironolactone and low-dose dexamethasone also increased the extinction of an aversive memory, whereas fludrocortisone impaired extinction. High-dose dexamethasone and mifepristone were found to have no effect in this model. Post-test spironolactone treatment impaired aversive memory extinction. These results indicate that MR and GR are related to extinction of aversive memories, and MR blockade may be a promising candidate for the treatment of stress-related memory disorders.



## 1. Introduction

Certain stress-related mental disorders are associated with memory symptoms. For example, posttraumatic stress disorder (PTSD), an anxiety disorder precipitated by experiencing a traumatic event (American Psychiatric Association, 2000), can present both hyper-memorization of the traumatic event that cannot be forgotten and memory deficits (Van Praag, 2004). Alternatively, an extinction deficit of fear memories rather than hyper-memorization has been proposed for PTSD (Cammarota et al., 2007).

The hypothalamic–pituitary–adrenal (HPA) axis and glucocorticoids contained therein can significantly influence an individual's response to stress (Juruena et al., 2004). Glucocorticoids are released during a stressful event and bind to glucocorticoid (GR) or mineralocorticoid (MR) receptors. GR is expressed in several regions of the brain, and they can bind both natural and synthetic glucocorticoids (Buckingham, 2006; De Kloet et al., 2008). Characteristically, GR has low affinity for corticosterone and are occupied primarily during stress and the circadian peak (Khaksari et al., 2007). In the brain, MR is capable of binding natural glucocorticoids but not some synthetic glucocorticoids (e.g., dexamethasone). MR is located mostly in limbic structures, such as the hippocampus, amygdala, and prefrontal cortex (De Kloet et al., 2008). Unlike GR, MR has high affinity for corticosterone and are almost saturated under basal conditions (Khaksari et al., 2007). GR and MR are nuclear receptors, and they both mediate the genomic actions of glucocorticoids. However, the existence of low-affinity membrane MR that mediates the initial phase of a stress response has recently been proposed (Joëls et al., 2008). Furthermore, membrane GRs have also been reported. These MR and GR membrane receptors are associated with rapid, non-genomic actions of glucocorticoids. For example, membrane MR activation enhances glutamate release in CA1 hippocampal neurons, reduces voltage-gated potassium conductance, and increases endocannabinoid release (Oitzl et al., 2010; Prager and Johnson, 2009), whereas GR activation has been associated with an increase in γ-aminobutyric acid-A receptor-mediated currents, a reduction in *N*-methyl-D-aspartate-mediated calcium currents, and a change in mitochondrial membrane potential that can result in the release of cytochrome C and apoptosis (Prager and Johnson, 2009; Zhang et al., 2006).

Glucocorticoids have been demonstrated to play an important role in aversive memory, an effect that depends of the specific memory phase (Roozendaal, 2002; Roozendaal et al., 2006). In studies using conditioned fear, Thompson et al. (2003) showed that rats treated with corticosterone before fear conditioning exhibited improvements in memory. Roozendal

*Abbreviations:* GR, glucocorticoid receptor; HPA, hypothalamic–pituitary–adrenal; MR, mineralocorticoid receptor; PTSD, posttraumatic stress disorder.

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and co-workers reported that systemic or intra-amygdala infusions of GR, but not MR, antagonists impaired memory consolidation of an inhibitory avoidance task in rats (Quirarte et al., 1997). These authors also reported, however, that glucocorticoids appeared to impair memory retrieval (De Quervain et al., 1998). Abrari et al. (2008) found that corticosterone administration after the reactivation of a fear memory reduced the expression of this memory, whereas Tronel and Alberini (2007) found that memory of an inhibitory avoidance task was interrupted by GR blockade immediately after memory reactivation. MR also appear to play a role in memory because MR blockade prevented corticosterone-induced impairment of memory retrieval (Khaksari et al., 2007). These preclinical studies indicate that glucocorticoids may be a potential pharmacological treatment for some stress-related memory symptoms. One small clinical pilot study of PTSD patients ($n=3$) showed that a small daily dose of cortisol reduced the frequency or intensity of feelings associated with a traumatic event (Aerni et al., 2004).

Fear conditioning in rodents may help elucidate some aspects of memory symptoms induced by stress. Fear conditioning is induced in laboratory animals by pairing a neutral (conditioned) stimulus, such as a context or tone, with a fear-inducing (unconditioned) stimulus, such as a footshock. Presentation of the context in which the conditioned stimulus (CS) occurred or presentation of the tone causes stereotypical responses in the animal, including fear responses and a glucocorticoid response (Pamplona et al., 2006; Tronel and Alberini, 2007). In rats, crouching behavior is typically used as a fear index (Blanchard and Blanchard, 1969; Pamplona et al., 2006). In contextual fear conditioning, the CS is an ill-defined multiple sensory stimulus that is continuously presented during the training and testing sessions. In contrast, in cued fear conditioning, a well-defined and unimodal stimulus (e.g., tone) predicts the occurrence of the unconditioned stimulus (e.g., footshock). Moreover, cued fear conditioning appears to be related to the lateral amygdala, whereas contextual fear conditioning has been related to the basal amygdaloid nucleus (Pamplona et al., 2006; Yaniv et al., 2004). In PTSD, both types of conditioning likely occur, which can differentially contribute to symptom development.

The objective of the present study was to evaluate the effects of the GR agonist dexamethasone, the GR antagonist mifepristone, the MR agonist fludrocortisone, and the MR antagonist spironolactone on the extinction of fear-related memory in rats. Propranolol, a β-blocker that has shown some clinical efficacy in PTSD patients and in preclinical animal models of fear conditioning, was used as a positive control (Do Monte et al., 2008; Kroon and Carobrez, 2009; Vaiva et al., 2003). We hypothesized that glucocorticoid manipulations may impact the extinction of aversive memories. Because memory extinction is related to a learning process (Cammarota et al., 2007), an effect resulting from administration of a drug before the retrieval test may be attributable to either impairment of memory retrieval or an increase in memory consolidation (i.e., the new association between the cue [context] and the absence of the unconditioned stimulus [footshock]). Thus, when a drug effect is detected following pre-test administration, evaluating the drug's effect following post-test administration is also important. Additionally, because an anxiolytic-like effect could alter immobility behavior in the chamber, we also evaluated the effect of a drug that enhances extinction in the elevated plus maze.

## 2. Experimental procedures

### 2.1. Animals

Adult male Wistar rats weighing between 220 and 300 g were used. The animals were housed in plastic cages (41×32×16.5 cm), with five rats per cage and food and water available *ad libitum*. They were maintained in a temperature-controlled room (23±2 °C) under a 12 h/12 h light/dark cycle (lights on at 7 am). The behavioral experiments were conducted during the light phase of the cycle (between 9 am and 2 pm). All experiments and procedures were performed according to the Guidelines of Animal Care established by the UFPR Ethics Committee of Experiments with Animals (protocol no. 349). These guidelines were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication no. 80-23, revised 1996). All efforts were made to minimize the number of rats used and their suffering.

### 2.2. Drugs and treatment

DL-propranolol (Sigma, St. Louis, MO, USA) was dissolved in 0.9% NaCl (saline) and administered subcutaneously at a dose of 10 mg/kg. Dexamethasone disodium phosphate (Hipolabor, Belo Horizonte, MG, Brazil) was also dissolved in saline and administered intraperitoneally (i.p.) at three different doses: 0.5, 1, and 5 mg/kg. Mifepristone (Sigma) was dissolved in propylene glycol and administered subcutaneously at doses of 10 and 25 mg/kg. Spironolactone (Sigma) was also dissolved in propylene glycol and administered subcutaneously (s.c.) at a dose of 10 mg/kg. Fludrocortisone (Sigma) was dissolved in 0.5% carboxymethylcellulose, 0.4% Tween-80, 0.9% benzilic alcohol, and saline and administered i.p. at doses of 5 and 10 mg/kg. All drugs were administered at a constant volume of 1.0 ml/kg.

### 2.3. Contextual fear conditioning

The conditioning chamber consisted of a box measuring 26×31.5×21 cm (Insight, Ribeirão Preto, SP, Brazil). Three sides of the box were made of steel, and the fourth side was made of Plexiglas, which allowed for the behavioral analysis of the animal. The bottom of the box consisted of metal rods connected to an electrical source. An electric footshock was delivered through this device.

Contextual fear conditioning (training session) was conducted as described by Pamplona et al. (2006). The rats were placed in the conditioning chamber for 3 min, and then received an electric footshock (1.5 mA, 1 s duration). The animals remained in the box for 1 min after the shock and were then returned to their home cages. For 3 consecutive days, the animals were reexposed to the conditioning chamber for 9 min without receiving the shock (test sessions 1–3).

The freezing behavior of each animal was used as an index of memory following non-reinforced reexposure to the context (test sessions). An animal was considered frozen when it presented a stereotypical crouching position with complete immobility, with the exception of respiration.

To evaluate the effect of each drug on memory extinction, the animals were treated with one of the drugs before each reexposure (test sessions). An exception was Experiment 5, in which animals received the drug after test sessions 1 and 2.

Six related treatment protocols were devised to compare the effects of selected GR and MR drugs on memory compared with vehicle controls. In Experiment 1, we evaluated the effect of propranolol on fear memory extinction by treating animals with DL-propranolol (10 mg/kg, s.c.) or saline solution 20 min before each extinction session. Experiment 2 similarly examined the effect of dexamethasone (0.5, 1.0, or 5.0 mg/kg, i.p.) or saline solution 20 min before each reexposure to the conditioning chamber. Experiment 3 studied the effect of mifepristone, a GR antagonist, on the extinction of fear memory. In this case, the animals were treated with mifepristone (10 or 25 mg/kg, s.c.) or propylene glycol 1 h before each extinction session. Experiment 4 evaluated the effect of spironolactone (10 mg/kg, s.c.), an MR antagonist, and propylene glycol on the extinction of fear memory 1 h before each extinction session. Experiment 5 was similar to Experiment 4, with the exception of the drug administration schedule. The animals were treated with spironolactone (10 mg/kg, s.c.) or propylene glycol, but the doses were administered 1–5 min after test sessions 1 and 2, such that studying the effects of this drug on the consolidation of new learning was possible. Experiment 6 was similar to Experiments 3 and 4, with the exception that fludrocortisone (5 and 10 mg/kg, i.p.), an MR