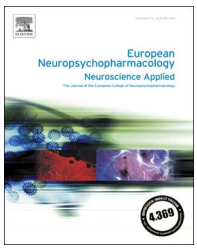
European Neuropsychopharmacology (▮▮▮▮) ▮, ▮▮▮–▮▮▮

www.elsevier.com/locate/euroneuro



Evidence for an expanded time-window to mitigate a reactivated fear memory by tamoxifen

Thiago R. da Silva[a], Reinaldo N. Takahashi[b], Leandro J. Bertoglio[b], Roberto Andreatini[a], Cristina A.J. Stern[a,*]

[a]Department of Pharmacology, Federal University of Paraná, Curitiba, PR, Brazil
[b]Department of Pharmacology, Federal University of Santa Catarina, Florianopólis, SC, Brazil

Received 17 June 2016; received in revised form 25 July 2016; accepted 5 August 2016

KEYWORDS
Memory reconsolidation;
Memory persistence;
Protein kinase C;
Tamoxifen

Abstract
The mechanisms underpinning the persistence of emotional memories are inaccurately understood. Advancing the current level of understanding with regards to this aspect is of potential translational value for the treatment of post-traumatic stress disorder (PTSD), which stems from an abnormal aversive memory formation. Tamoxifen (TMX) is a drug used in chemotherapy for breast cancer and associated with poor cognitive performances. The present study investigated whether the systemic administration of TMX (1.0–50 mg/kg) during and/or beyond the reconsolidation time-window could attenuate a reactivated contextual fear memory in laboratory animals. When administered 0, 6 or 9 h (but not 12 h) post-memory retrieval and reactivation, TMX (50 mg/kg) reduced the freezing behavior in male rats re-exposed to the paired context on day 7, but not on day 1, suggesting a specific impairing effect on memory persistence. Importantly, this effect lasts up to 21 days, but it is prevented by omitting the memory retrieval or memory reactivation. When female rats in the diestrous or proestrous phase were used, the administration of TMX 6 h after retrieving and reactivating the fear memory also impaired its persistence. Altogether, regardless of the gender, the present results indicate that the TMX is able to disrupt the persistence of reactivated fear memories in an expanded time-window, which could shed light on a new promising therapeutic strategy for PTSD.

1. Introduction

The mechanisms underlying the persistence of aversive post-acquisition memories have long been studied (Bekinschtein et al., 2007; Shema et al., 2009; Katche et al., 2010; Migues et al., 2010). Convergent evidence has implicated a delayed

*Correspondence to: Departamento de Farmacologia – Anexo I, Centro Politécnico, Universidade Federal do Paraná, Rua Coronel Francisco Heráclito dos Santos 100, Jardim das Américas, 81531-980, Curitiba, PR, Brazil. Fax: +55 41 3266 2042.
E-mail address: cristinastern.cs@gmail.com (C.A.J. Stern).

http://dx.doi.org/10.1016/j.euroneuro.2016.08.005
0924-977X/

Please cite this article as: da Silva, T.R., et al., Evidence for an expanded time-window to mitigate a reactivated fear memory by tamoxifen. European Neuropsychopharmacology (2016), http://dx.doi.org/10.1016/j.euroneuro.2016.08.005

wave of protein synthesis (Bekinschtein et al., 2007; Katche et al., 2010), and the brain-specific protein kinase C isoform PKM-zeta in this process (Laferrière et al., 2011; Sacktor, 2012). At the post-retrieval stage, the disrupting effect of protein synthesis inhibition in memory persistence has also been reported (Nakayama et al., 2013). However, it is still unknown whether PKC has a corresponding role in the persistence of aversive memories following their retrieval and reactivation. Advancing the current understanding about the latter aspect is of a potential translational value for the post-traumatic stress disorder (PTSD) treatment because it may highlight a target for intervention during, and even beyond, the theoretical time-window (~6 h; Nader et al., 2000) in which aversive memory reconsolidation takes place.

Pharmacological interventions after memory retrieval have shown to be able to attenuate abnormal memories present in psychiatric conditions such as PTSD (Parsons and Ressler, 2013). PTSD symptoms are often associated with breast cancer and its treatment (Hermelink, 2015). Women receiving adjuvant therapy with tamoxifen (TMX) demonstrate poor memory performance (Boele et al., 2014), but to the best of our knowledge, no preclinical study has evaluated the effects of TMX on reactivated aversive memories. TMX is a selective estrogen-receptor modulator: depending on the tissue, it acts as an agonist or as an antagonist of alpha- and beta-estrogen receptors (Wang et al., 2002; Eigeliene et al., 2016). TMX is also able to inhibit the activity of PKC isoforms in rodents and humans in a dose ranging of 1 to 200 mg/kg (Abrial et al., 2013; Armani et al., 2014).

Based on the above mentioned, and on the safe profile of TMX use in humans (Armani et al., 2014), in the present study we tested the hypothesis that the administration of TMX at certain time points after retrieval and reactivation of a fear memory would promote a specific delayed effect on its persistence either in male and female rats. We showed that: (1) TMX impaired memory persistence when administered 0, 6 or 9 h markers but not at the 12 h marker, after memory reactivation; (2) when administered immediately after memory reactivation, TMX does not impair memory reconsolidation; (3) the TMX-delayed effect lasts up to 21 days; (4) The TMX effect on memory persistence depends on memory retrieval and reactivation; (5) The effect of TMX on fear memory persistence is not gender-specific.

2. Experimental procedures

2.1. Animals

Experimental animals were male and female Wistar rats weighing from 250 to 300 g provided by the Biological Sciences Sector of The Federal University of Paraná. They were housed in groups of five in plexiglas cages measuring $60 \times 25 \times 25$ cm, kept at a controlled temperature of 23 ± 2 °C, exposed to a light/dark cycle of 12 h and with food and water provided *ad libitum*. The experiments were conducted after the approval from the experimental protocol branch for the Ethical Committee for the care and use of laboratory animals located in the Biological Sciences Sector of The Federal University of Paraná (CEUA 856), in compliance with Brazilian legislation.

2.2. Drugs

TMX (1.0–50 mg/kg, Sigma, USA) and nimodipine (16 mg/kg, Sigma USA), an antagonist of the L-type voltage-gated calcium channels (LVGCCs), were dissolved in a 5% mixture of polyoxyethylene sorbitan monooleate (Tween 80) and saline (Einat et al., 2007; Steckert et al., 2012; Abrial et al., 2013; Crestani et al., 2015). The benzodiazepine midazolam (1.5 mg/kg, Cristalia, Brazil) was diluted in saline and utilized as a memory reconsolidation blocker (Stern et al., 2012). All drugs were administered through intraperitoneal injection in a volume of 1.0 ml/kg.

2.3. Apparatus

The contextual fear conditioning session was held in a chamber named Context A ($26 \times 31.5 \times 21$ cm; Insight, Brazil) which was constructed of aluminum sidewalls, and incorporated a plexiglas front wall and top cover. The floor consist of stainless-steel bars (3 mm diameter and spaced 0.9 mm apart) which are connected to a shock generator (Insight, Brazil). A neutral chamber named Context B ($34 \times 26 \times 33$ cm) had transparent plexiglas walls and a black lid in order to provide contextual cues as different as possible from those of Context A. Context B was used to evaluate fear generalization or as a context unable to induce fear memory reactivation.

2.4. General procedures and data collecting

Following international standards for care and handling of scientific experimental animals, all experimental procedures were designed in order to minimize the number of animals used. Experiments were performed during the same period of the day; between12:00 and 5:00 PM, in order to minimize the circadian behavioral changes in rodents. All animals were acclimatized to the experimenter and experiment room for 30 min before each experimental session. The trial rooms were maintained at a controlled temperature of (22 ± 2 °C) and maintained a 78 lux brightness throughout the experiment. During the first day, the animals were familiarized in the Context A for 3 min. After 24 h, the animals were submitted to the conditioning session in Context A. After the initial 30 s, the animals received three footshocks (0.6 mA/3 s and 30 s intervals), the unconditioned stimulus (US). After the last shock, the animal remained for 30 s in the conditioning chamber. One day later, the animals were re-exposed to Context A for 3 min, without the US being administered, to induce memory reactivation. Pharmacological treatment was given 0, 6, 9 or 12 h after this session. After 24 h, the animals were exposed to Test A_1 (context A for 3 min). Seven days later, they were re-exposed to Context A for 3 min in a session called Test A_2. When necessary, the animals were exposed to Test A_3, 21 days after memory reactivation (experiment 3). The unpaired and neutral Context B was used to avoid memory reactivation (experiment 4). In all experiments, animals were exposed to a neutral and unpaired Context B (Test B) for 3 min 24 h after Tests A_1 and A_2, aiming at investigating the possibility of generalized fear expression. Since no fear generalization was observed in any case, Test

Please cite this article as: da Silva, T.R., et al., Evidence for an expanded time-window to mitigate a reactivated fear memory by tamoxifen. European Neuropsychopharmacology (2016), http://dx.doi.org/10.1016/j.euroneuro.2016.08.005