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Long-term effects of traumatic stress on subsequent contextual fear conditioning in rats

Rie Ryoke, Kazuo Yamada *, Yukio Ichitani

Department of Psychology and Behavioral Neuroscience, University of Tsukuba, Tsukuba, Ibaraki 305-8577, Japan

HIGHLIGHTS

- Exposure to stressful events affects subsequent sensitivity to fear.
- Long-term effect of multiple stress on subsequent fear conditioning was examined.
- A single multiple stress enhanced subsequent conditioned fear for at least 30 days.
- Stress-induced sensitization of fear was enhanced by a situational reminder.
- Pretreatment with metyrapone did not affect the sensitization of fear.

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ABSTRACT

Exposure to stressful events affects subsequent sensitivity to fear. We investigated the long-term effects of a traumatic experience on subsequent contextual fear conditioning and anxiety-like behaviors in rats (Experiment 1). In addition, we tested whether the administration of the glucocorticoid synthesis inhibitor metyrapone (MET) attenuated the sensitization of fear induced by traumatic stress (Experiment 2). Male rats were subjected to a multiple stress (MS) session, which consisted of 4 foot shocks (1 mA, 1 s) and forced swimming for 20 min, followed by exposure to a situational reminder 7 days after the MS session. MET (25 or 100 mg/kg, intraperitoneal) was administered 30 min before MS. The contextual fear conditioning was performed 14 days after MS. MS enhanced the conditioned fear response for at least 14 days after the conditioning, and pretreatment with MET did not affect the enhancement of conditioned fear. These results suggest that glucocorticoid secretion triggered by MS is not involved in regulating the long-term stress-induced sensitization of fear.

1. Introduction

It is well recognized that stressful or emotionally arousing experiences enhance learning and memory. These reactions represent adaptive phenomena aimed at increasing the ability to avoid life threat that has been previously encountered. However, the memory of a danger may lead to long-lasting behavioral changes. Individuals who have faced traumatic events can often vividly retrieve the traumatic experiences to the extent that fright and anxiety are sustained. For example, posttraumatic stress disorder (PTSD) is an anxiety disorder that may develop after exposure to a strongly traumatic event.

Stress, fear, and anxiety are associated with learning and memory processes in animals [1]. For example, Pavlovian fear conditioning is a behavioral paradigm typically used to evaluate the strength of aversive memory in rodents [2] and it has been reported that previous exposure to a stressful treatment enhances subsequent fear conditioning [3–5]. This phenomenon may correspond to the hyper-sensitized reaction observed in patients with PTSD, in whom the magnitude of the response is more appropriate to the original traumatizing event than it is to current conditions [6].

Long-term effects of exposure to a single stressor have been reported previously and, the search for putative animal models of PTSD has focused on these long-term consequences [7]. A few previous studies have reported that a single session of stress can have long-lasting effects, including development of anxiety-like behaviors and neurobiological changes. For example, a social stress was able to induce long-lasting behavioral changes suggestive of enhanced anxiety and depression-like symptoms [8]. In a different study, ketoconazole, an inhibitor of steroid synthesis, prevented the long-lasting effects of predator stress on an anxiety-like behavior [9]. Pynoos et al. [10] reported that, after a severe shock, mice exposed to a situational reminder (without shocks) showed enhanced startle reflexes in an acoustic startle response test. This experimental paradigm was based on the finding that, with the exception of

* Corresponding author. Tel./fax: +81 29 853 2961.
E-mail address: kayamada@human.tsukuba.ac.jp (K. Yamada).

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repeated trauma, traumatized individuals are often confronted with reminders of the traumatic event, but not with the traumatic event itself. The authors suggested that situational reminders of the traumatic event contributed to the reactivity in, and chronic aspects of, PTSD [10].

The biological mechanisms responsible for the stress-induced enhancement of conditioned fear have not yet been elucidated. Interestingly, Rau et al. [4] demonstrated that the stress-induced enhancement of conditioned fear was not simply the summation or generalization of fear, because pre-stress administration of an *N*-methyl-D-aspartate (NMDA) receptor antagonist (which generally prevents memory consolidation and retrieval) did not prevent stress-enhanced fear learning. Therefore, it was suggested that enhanced fear memory might not depend on associative memory of the traumatic event.

Corticosterone (CORT) is a glucocorticoid that has complex effects on cognitive and emotional functions. For example, research in learning and memory has reported that CORT has multifaceted actions on memory processes, such as enhancing the acquisition of new information and impairing the retrieval of memory [11]. In addition, many studies have indicated that administration of CORT can facilitate fear conditioning, while a pre- and post-training injection of either a CORT synthesis inhibitor or a glucocorticoid receptor antagonist impaired fear conditioning [3,12]. Multiple studies have demonstrated that administration of either a glucocorticoid synthesis inhibitor or a glucocorticoid receptor antagonist before exposure to restraint stress prevented the anxiogenic effects of the stressor [13,14].

It is well established that exposure to stress activates the hypothalamus–pituitary–adrenal (HPA) axis, which results in CORT secretion. Therefore, the first aim of the present study was to examine whether a multiple stressor (MS), consisting of foot shocks and forced swimming, had long-term effects on anxiety-like behavior and contextual fear conditioning. Compared to just foot shocks or forced swimming, MS strongly affected subsequent conditioned fear in our preliminary experiments. The hypothesis was that the stress-induced secretion of CORT during a traumatic event contributes to the post-trauma enhancement of conditioned fear. An additional aim was to investigate whether a situational reminder of the traumatic event enhanced the effects of the MS. A final goal was to investigate whether pre-MS treatment with metyrapone (MET), a glucocorticoid synthesis inhibitor, affected the MS-induced enhancement of conditioned fear. The current results suggest that glucocorticoid secretion, triggered by MS, is not involved in regulating long-lasting stress-induced behavioral changes.

2. Material and methods

2.1. Animals

Adult male Wistar–Imamichi rats (age range 8–12 weeks) were purchased from the Institute for Animal Reproduction (Ibaraki, Japan). Rats were housed in individual cages and maintained on a 12 h/12 h light/dark cycle at a controlled ambient temperature (23 ± 1 °C). All experiments were carried out according to the guidelines for the Care and Use of Animals approved by the University of Tsukuba Committee on Animal Research.

2.2. Drugs

The glucocorticoid synthesis inhibitor, 2-methyl-1,2-di-3-pyridyl-1-propanone (metyrapone (MET); Sigma-Aldrich, St. Louis, MO, USA), was dissolved in a solution of 45% 2-hydroxypropyl-β-cyclodextrin (HBC; Sigma-Aldrich). MET inhibits enzymatic conversion, by 11-beta-hydroxylase, of deoxycorticosterone to CORT, thus inhibiting CORT synthesis and subsequent release into the bloodstream. MET (dose: 25 or 100 mg/kg, volume: 1 mL/kg, IP) was injected 30 min before the rat was placed in a foot-shock chamber, used to deliver MS. The MET doses were chosen based on previous studies performed in rats exhibiting the effects of fear conditioning and anxiety-like behavior [15,16]. Control rats received a similar volume of 45% HBC solution.

2.3. Apparatuses

2.3.1. Multiple stress

In this study, 2 different shock chambers were used to deliver the electric foot shocks (Context A: 30 × 25 × 30 cm, O'Hara & Co., Ltd., Tokyo, Japan) and to conduct the fear conditioning (Context B). These chambers differed in their context, including the illumination, background, and sound. Electric shocks (used as the MS) were delivered in Context A, which was positioned inside a sound-attenuating chamber (100 × 45 × 60 cm, Tech Serv. Inc., MD). The sidewalls consisted of opaque black acrylic (Plexiglas, Dow Chemical, Philadelphia, USA) and the back and the front walls consisted of clear acrylic (Plexiglas, Dow Chemical) attached to a sheet of paper with a black stripe. The lid consisted of clear acrylic placed below an incandescent bulb. For the delivery of scrambled shocks, the floor consisted of 19 steel rods (diameter: 5 mm) spaced 1.5 cm apart and wired to a shock generator (O'Hara & Co., Ltd.). Forced swimming was performed in an opaque-blue plastic bucket (height: 50 cm, diameter: 40 cm). The bucket was filled with water to a depth of 30 cm.

2.3.2. Open field

The locomotor behavior of rats was videotaped in an open field test (OFT) chamber. The chamber was an open-top box (90 × 90 × 45 cm) that consisted of black polyvinylchloride walls and a gray floor (O'Hara & Co., Ltd.). The box was illuminated by 4 incandescent bulbs installed on the ceiling. The brightness of the center of the floor was 52.5 lx. A video camera was placed above the chamber.

2.3.3. Fear conditioning

An automated computer-controlled system (O'Hara & Co., Ltd.) was used in the habituation, conditioning, and retention test phases. The fear-conditioning chamber (Context B: 25 × 20 × 30 cm) was constructed of clear acrylic walls and included a lid with a hole in the center (O'Hara & Co., Ltd.). For delivering electric shocks, the Context B chamber was equipped with a grid floor made of 16 stainless-steel rods (diameter: 5 mm, 10 mm apart). The chamber was located within a sound-attenuating box (70 × 60 × 60 cm; Muromachi Kikai Co., Ltd., Tokyo, Japan) with white inside walls and a ventilation fan that provided fresh air, background noise (50 dB), and illumination (200 lx).

2.4. Procedure

2.4.1. Experiment 1

2.4.1.1. Multiple stress (MS). Rats were randomly assigned to either an MS group or non-stressed (NMS) group. Rats were gently transported to the behavioral experimentation room in a stainless-steel black box (transportation box, 20 × 40 × 20 cm) that consisted of opaque black walls and lid and an opaque-black smooth polypropylene floor. Rats were left in the transportation box for 20 min after transportation, prior to placement in the respective testing apparatuses. Rats in the MS group were first placed into the Context A chamber. They were left in that for approximately 4 min, and then received 4 electric foot shocks (1 mA, duration: 1 s) with an inter-shock interval that varied from 4 to 6 min (total time in Context A: 25 min). The rats in the MS group were then placed in the forced swimming chamber and were subjected to forced swimming for 20 min immediately after footshocks. Rats in the NMS group were merely exposed to the Context A chamber, but did not receive the shocks (total time in Context A: 25 min). Following Context A, NMS rats were then placed into a plastic cage with new bedding (Waiting period, duration: 20 min). After either forced swimming (MS group) or the waiting period (NMS group), all animals were returned to their room in the transportation box. The procedure for this experiment is summarized in Fig. 1.