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Post-reexposure administration of riluzole attenuates the reconsolidation of conditioned fear memory in rats

Kie Akagi [a, b], Misa Yamada [a], Akiyoshi Saitoh [a], Jun-Ichiro Oka [b], Mitsuhiko Yamada [a, *]

[a] Department of Neuropsychopharmacology, National Institute of Mental Health, National Center of Neurology and Psychiatry, Tokyo, Japan
[b] Laboratory of Pharmacology, Faculty of Pharmaceutical Science, Tokyo University of Science, Chiba, Japan

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ABSTRACT

Recently, we demonstrated that riluzole, which has been shown to block the glutamatergic system, facilitates fear extinction in rats. Here, we undertook experiments on contextual fear conditioning to clarify the actions of riluzole on the reconsolidation of fear memory in rats. We used the fast-acting benzodiazepine midazolam as a reconsolidation-inhibiting control drug. We demonstrated that riluzole (3 mg/kg) and midazolam (1 mg/kg) impaired the reconsolidation of contextual fear memory. Results from spontaneous recovery experiments also suggested that riluzole attenuated reconsolidation. Indeed, conditioned fear did not recover spontaneously 4 weeks after a short (3 min) reexposure and riluzole administration, whereas it recovered after a long (10 min) reexposure. Using western blotting, we demonstrated that a short reexposure increased the phosphorylation of cyclic adenosine monophosphate response element binding protein significantly in the dorsal part of hippocampus, but not in the medial prefrontal cortex. Interestingly, this phosphorylation was attenuated by riluzole with short reexposure. In addition, bilateral microinjection of riluzole (2 μM/0.2 μl/side) directly into the dorsal hippocampus clearly attenuated the reconsolidation. These findings suggested that the attenuating effect of riluzole on the reconsolidation of fear memory involves, at least in part, the dorsal part of the hippocampus. In conclusion, we demonstrated that riluzole attenuates the reconsolidation of fear memory in rats.

1. Introduction

Previously, we demonstrated clearly that riluzole, which has been shown to block the glutamatergic system, shows anxiolytic-like effects in behavioral tests for anxiety (including the elevated plus-maze test, light–dark test and open-field test) in rats (Sugiyama et al., 2012). Recently, we demonstrated that riluzole facilitates fear extinction using the contextual fear-conditioning test in rats (Sugiyama et al., 2015). Contextual fear conditioning in experimental animals is a well-studied preclinical model of the aversive expectations of danger that characterize anxiety in patients (Grillon, 2002).

Another process has an opposing action on the fate of fear memory: reconsolidation. Studies using experimental animals have demonstrated that, depending on the duration of the exposure to the context, reexposure to the context induces the extinction learning or the reconsolidation of fear memory. A longer reexposure to the context without an aversive stimulus can induce extinction learning of fear memory (Myers and Davis, 2002). Conversely, a short reexposure to the context elicits characteristic fear responses, including conditioned freezing. The consolidated memory is then destabilized and reconstructed into a more stable state: reconsolidation (Nader et al., 2000; Sara, 2000). Reconsolidation serves to stabilize or strengthen the memory. Previously, we reported that riluzole may attenuate reconsolidation of fear memory if administered before a short reexposure to the context in rats (Sugiyama et al., 2015). However, whether riluzole truly attenuates reconsolidation of fear memory is not known.

To answer this important question, we undertook experiments on contextual fear conditioning with post-reexposure administration of drugs in the present study. As described above, riluzole has an anxiolytic-like effect in rats (Sugiyama et al., 2012). Therefore, to avoid some possible influences of riluzole on the basal anxiety level or reactivation process, rather than on the processes of

* Corresponding author. Department of Neuropsychopharmacology, National Institute of Mental Health, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashimachi, Kodaira, Tokyo 187-8553, Japan.
E-mail address: mitsu@ncnp.go.jp (M. Yamada).

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reconsolidation, it was necessary to administer the drug after reexposure to the context.

In addition, we carried out experiments on contextual fear conditioning using a relatively mild protocol for fear conditioning to clarify the actions of riluzole on the reconsolidation of fear memory. The potential reconsolidation–extinction interface could be affected by the strength of an association between context and fear. In our previous study, we conducted 2 days of fear conditioning (60 shocks over 2 days), as opposed to more traditional protocols (Sugiyama et al., 2015). Thus, the large prediction error during short-reexposure to the context could lead to alteration of any potential extinction–reconsolidation interface towards faster extinction learning. Therefore, to avoid the possible influence of conditioning levels on the potential extinction–reconsolidation interface, it was necessary to employ a relatively mild protocol for fear conditioning.

Furthermore, to distinguish the effect of riluzole on reconsolidation from that on fear extinction, we employed two additional experiments. First, we conducted spontaneous recovery tests. With the passage of time, a reduced fear response to a conditioned stimulus by extinction learning recovers spontaneously, which indicates that extinction learning does not erase or delete the conditioned memory, but that a new inhibitory association is formed, which competes with the original fear memory (Mamiya et al., 2009; Monfils et al., 2009; Quirk and Mueller, 2008). However, it has been reported that disrupting reconsolidation prevents memory restoration and produces amnesia of the original conditioned fear memory (Monfils et al., 2009). Therefore, if riluzole attenuates reconsolidation of fear memory in rats, a reduced fear response to the context would not recover with the passage of time. Then, to understand the attenuating effect of riluzole on the reconsolidation at the molecular level, we examined the phosphorylation of cyclic adenosine monophosphate response element binding protein (CREB) in the dorsal part of the hippocampus and compared the data with those obtained in the medial prefrontal cortex. It has been reported that a CREB-dependent process is engaged in the dorsal part of the hippocampus after brief reexposure to the context, but not after the prolonged reexposure (Mamiya et al., 2009). If riluzole attenuates the reconsolidation of fear memory in rats, increased phosphorylation of CREB would be attenuated upon riluzole treatment. Finally, we microinjected riluzole directly into the dorsal hippocampus to confirm the possible involvement of this region of the brain in the reconsolidation process.

Here, we clarified the effects of riluzole on the reconsolidation of fear memory in rats and compared them with those on fear extinction.

2. Materials and methods

2.1. Ethical approval of the study protocol

The present study was conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee of the National Center of Neurology and Psychiatry (approval number, 2017014; Tokyo, Japan).

2.2. Animals

Male Wistar/ST rats, aged 8 weeks at the time of conditioning, were used (SLC, Shizuoka, Japan). The rats had free access to food and water in an animal room maintained at 23 ± 1 °C with a 12-h light–dark cycle (lights switched on automatically at 8 a.m.). Rats were maintained in this environment for ≥1 week before experimentation.

2.3. Drugs

We used riluzole hydrochloride (Tocris Bioscience, Ellisville, MO, USA) and compared it with a benzodiazepine midazolam (Sandoz Japan, Tokyo, Japan) which are known to be inhibitory agents for the reconsolidation of contextual fear memory. Riluzole at 0.1, 0.3, 1, and 3 mg/kg (s.c.) was dissolved in saline (Otsuka Pharmaceuticals, Tokushima, Japan) and used for systemic injection. Midazolam was dissolved in saline. The dose of midazolam (1 mg/kg, s.c.) was determined based on a previous study (Bustos et al., 2006).

For the microinjection study, riluzole was dissolved in phosphate buffered saline (PBS) solution (pH 7.4). The dose of riluzole (2 μM/0.2 μl/side) was based on our previous study (Sugiyama et al., 2017, 2018). Medetomidine (0.4 mg/kg, s.c., ZENOAQ, Tokyo, Japan), midazolam (2.0 mg/kg, s.c., SANDOZ, Tokyo, Japan), and butorphanol (2.5 mg/kg, s.c., Meiji Seika, Tokyo, Japan) were used as a mixed anesthetic agent (Gotoh et al., 2017). Benzylpenicillin potassium (2×10^4 U/kg, i.m., Meiji Seika, Tokyo, Japan) and atipamezole (0.75 mg/kg, s.c., ZENOAQ, Tokyo, Japan) were used after the surgery.

2.4. Conditioning apparatus

The training, reactivation, and testing of contextual fear conditioning took place in a fear-conditioning chamber (MK-450RSQ; Muromachi Kikai, Tokyo, Japan) of dimension 28 × 22 × 50 cm located in a sound-attenuating room (MC-050; Muromachi Kikai). The illumination on the floor of the chamber was 40 ± 15 lux. The front wall and ceiling of the chamber were made of clear acrylic, whereas the lateral and rear walls were made of opaque plastic. The floor of the chamber consisted of 32 stainless-steel rods (diameter, 4 mm), spaced 1 cm apart and wired to a shock generator (SGS-003DX; Muromachi Kikai). The chamber was cleaned with 70% ethanol before and after each experiment.

2.5. Behavioral procedure

The contextual fear-conditioning test consisted of three procedures carried out on respective days: conditioning, reactivation, and test session. At the beginning of each experimental day, rats were replaced from the animal room to the experimental room and habituated for ≥1 h.

2.5.1. Contextual fear-conditioning

In the fear-conditioning trial, each animal experienced a single session of fear conditioning, as described previously (Ribeiro et al., 2013; Saitoh et al., 2017) with minor modifications. In brief, each rat was placed in the fear-conditioning chamber and habituated to the apparatus for 3 min (pre-shock period), and 3 footshocks (1 s, 0.4 mA) were delivered subsequently, 40 s apart. After the final footshock, rats remained in the chamber for an additional 60 s (post-shock period) before being returned to their home cages.

2.5.2. Reactivation session

Twenty-four hours after conditioning, the rats were reexposed to the same apparatus for 3 min or 10 min. During each reactivation session, a footshock was not given, and the rat was monitored every 1 min by a trained observer to assess its freezing behavior via a monitor connected to a video-camera system mounted over the experimental chamber. Reexposure to the context induces reconsolidation or the extinction of fear memory, depending on the duration of the reexposure. The length of the reactivation session was determined by previous studies (Ribeiro et al., 2013; Saitoh et al., 2017). Immediately after the reactivation session, the animals were injected with drugs before being returned to their home