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MIDAZOLAM DISRUPTS FEAR MEMORY RECONSOLIDATION

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Abstract—The current research examines the influence of midazolam (MDZ) on memory reconsolidation using a contextual fear paradigm in rats, based on three context-shock training trials (0.7 mA, 3 s). First, we evaluate the effect of MDZ (1 mg/kg, i.p.) injected shortly after the training procedure. Second, we examined the influence of MDZ after a brief exposure (90 s) either in the training context (reactivation procedure) or in a neutral environment (no reactivation procedure) and one day later, freezing behavior was scored when rats were re-exposed to the training environment. Third, we investigate both the effect of MDZ administered at different times following reactivation on fear memory and the persistence of such effect 10 days after reactivation. Finally, we test whether the MDZ effect could be reverted by a single weak training trial (0.2 mA, 3 s) or by the presentation of the same unconditioned stimulus in the absence of the conditioned stimulus as a reminder which proves to induce significant freezing in rats not previously trained. Results show that MDZ interferes with the formation of a contextual fear memory only when administered after the reactivation procedure but not after the training procedure. This interference was effective up to 60 min after reactivation and not at a later time. No spontaneous recovery of freezing behavior was observed 11 days after MDZ injection which was not reverted by a weak training trial and by the unconditioned stimulus alone. All these data support the idea that stimulating GABA A receptor sites via MDZ selectively disrupts the reconsolidation process of a contextual fear memory. © 2006 Published by Elsevier Ltd on behalf of IBRO.

Key words: benzodiazepine, retrograde amnesia, GABA-A receptors, contextual aversive conditioning, reminder.

The formation of new memories requires a cascade of intracellular events which result in the passage of transient modifications into a stable memory trace. Immediately or shortly after learning, the new memory is vulnerable to agents that can block such process (McGaugh, 2000), whereas once this process is complete, this memory is consolidated and becomes insensitive to further manipulation, implying a time-limited role for memory interference (McGaugh, 1966).

In the last years, several studies have proposed the idea that recalling a previously consolidated aversive memory by a reminder—usually a non reinforced re-exposure to the conditioned stimulus (CS)—renders this memory susceptible to disruption again through a variety of amnesic procedures including different types of pharmacological agents (Przybyslawski and Sara, 1997; Przybyslawski et al., 1999; Nader, 2003; Debiec and Ledoux, 2004; Duvarci and Nader, 2004). The process involved after retrieval is usually referred to as reconsolidation (Przybyslawski and Sara, 1997; Nader et al., 2000; Sara, 2000; Nader, 2003) and as in the case of the consolidation process, the vulnerability of this reactivated information decreases as the interval between the reminder presentation and the amnesic procedure increases. For instance, inhibition of protein synthesis shortly after retrieval of an early aversive memory interferes with the expression of that memory, which remains insensitive to the same treatment following several hours (Nader et al., 2000). Although there is controversy regarding the reconsolidation hypothesis (Miller and Matzel, 2000; Lattal and Abel, 2004), the phenomenon of memory reconsolidation has been tested and confirmed using different learning paradigms (Przybyslawski et al., 1999; Sara, 2000; Nader et al., 2000; Suzuki et al., 2004; Gruest et al., 2004) and in a wide variety of species (Anokhin et al., 2002; Pedreira et al., 2002; Suzuki et al., 2004). According to the reconsolidation hypothesis, reactivating a previously consolidated memory by using an appropriate reminder would allow to selectively modify such memory by means of amnesic agents administered shortly after the reactivation procedure. Clinical and experimental findings have demonstrated the amnesic property of benzodiazepine agents (BDZ) apart from their well-known anxiolytic, sedative and muscle relaxant effects (Thiebot, 1985; Venault et al., 1986; McNamara and Skelton, 1991; McNamara et al., 1993). In fact, a vast amount of evidence has shown that these compounds administered pre-training and post-training induced amnesia in different species and in a variety of experimental learning procedures including fear conditioning (Harris and Westbrook, 2001). Depending on the particular behavioral paradigm used and the time of drug administration, deficits in acquisition, retention and/or expression of the learned response or in the expression of extinction have been observed following BDZ administration (Brioni, 1993; Barros et al., 1998; Harris and Westbrook 1998, 1999, 2001; Chapouthier and Venault, 2002).

A potential amnesic effect of these pharmacological agents acting on memory reconsolidation could not only have implications for the comprehension of the neural mechanisms associated with the reconsolidation process,

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Abbreviations: BDZ, benzodiazepine; CA, context A, conditioned context; CB, context B, novel context; CC, context C, different conditioned context; CS, conditioned stimulus; GABA-A, GABA A receptor; MDZ, midazolam; SAL, saline; US, unconditioned stimulus.

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but also can have potential clinical relevance, particularly for individuals suffering from emotional disorders. Thus, a pharmacological agent that would block "traumatic memories" reactivated by selective reminders could potentially provide an important therapeutically tool for the treatment of these disorders. Furthermore, in contrast to other procedures that disrupt memory reconsolidation (protein synthesis inhibitors, hypothermia, *N*-methyl-D-aspartate antagonists) which cannot obviously be used in humans, BDZ are widely prescribed for a number of disorders without major side effects when administered at appropriate doses and for relatively short lasting treatments.

In order to explore the influence of BDZ on memory reconsolidation, the present study evaluated the effect of midazolam (MDZ), a fast acting BDZ agent, on the reconsolidation process using a contextual fear conditioning paradigm. In this model, the unconditioned aversive stimulus (footshock) is associated with a complex environmental representation (context) in which the shock occurred. When placed back into this context, animals exhibit a number of conditioned responses, including freezing (Antoniadis and McDonald, 1999). Moreover, to determine the time window of efficacy of this potential amnesic procedure, MDZ was administered at different times following re-exposure to the context used for learning (reactivation procedure). In addition, MDZ-treated animals were assessed again in their freezing response 10 days after the test in the same context as that used during learning. True amnesia presumably would result in the lack of spontaneous recovery. Finally, in order to test if the potential amnesia after MDZ could be overcome by the exposure to a reminder cue, MDZ-treated animals were subsequently re-exposed to a weak, single footshock session in the training context. In an additional experiment, a shock reminder cue was used in a different chamber (context C, CC) and fear memory was tested one day later in the originally conditioned context. Finally, trained MDZ and SAL animals were subjected to another shock training in a different context and later tested in this context to evaluate whether MDZ could affect subsequent associative learning.

EXPERIMENTAL PROCEDURES

Animals

Adult male Wistar rats provided by the Facultad de Veterinaria, Universidad Nacional de La Plata weighing 270–300 g at the start of the experiments were used. All animals were housed in standards laboratory Plexiglas cages in groups of three per cage at the animal colony of the Department of Pharmacology of the Facultad de Ciencias Químicas, and left without manipulation for 2 weeks for acclimation. Food and water were available *ad libitum*. Animals were maintained on a 12-h light/dark cycle (lights on at 07:00–19:00 h) and a room temperature of 21–23 °C.

The protocols used were approved by the Animal Care Committee of the Facultad de Ciencias Quìmicas, Universidad Nacional de Córdoba consistent with the standards for the care and use of laboratory animals as outlined in the NIH Guide for the Care and Use of Laboratory Animals. The number of animals used, as well as their suffering, was kept to the minimum possible needed to accomplish the goals of this study.

Drugs

MDZ (Gobbi Novag S.A., Buenos Aires, Argentina) was diluted in sterile isotonic saline (SAL) (0.9% w/v) to a concentration of 1 mg/ml. MDZ was administered intraperitoneally (i.p.) at a dose of 1 mg/kg. The total volume of drug or an equivalent amount of SAL was 1.0 ml/kg in all cases. The dose of MDZ used has been previously shown to induce amnesia in an inhibitory avoidance paradigm (Barros et al., 1998).

Apparatus

The conditioning environment was designated as context A (CA); made of gray plastic (20×23×20 cm) with clear lid and the floor consisted of 10 parallel stainless steel grid bars, each measuring 4 mm in diameter and spaced 1.5 cm apart (center to center), enclosed within a sound attenuating chamber. The grid floor was attached to a scrambled shocker (Ugo Basile Biological Research Apparatus, Italy) to provide footshock. Background noise was supplied by ventilation fans and shock scramblers. A second distinctive environment designated as context B (CB), was made as different as possible from the context used for training. CB was made of wood and had a transparent plastic lid, black walls and black rubber floor, dimensions being 33×25×33 cm. The illumination in both contexts was provided by a 2.5 W white light bulb. Both chambers were cleaned with 0.5% acetic acid before and after utilization. Experiments were always performed between 11:00 and 14:00 h with the experimenters unaware of the treatment condition.

Another chamber, designated as CC was used in experiment 3B. This chamber was located in a different room and consisted of a distinct Plexiglas chamber (60×20×20 cm) with orange transparent walls and the floor of which consisted of stainless steel rods separated by 1.0 cm (center to center). The chamber was brightly illuminated by three lights and. was cleaned with water before and after utilization.

Behavioral procedure

Contextual fear conditioning. The conditioning procedure used was similar to the one previously described in Isoardi et al. (2004). Rats were habituated to handling and injected with SAL for at least 2 days prior to the start of each experiment. At the beginning of each experimental day, animals were transported from the colony room to the experimental room, where they remained in their cages during the running of the conditioning, reactivation and testing sessions. A different acoustically isolated room was used to run the training and the other phases of the experiment.

Conditioning. Training consisted in placing the rat in the chamber (CA) and allowing a 3 min acclimation period (preshock period). After this period, rats received three footshocks (0.7 mA, 3 s duration and intershock interval 30 s; unconditioned stimuli). They remained in the chamber for an additional 2 min (postshock period) and after this period, rats were placed in their home cages and returned to the colony room.

Reactivation. Twenty-four hours after training, subjects were placed in the training context without shocks for 90 s. Freezing was video recorded during the 90 s exposure period.

Test session. Contextual fear conditioning was assessed 24 h after the exposure to CA by placing the rats in the training environment for 5 min. Memory was assessed and expressed as the percentage of time that rats spent freezing. Such behavior, commonly used as an index of fear in rats (Blanchard and Blanchard, 1969) was video recorded during each observation period. An animal was considered to be freezing when it was crouching without movement of the body and the head except that associated with breathing.