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Diazepam effects on aversive memory retrieval and extinction: Role of anxiety levels

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ABSTRACT

Benzodiazepines (BDZs) are anxiolytic drugs that impair memory acquisition. Previous studies using the plus-maze discriminative avoidance task (PMDAT, which assesses memory and anxiety concomitantly) indicated that the effects of BDZs on anxiety and acquisition are related to each other. The possible influence of the anxiolytic action of BDZs on their effects on memory retrieval and extinction are poorly understood. This is relevant considering the relationship between aversive memories and anxiety disorders. We designed a modified protocol of PMDAT that evaluates anxiety during retrieval and extinction of the task. Male Wistar rats were trained in the PMDAT (plus-maze with two open and two enclosed arms) using a standard or a modified protocol. In the standard protocol, the aversive stimuli were presented in one of the enclosed arms during training, and the animal had free access to the whole apparatus. In the modified protocol, the open arms were blocked with glass walls. Twenty-four hours after training, the animals subjected to each of the protocols were treated with saline or 2.0 mg/kg of diazepam (DZP) 30 min before the test. There was a third session in the maze (retest) 24 h after the test. During the test, DZP impaired and improved retrieval in rats that had been trained in the standard and the modified protocol when compared to the respective saline-treated groups. In addition, treatment with DZP prior to the test induced anxiolysis, but only in the animals that were not pre-exposed to the open arms of the apparatus (modified protocol). In these animals, DZP impaired extinction, which was evaluated during retest session. The impairing effect of DZP on extinction seems to be related to its anxiolytic action during the test (extinction learning). Further, we suggest that aversive memory retrieval depends on both the treatment and the arousal elicited by exposure to the apparatus.

1. Introduction

Some anxiety disorders, such as post-traumatic stress disorder (PTSD), panic disorder and phobias, involve some extent of maladaptive mnemonic features (Ferreri et al., 2011; Milad et al., 2006; Rubin et al., 2008). In these disorders, a trigger stimulus, which might be conscious or unconscious, associated to a past traumatic episode — evokes an abnormal and/or excessive anxiety response (American Psychiatric Association, 2013). Indeed, even generalized anxiety disorder patients presents underlying negative implicit associations with neutral or negative attributes (Reinecke et al., 2010). Thus, understanding the relationship between aversive memories and anxiety is imperative to stablish the appropriate therapeutic approach to each anxiety disorder.

Although other pharmacological strategies have emerged to treat such disorders, the benzodiazepines (BDZs) are still widely used for alleviating acute distress symptoms or in combination with other anxiolytics, such as selective serotonin reuptake inhibitors (Bandelow et al., 2008). However, BDZs impair aversive memory extinction in PTSD patients (Gelpin et al., 1996; Matar et al., 2009; Mellman et al., 2002; Zohar et al., 2011). Also, memory deficits and cognitive impairment are observed in patients chronically treated with BDZs (CADTH, 2014).

Studies have shown that BDZs impair memory acquisition in humans and in rodent models (Beracochea, 2006; McNamara and Skelton, 1991; Savić et al., 2005). However, the effects of BDZs on aversive memory retrieval are poorly understood. Previous literature reports contradictory results, showing that BDZs enhance (File et al., 1999; Obradović et al., 2004), impair (Borde et al., 1997, 1996) or have no effects (Chapouthier and Venault, 2002; Venault et al., 1986) on memory retrieval. On the other hand, BDZs have been clearly shown to impair aversive memory extinction (Hart et al., 2009; Matar et al., 2009).

Abbreviations: BDZ, benzodiazepine; EPM, elevated plus-maze; MOD, modified protocol; PMDAT, plus-maze discriminative avoidance task; TAV%, percent time spent in the aversive arms; TOA%, percent time spent in the open arms; STD, standard protocol.

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Further, most of the studies do not take into consideration that BDZs act on both emotional and mnemonic processes, and the relevance of the anxiolytic effects of BDZs to mnemonic processes is unknown. Indeed, the interaction between these two effects could be relevant to the interpretation of the results in aversive conditioning tasks (Pain et al., 2002; Silva and Frussa-Filho, 2000). Thus, further research to understand how the anxiolytic effect of BDZ interacts with aversive memory retrieval and extinction is necessary to advance the current knowledge on conditioned fear-related disorders.

The plus-maze discriminative avoidance task (PMDAT) is an adaptation of the conventional elevated plus-maze that has been used to assess anxiety and memory-related behaviors concomitantly (Calzavara et al., 2004; Kameda et al., 2007; Munguba et al., 2011; Ribeiro et al., 2011; Silva and Frussa-Filho, 2000). During the first trial (training) of this two-trial task, one of the enclosed arms is paired with aversive stimuli every time the animal enters it. The stimuli are light and noise, which are not presented in the second trial (test session). Retrieval of the task is evaluated by comparing the time spent in each enclosed arm (aversive and non-aversive) during the second trial. Amnestic (Patti et al., 2006; Silva et al., 1999) and memory-enhancing (Claro et al., 1999; Silva et al., 1997) manipulations decrease and increase the avoidance of the aversive arm in the test session, respectively. Concomitantly, anxiety-like behavior is evaluated by the time spent in the open arms of the apparatus during the training session (Silva and Frussa-Filho, 2000). Therefore, the paradigm enables the study of the possible relationships between memory and anxiety. Pre-training administrations of chlordiazepoxide and caffeine in rodents have anxiolytic and anxiogenic effects, respectively, and both manipulations impair learning in this task, suggesting that aversive memory acquisition is dependent on an optimal anxiety level during the conditioning phase (Silva and Frussa-Filho, 2000). Further, previous studies have indicated that the impairment in acquisition induced by BDZs is highly dependent on the presence of their anxiolytic effect during the training in the PMDAT (Calzavara et al., 2004; Silva and Frussa-Filho, 2000).

One important limitation of the use of PMDAT is that the anxiolytic effect of some pharmacological agents (mainly BDZs) is weakened, or even absent, during the second trial in the apparatus. This is a consequence of the one-trial tolerance (OTT) that is induced by the exposure to the apparatus in the training session (Silva and Frussa-Filho, 2000). This phenomenon is also described to the conventional elevated plus-maze (EPM), and defined as the loss of the anxiolytic effect of benzodiazepines (BDZs) on rodents previously exposed to the EPM (File, 1990; File et al., 1990).

The absence of an anxiolytic action of BDZs on rodents pre-exposed to the PMDAT precludes the investigation of possible relationships between the effects of BDZs on anxiety and retrieval or extinction. In this sense, studies support the view that one-trial tolerance depends on the prior exploration of relatively safe areas of the maze and the retention of information from the first trial. Such learning would prevent the presence of novelty, and the loss of BDZ efficacy on the second trial would reflect absence of an approach/avoidance conflict (Bertoglio and Carobrez, 2000; Rodgers and Shepherd, 1993; Roy et al., 2009). Therefore, a modification of the original protocol that allows the reliable assessment of anxiety-like behavior during the second exposition in the PMDAT would be useful to the investigation of possible relationships between retrieval/extinction and anxiety.

This investigation is required because the anxiolytic activity of a compound is often measured by the ability to prevent aversive conditioned responses. That is the case of tests such as fear-potentiated startle (Brown et al., 1951; Davis, 1986), Vogel water-lick conflict (Petersen and Lassen, 1981; Vogel et al., 1971) and context-conditioned freezing (Beck and Fibiger, 1995; Fanselow and Helmstetter, 1988; Malkani and Rosen, 2000; Resstel et al., 2006). These tests do not dissociate innate from learned fear responses, because the same behavioral outcome is expected in both cases. Therefore, a specific activity upon innate fear cannot be dissociated from the impairing effects on conditioned fear responses. An animal model able to address both innate and learned fear responses could be useful to the screening for novel therapeutic agents as well as to the understanding of the pathophysiology of anxiety disorders.

Briefly, the PMDAT address the effects of treatments on both innate and learned fear responses, evaluated by different behavioral outcomes. This dissociation is relevant because these responses rely on different neural pathways (for review, see (Gross and Canteras, 2012)). Indeed, as demonstrated in the study by Ribeiro et al. (2011), the inactivation of the basolateral amygdala (BLA) impaired aversive memory acquisition, but not the innate fear response in the PMDAT.

Furthermore, the PMDAT has an ethical advantage compared to other aversive conditioning paradigms. The conditioning in the training session is conducted through the presentation of mild stimuli (i.e. light and noise), while most of other associative learning paradigms comprise painful stimuli (Davis, 1986; Resstel et al., 2006; Vogel et al., 1971). As well, the nature of the aversive stimuli in the PMDAT is closer to rodent's natural aversive behavioral repertory. That is, strong light and loud noises relate closer to aversive environmental expositions than electrical shocks or other painful stimuli in rodents. Hence, the PMDAT also relates closer to a natural aversive condition in humans, which is a desirable feature to emulate disorders with aversive associations.

The aim of the present study was to verify the effects of the BDZ diazepam (DZP) on aversive memory retrieval and extinction in rats subjected to the PMDAT. In order to evaluate anxiety-related behaviors on the second trial of the PMDAT, we designed a protocol to prevent the occurrence of one-trial tolerance. In the modified protocol, the open arms were blocked with transparent glass walls during the training session, precluding the exploration of these areas of the maze. The test was carried out without protocol modifications, and we added a third session (identical to the test session) at the end of the experiment to assess aversive memory extinction.

2. Materials and methods

2.1. Animals

Three-month-old Wistar male rats (250–300 g) were housed in groups of four in plastic-walled cages (45 × 35 × 15 cm), under controlled temperature (23 ± 1 °C) and lighting (12:12 h light–dark cycle; lights on at 6:00 am). The animals had free access to food and water, and were exposed to 5 min of gentle handling for 7 days prior to the experiment. All tests were performed during the light period (1:00 to 5:00 p.m.), and animals were pseudo-randomly assigned to each group in order to include at least one animal of each group per cage.

All procedures were approved by the Committee on Animal Research and Ethics of the Federal University of Rio Grande do Norte and are in accordance with the Brazilian law for animal experimentation (Law 11.794). All efforts were taken to minimize pain, suffering, discomfort, and the number of animals used.

2.2. Plus-maze discriminative avoidance task (PMDAT)

The PMDAT was conducted in a modified elevated plus-maze that was made of wood, with two enclosed (50 × 15 × 40 cm) opposing two open (50 × 15 cm) arms. A 100 W lamp and a speaker were placed over one of the enclosed arms (aversive enclosed arm).

The behavioral protocol is schematized in Fig. 1. The rats were exposed to three trials. During the training session, each rat was placed individually in the center of the apparatus, facing the intersection between the open arms, and allowed 10 min of free exploration. Every time an animal entered with all four paws into the aversive enclosed arm, the 100 W light (1500 lx at the maze floor level) and a digitally produced 80 dB white noise were turned on until the animal left the arm. In