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Enhancing a declarative memory in humans: The effect of clonazepam on reconsolidation☆

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ABSTRACT

A consolidated memory recalled by a specific reminder can become unstable (labile) and susceptible to facilitation or impairment for a discrete period of time. This labilization phase is followed by a process of stabilization called reconsolidation. The phenomenon has been shown in diverse types of memory, and different pharmacological agents have been used to disclose its presence. Several studies have revealed the relevance of the GABAergic system to this process. Consequently, our hypothesis is that the system is involved in the reconsolidation of declarative memory in humans. Thus, using our verbal learning task, we analyzed the effect of benzodiazepines on the re-stabilization of the declarative memory. On Day 1, volunteers learned an association between five cue- response-syllables. On Day 2, the verbal memory was labilized by a reminder presentation, and then a placebo capsule or 0.25 mg or 0.03 mg of clonazepam was administered to the subjects. The verbal memory was evaluated on Day 3. The volunteers who had received the 0.25 mg clonazepam along with the specific reminder on Day 2, exhibited memory improvement. In contrast, there was no effect when the drug was given without retrieval, when the memory was simply retrieved instead of being reactivated or when short-term memory testing was performed 4 h after reactivation. We discuss the GABAergic role in reconsolidation, which shows a collateral effect on other memories when the treatment is aimed at treating anxiety disorders. Further studies might elucidate the role of GABA in the reconsolidation process associated with dissimilar scenarios.

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1. Introduction

The consolidation theory establishes that memories are labile during a time window after acquisition, but as time progresses, memories become stable and resistant to amnesic agents. Several studies using behavioral, pharmacological and molecular approaches in diverse species, from nematodes to humans, have shown that consolidation is an evolutionarily conserved process that initially requires RNA and protein synthesis (Bailey et al., 1996; Davis and Squire, 1984; Dudai, 2002; Kandel, 2001; McGaugh, 2000; Squire and Alvarez, 1995). However, the notion of immutable memories after consolidation has been challenged. Since the pioneer study of Misanin et al. (1968), a growing number of reports have shown that old memories become labile and again become susceptible to amnesic agents after a specific reminder is presented. Such susceptibility decreases over time and leads to a re-stabilization phase, usually referred to as reconsolidation.

In humans, reconsolidation has been reported in a procedural motor-skill task (Walker et al., 2003), Pavlovian fear conditioning (Kindt et al., 2009; Schiller et al., 2010) and in a verbal learning task (Forcato et al., 2007; Hupbach et al., 2007). Previously, our group has not only reported that declarative human memories undergo reconsolidation (Forcato et al., 2007), but we have also described boundary conditions necessary to trigger labilization (Forcato et al., 2011, 2010, 2009). Our paradigm consists of learning a verbal material (lists of five pairs of nonsense syllables) acquired by a training process (L1-training) on Day 1. After this declarative memory is consolidated, it can be labilized when a specific

☆ In memoriam of my science mentor Dr Héctor Maldonado.

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[1] We regret to announce that our colleague and friend, Prof Maldonado, passed away during the publication of this study.

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reminder is presented. Then, this memory passes through a stabilization process. To reveal the presence of this process, the method selected was a second learning process (L2-training), which interfered with the re-stabilization phase. The time window for the interference was determined by demonstrating that at 6 h after labilization, the memory was still sensitive to an amnesic agent. Conversely, 10 h after reactivation, the memory was impervious to the interfering agent (Forcato et al., 2007). Furthermore, the labilization–reconsolidation was only triggered under certain circumstances. When the reminder was formed by the context cues and one cue syllable, without giving the subjects the opportunity to write down the response syllable (cue-reminder), the labilization–reconsolidation was triggered. In contrast, when the subjects had the possibility to write down the response syllable (cue-response reminder), the memory was evoked but not labilized. Thus, as in other paradigms, the presence of a mismatch and the discrepancy between expected and current events in the reminder determine the occurrence or absence of reconsolidation (Lee, 2009; Pedreira et al., 2004).

Furthermore, it is well known that Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in both the central nervous system (CNS) and the peripheral nervous system (Erdo et al., 1986). A considerable amount of evidence from different studies, using a variety of paradigms and tasks, supports a role for the GABAA receptor in diverse behavioral outcomes (Chapouthier and Venault, 2002; Paredes and Agmo, 1992). A large body of evidence from studies of human memory indicates that the use of benzodiazepines produces anterograde amnesia (Brown et al., 1982; Curran, 1991; Uzun et al., 2010; Venault et al., 1986;). In addition, there is general consensus that benzodiazepines do not produce retrograde amnesia (Ghoneim and Mewaldt, 1975; McNamara and Skelton, 1991; Savic et al., 2005). Moreover, in humans, retrograde memory-enhancing effects have been found (Hinrichs et al., 1984), and declarative memory retrieval has been improved by low doses of benzodiazepines (Delgado et al., 2005; File et al., 1999; Fillmore et al., 2001).

Regarding the role of GABA in the reconsolidation phase, the results obtained with different paradigms and animal models reveal the relevance of the GABAergic system to this process (Bustos et al., 2009, 2006; Carbo Tano et al., 2009; Zhang and Cranney, 2008). Thus, in mammals, previous researchers have demonstrated an amnesic effect following midazolam (MDZ) administration during the labilization–reconsolidation process of a contextual fear conditioning paradigm in rats (Bustos et al., 2006). These results support the view that stimulating $GABA_A$ receptor sites via this short-acting benzodiazepine selectively disrupts the reconsolidation process of a contextual fear memory. In line with these results and using the same paradigm, Zhang and Cranney (2008) revealed a reconsolidation impairment induced by the systemic administration of midazolam immediately after reactivation. Additionally, in this case, the effect of the drug did not differ between high- and low-anxiety rats. Interestingly, based on the well-known pharmacological actions of ethanol as a positive modulator of $GABA_A$ receptors (Lister, 1987; Weiss and Porrino, 2002), a recent study showed that ethanol, administered after the reactivation of a contextual fear memory, enhanced the performance of treated animals at testing (Nomura and Matsuki, 2008). Moreover, this effect appeared to be mediated by the GABAergic system because the administration of picrotoxin, a $GABA_A$ receptor antagonist, inhibited the memory enhancement produced by ethanol. Thus, this study supports the hypothesis that ethanol enhances contextual fear memories following labilization via the activation of $GABA_A$ receptors.

Therefore, it can be strongly argued that GABA transmission is implicated in memory reconsolidation. In this framework, we hypothesized that the GABAergic system is involved in the reconsolidation of a declarative memory in humans.

Taking into consideration that benzodiazepines, widely used for the treatment of anxiety disorders, increase the activity of $GABA_A$ receptors, we determine the role of the GABAergic system by analyzing the effect of benzodiazepines on the re-stabilization of declarative memory.

To accomplish the main goal of this study, we selected clonazepam, a long-acting benzodiazepine (half-life 20–50 h), which has a fast onset, high effectiveness, low toxicity and does not produce adverse reactions. The selected doses were low enough to avoid undesirable effects, such as excessive sedation during the course of the experiment.

Taking into account the absence of an emotive charge in our paradigm and the doses selected, we wondered if this type of modulation during re-stabilization would induce a memory enhancement, as has been shown in other reports (Nomura and Matsuki, 2008).

Thus, in this study, the volunteers learned an association between five cue-syllables and five respective response-syllables. Twenty-four hours later, the paired associated verbal memory was labilized by exposing the subjects to the cue-reminder and by administering a capsule of 0.25 mg or 0.03 mg clonazepam (CLZ) or a placebo (PLC). On Day 3, the list-memory was evaluated by presenting the 5 cue-syllables twice and allowing the subjects to respond with the response syllables. Memory improvement was observed when the volunteers received 0.25 mg of CLZ in conjunction with the specific reminder on Day 2. In contrast, there was no effect when the drug was administered without retrieval, when the memory was simply retrieved instead of being reactivated or when short-term memory testing was performed 4 h after reactivation.

We have therefore demonstrated, for the first time, that the daily dose of benzodiazepine prescribed to treat anxiety (0.25 mg of CLZ) enhances a reactivated declarative memory in humans. These results strongly suggest that the positive modulation of GABAsites was the factor that changed the strength of the previous consolidated memory.

Our results add new evidence regarding the role of the GABAergic system on mnemonic processes, showing that the effects critically depend on the characteristics and parametric conditions of the paradigm and agents used.

2. Materials and methods

2.1. Subjects

Two hundred and four undergraduate and graduate students volunteered for the study. To evaluate clonazepam's strengthening effect on declarative memory reconsolidation, only the subjects that achieved at least 45% of correct responses during the last four trials of the training session (9/20 correct responses) were included. Additionally, subjects were excluded for any of the following reasons: those who drank alcohol during the period of the experiment, those who wrote the syllables down, those who slept during the daytime after the reminder and drug administration, and/or those who missed some step in the protocol of the experiment.

The final sample was composed of 104 volunteers, 42 (40%) men and 62 (60%) women, with ages ranging between 20 and 35 and with a mean of 23 years old.

Before their participation in the experiment, subjects signed a written informed consent form approved by the Ethics Committee of the Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia (FLENI).

2.2. Procedure

The experiments were conducted in a dark room using a personal computer. Each subject was provided with earphones and seated facing a monitor placed in front of a large screen on the wall.

The subjects were required to learn a list of five pairs of nonsense syllables presented on the monitor screen. The List was associated with a specific context (light projected on the large screen, an image on the monitor screen; and sound coming through the earphones). The selection of this enriched context was based on previous reports. That is, it was demonstrated by Forcato et al. (2007) that the presentation of contextual cues enhanced performance during the testing session.