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MEMORY CONSOLIDATION AND RECONSOLIDATION IN AN INVERTEBRATE MODEL: THE ROLE OF THE GABAergic SYSTEM

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Abstract—Consolidation theory assumes that memories are labile during a limited time window after acquisition, but as time passes, memories become stable and resistant to amnesic agents. However, the vision of immutable memories after consolidation has been challenged. Thus, after the presentation of a reminder, the reactivated old memories become labile and again susceptible to amnesic treatments. This process implies a re-stabilization phase, usually referred to as reconsolidation.

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter both in the Central nervous system (CNS) and in the periphery. A considerable amount of evidence has arisen from different studies regarding the role of the $GABA_A$ receptor in diverse behavioral paradigms and tasks. Here, we investigate the role of the GABAergic system on both memory consolidation and reconsolidation phases by using the memory paradigm of the crab *Chasmagnathus*. In order to achieve such a goal, we design pharmacological-behavioral experiments, which include the administration of classic agonist (muscimol) and antagonist (bicuculline) of the mammals $GABA_A$ receptors. The current results show that the systemic administration of muscimol impairs the consolidation and reconsolidation processes. In contrast, the administration of bicuculline improves the consolidation and reconsolidation processes. Furthermore, the co-administration of both drugs blocks the agonist amnesic effect on the consolidation phase.

The ubiquity of the neurotransmitter and its receptors in the animal taxa allows us to use the classic agonist-and-antagonist administration procedure in this invertebrate. Thus, all the results reported in this paper can be judged as a result of the modulation exerted by the functional state of the GABAergic system in the CNS.

To conclude, the results obtained in this report with an invertebrate model represent additional evidences supporting the view that some molecular mechanisms subserving different memory phases could be the basic tools employed by phylogenetically disparate animals.

Key words: *Chasmagnathus*, GABA, memory, facilitation, CNS.

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Abbreviations: ANOVA, analysis of variance; BMI, bicuculline; BMI15, injected with 15.4×10^{-3} μg/g of bicuculline; BMI15-VDS, injected with 15.4×10^{-3} μg/g of bicuculline and confronted with the visual danger stimulus during the last minute; COC, cocktail; CSM, context-signal memory; MUS, muscimol; SAL, crustacean saline solution; ST, strong training; T, trained groups; T-BMI1.5, trained group that received bicuculline doses of 1.54×10^{-3} μg/g; T-BMI15, trained group that received bicuculline doses of 15.4×10^{-3} μg/g; T-MUS1.5, trained group that received 1.5 μg/g of muscimol; T-SAL, trained group treated with saline; U, untrained groups; U-BMI15, untrained group receiving 15.4×10^{-3} μg/g of bicuculline; U-MUS1.5, untrained group that received 1.5 μg/g of muscimol; U-SAL, untrained saline-control group; VDS, visual danger stimulus; WT, weak training.

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Consolidation theory assumes that memories are labile during a limited time window after acquisition, but as time passes, memories become stable and resistant to amnesic procedures, including pharmacological intervention (McGaugh, 2000). This passage from a labile to a stable state requires a cascade of intracellular events which result in the passage of transient to persistent modifications (McGaugh, 2000; Dudai, 2004). Evidence from invertebrate species (Tully et al., 1994; Abel and Kandel, 1998; Tully, 1998; Alberini, 1999; Muller, 2000; Menzel, 2001; Maldonado, 2002), supports the view that both memory consolidation and the cascade of intracellular events underlying this process are preserved across evolution (Carew, 2000).

Since the early work of Misanin et al. (1968) more than three decades ago, there has been renewed interest in another memory phase. Thus, the vision of immutable memories after consolidation has been substantially challenged as a result of several studies (Przybyslawski and Sara, 1997; Nader et al., 2000; Debiec et al., 2002; Suzuki et al., 2004). These reports show that after the presentation of a specific reminder, reactivated old memories become labile and again susceptible to amnesic treatments. Such vulnerability diminishes with the progress of time (Nader et al., 2000; Sara, 2000) and implies a re-stabilization phase, usually referred to as reconsolidation (Przybyslawski et al., 1999). Moreover, it has been proposed that reconsolidation shares many of the cellular and molecular mechanisms used during consolidation (Dudai and Eisenberg, 2004; Alberini, 2005).

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter both in the Central nervous system (CNS) and in the periphery (Erdo et al., 1986). It was first identified as the neurotransmitter located in the neuromuscular junction of the crayfish (Iwasaki and Florey, 1969). It acts on $GABA_A$ and $GABA_C$ ionotropic receptors (Macdonald and Olsen, 1994; Zhang et al., 1995), as well as on $GABA_B$ metabotropic receptors (Kerr and Ong, 1995). In recent years a considerable amount of evidence has arisen from different studies regarding the role of the

$GABA_A$ receptor in diverse behavioral outcomes using a variety of paradigms and tasks (Paredes and Agmo, 1992; Chapouthier and Venault, 2002).

Findings from those studies using different memory paradigms such as inhibitory avoidance (Jensen et al., 1979; Bonetti et al., 1982), spatial water maze (McNaughton and Morris, 1987; McNamara and Skelton, 1991) and radial arm maze (Hodges and Green, 1986; Stackman and Walsh, 1995) indicate that systemic administration of benzodiazepines (a positive modulator of $GABA_A$) induces amnesia in rats and mice. Moreover, systemic injection of muscimol (MUS) (a $GABA_A$ agonist) produces a dose-dependent impairment of memory retention in diverse tasks (Brioni, 1993).

Breen and McGaugh (1961) were the first to investigate the effect of picrotoxin, a $GABA_A$ antagonist, on memory processes. These authors observed that the post-training administration of this drug facilitated memory formation. In other studies, memory storage was positively modulated when the antagonists were administered either shortly before or shortly after the training phase (Brioni et al., 1989; Clements and Bourne, 1996; Chapouthier and Venault, 2002; Luft et al., 2004).

Regarding the reconsolidation phase Bustos et al. (2006) showed an amnesic effect following the administration of a short-acting benzodiazepine, midazolam, during the labilization–reconsolidation process using a contextual fear conditioning paradigm in rats. However, to the best of our knowledge, there are no reports evaluating the influence of the antagonism of $GABA_A$ sites on memory reconsolidation.

In summary, compounds that augment GABAergic transmission acting at the $GABA_A$ receptor complex such as MUS or benzodiazepines, impair the consolidation or reconsolidation processes. In contrast, compounds which block or diminish GABAergic transmission enhance the memory consolidation process (Paredes and Agmo, 1992; Chapouthier and Venault, 2002).

It is necessary to take into consideration two issues which have already been mentioned. First, the initial description of GABA as an inhibitory neurotransmitter appeared in the giant neurons of the crayfish (Iwasaki and Florey, 1969). After that, a large number of reports showed the role of GABA in a wide number of systems and species (Roberts, 2000; Florey, 1991; Del Castillo et al., 1967; Otsuka et al., 1966; Kravitz et al., 1963).

Second, results obtained with invertebrate species lend support to the view that both the memory consolidation phase after acquisition and the cascade of intracellular events subserving consolidation are preserved across evolution (Abel and Kandel, 1998; Alberini, 1999; Tully et al., 1994; Tully, 1998; Muller, 2000; Menzel, 2001; Maldonado, 2002). This persistence does not necessarily imply that the receptors share the same subunits, similar circuit organization or identical intracellular events to consolidate the memory.

Moreover, in mammals, the role of excitatory neurotransmitters as glutamate and noradrenaline has been well described in memory consolidation. Indeed, the administration of agonists and antagonists enhances and impairs, respectively, this memory phase (Breen and McGaugh, 1961; Brioni and McGaugh, 1988; Brioni et al., 1989; Castellano et al., 1989). Other neuromodulatory systems, including the GABAergic system, also modulate memory consolidation by regulating the release of the excitatory neurotransmitters. In this case, administration of agonists and antagonists impairs and facilitates memory consolidation, respectively. Regarding our previous research into *Chasmagnathus*, none of our studies has intended to determine the role of an inhibitory neurotransmitter in the mnemonic process.

Thus, the purpose of the present paper is to investigate the role of the GABAergic system on both memory consolidation and reconsolidation phases by using the memory paradigm of the crab *Chasmagnathus*, which has been extensively studied at behavioral and mechanistic levels (Maldonado, 2002).

In brief, the crab's associative learning paradigm is based on its escape response elicited by the presentation of a visual danger stimulus (VDS), an opaque rectangle passing over the animal. Upon the iterative presentation of VDS, the crab's escape-response declines and a strong freezing-response is built up (Pereyra et al., 1999). The response decrement lasts for at least 5 days (Lozada et al., 1990; Pedreira et al., 1995). The memory formed using this paradigm is based on the association between the environmental features of the training site (the context) and the features of the screen moving over the animal (the signal) (Tomsic et al., 1998), such memory was termed as the context-signal memory (CSM). Studies performed on the mechanisms underlying consolidation have shown that CSM consolidation is blocked by protein synthesis inhibitors (Pedreira et al., 1995, 1996; Hermitte et al., 1999); positively modulated by angiotensins (Delorenzi et al., 1996, 2000); selectively regulated by a muscarinic cholinergic mechanism (Berón de Astrada and Maldonado, 1999) and crucially mediated by N-methyl-D-aspartic acid (NMDA)-like glutamatergic receptors (Troncoso and Maldonado, 2002).

At the molecular level, it was demonstrated the cAMP signal pathway (Romano et al., 1996a,b; Locatelli et al., 2002), MAP kinases pathway (Feld et al., 2005) and the NFκ-B transcription factor (Freudenthal et al., 1998; Freudenthal and Romano, 2000; Merlo et al., 2002) are required during CMS consolidation. Findings from studies done to investigate the mechanisms underlying reconsolidation showed a reliable CSM labilization by re-exposing the animals 5 min to the learning context 24 h after training. This labilized memory is cycloheximide sensitive (Pedreira et al., 2002; Pedreira and Maldonado, 2003); positively modulated by angiotensins (Frenkel et al., 2005); mediated by the NFκ-B transcription factor (Merlo et al., 2005) and by NMDA-like glutamatergic receptors (Pedreira et al., 2002).

It is important to note that in accordance with the interpretive model of CSM retrieval (Tomsic et al., 1998; Hermitte et al., 1999; Maldonado, 2002), re-exposure of a trained crab to the learning context could evoke a CSM representation that induces a freezing response as soon

as the animal is faced with the VDS. Until the experimental designs to evaluate the CSM reconsolidation no previous evidence had shown that such memory representation was already present before VDS display. However, the outcome obtained in reconsolidation experiments supported this proposal. As a result, the mere reexposure to the original learning context, even in the absence of VDS presentation, is quite enough for an amnesic agent to impair reactivated memory. Finally, two other lines of experiments designed to evaluate the context specificity and the circadian specificity suggested the relevance of other components in the CSM. Indeed, a training-to-testing context shift abolished the CSM retention; and, the retention-test performance was impaired in crabs tested at a time of the day that differed from that of their original training, regardless of the daily phase of the training and testing session or the extension of the intersession interval.

Thus, these findings strongly support the associative nature of CSM and, specifically, the existence of an associative link between the signal and the context as a distinctive feature of this memory process.

In particular, the current experiments are aimed to test whether the crab's CSM could be modulated by changes in the functional tone of the GABAergic system during memory consolidation and reconsolidation. Hence, the demonstration that similar molecular mechanisms subserve both memory phases along the phylogenetic-tree supports the view that such mechanisms could be common tools employed across evolution to promote adaptive changes in phylogenetically very disparate animals (Carew, 2000).

EXPERIMENTAL PROCEDURES

Animals

Animals were adult male *Chasmagnathus granulathus* crabs 2.7–3.0 cm across the carapace, weighing around 17.0 g, collected from water less than 1 m deep in the rías (narrow coastal inlets) of San Clemente del Tuyú, Argentina, and transported to the laboratory, where they were lodged in collective tanks (20 animals each). Water used in tanks and other containers during experiments was artificial seawater prepared with hw-Marinex salt (Winex, Germany; pH 7.4–7.6), salinity 10–14 ‰, and maintained within a range of 22–24 °C. The holding and experimental rooms were maintained on a 12-h light/dark cycle (light on 07:00–19:00 h). Animals were fed with rabbit pellets (Nutrientes S.A., Argentina) every 3 days and after feeding the water was changed. Experiments were carried out within the first week after the animals' arrival, from January to August, and between 08:00 and 18:00 h. Each crab was used only in one experiment. Experimental procedures are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (USA), and the Argentinean guidelines on the ethical use of animals. All the experiments performed in this work were planned minimizing the number of animals used and their suffering.

Apparatus

The apparatus is described in detail elsewhere (Maldonado, 2002). Briefly, the experimental unit was the actometer: a bowl-shaped opaque container with a steep concave wall 12 cm high (23 cm top diameter and 9 cm floor diameter) covered to a depth of 0.5 cm with marine water. The crab was lodged in the container, which was suspended by three strings from an upper wooden framework (23×23×30 cm) and illuminated with a 5-W lamp placed 30 cm above the animal. A motor operated screen (an opaque rectangular strip of 25.0×7.5 cm) which was moved horizontally over the animal cyclically from left to right and vice versa. A trial lasted nearly 9 s and comprised two successive cycles of movement. Screen displacements provoked a running response of the crab and subsequent container vibrations. Four microphones were centrally attached to the outside wall of the container, and these piezoelectric components recorded the vibrations produced by the animals' response. These signals were amplified, integrated during each 9-s trial and translated into arbitrary numerical units ranging from zero to 5000. The activity of every crab was recorded during each entire trial time. The experimental room had 40 actometers, separated from each other by partitions. A computer was employed to program trial sequences, trial duration and intertrial intervals, as well as to monitor experimental events.

Experimental procedure and design

Experiments to determine the effective dose for each drug. Each experiment was conducted to test the effective dose on the consolidation process, which lasted 2 days and included two phases (training and testing sessions), each corresponding to 1 day. Thirty to 40 crabs were included in each group. *Day 1. Training session.* Each experiment included one untrained group (U) that was kept in the actometers during the entire training session (between 20 and 50 min) but without being trained, i.e. without being presented the VDS; and three trained groups (T) that after being 5 min in the actometer without VDS (adaptation time), received 6 (weak-training, WT) or 15 (strong-training, ST) trials with VDS separated by an intertrial interval of 3 min. The actometer used during training session is referred to as the standard context. Immediately after training an injection with saline solution was administered to the U and to one of the T. Each of the other two T received a dose of the drug under study. During the intersession interval crabs were moved from the standard context to be housed individually in the resting containers, i.e. plastic boxes covered to a depth of 0.5 cm with water and kept inside dimly lit drawers, for 24 h. *Day 2. Test session.* Twenty-four hours after training, all crabs were placed again in the standard context for 5 min followed by the test trial, i.e. the VDS presentation.

Experiments for the consolidation and reconsolidation processes. Each experiment for the consolidation process included two phases (training and testing session) and lasted 2, 3 or 5 days. The experiments for reconsolidation included three phases (training, treatment and test session), each corresponding to 1 day; two or three pairs of U–T were formed in each experiment, the protocols of which differed basically in the pharmacological treatment. *Day 1. Training session.* Each pair included one U that was kept in the actometers during the entire training session (between 20 and 50 min) but without being trained; and one T that after being 5 min in the actometer without VDS (adaptation time), received 6 or 15 VDS-trials—a WT or a ST respectively. During the intersession interval crabs were moved from the standard context to be housed individually in the resting containers for 24 h or 72 h. An injection with saline or drug solution was given at diverse times relative to the end of training for the consolidation experiments. *Day 2. Treatment session.* This session is only included in the reconsolidation experiments. The core of this phase was the crab's re-exposure for 5 min to the standard context without VDS presentation. In other experiments handling has implied either the presentation of a VDS in the last minute or the exposure to a context unlike that of the training session referred to as the different context, consisting of a cylinder 15 cm high, 15 cm in diameter, whose wall consisted of vertical black and white bands, and illuminated as actometers. An injection with saline or drug solution was given at diverse times regarding one of the maneuvers previously described, during the same day 2. *Test session.* Twenty-

four hours or 48 h after training or treatment session, all crabs were placed again in the standard context for 5 min time followed by the test trial, i.e. the VDS presentation.

Selection test

Before animals were placed in the actometers to start an experiment, they underwent a selection test: Each crab was turned on its back and only those that immediately returned to their normal position were used. The rationale behind this selection is that crabs with a slow correction reaction show a low responsiveness to a large diversity of stimuli and, at a later time, they usually present unhealthy symptoms. No more than 5% of tested crabs were eliminated.

Drugs and injection procedure

Crustacean saline solution (SAL) (Hoeger and Florey, 1989) was used as a vehicle. Fifty microliters of saline or drug solution was given through the right side of the dorsal cephalothoracic–abdominal membrane, by means of a syringe fitted with a sleeve to control depth of penetration to 4 mm, thus ensuring that the injected solution was released in the pericardial sac.

We used MUS, a GABAergic agonist; it is a structural GABA analog, which bound to the same site of GABA (DeFeudis, 1981); and bicuculline (BMI) a competitive antagonist of GABA-receptor (Bormann, 2000). Both drugs were purchased from Sigma Co.

Data analysis

CSM retention was assessed by focusing data analysis on test trial scores, i.e. by estimating the difference between the response level of the T and that of the respective U of each pair. A T is said to show memory retention when its mean response level at test trial is statistically lower than that of the respective U. Rescorla (1988) convincingly argued in favor of using this sort of analysis instead of a paired training-testing comparison, stressing the need to clearly distinguish between time of input (training session) and time of assessment (testing session).

In previous experiments at our laboratory, a significant difference (*t*-test, $\alpha<0.05$) between T and U was invariably disclosed at test trial (T<U), 24 h after training, proving that each group consisted at least of 30 crabs each and that they were given 15 or more training trials with 3 min of intertrial interval. Accordingly, prediction has been feasible for a significant difference (T<U) at test trial. In contrast, no significant differences were found between a U–T pair when the T-group received a WT, six trials with 3 min of intertrial interval. Therefore, results have been analyzed using a priori planned comparisons (LSD; Rosenthal and Rosnow, 1985; Howell, 1987). For each experiment, which included two or three U–T pairs of groups, three or five comparisons were carried out: between the two or three U, and between the U–T pairs for each treatment. The set of planned comparisons was performed following a one-way analysis of variance (ANOVA) ($\alpha<0.05$). All response scores were represented as the normalized means±the standard error of those means with respect to the untrained saline-control group (U-SAL). We analyzed the data using Statistica '99 Edition Windows 6.1 software package.

RESULTS

MUS impairs both consolidation and reconsolidation processes

These first series of experiments were conducted to analyze the effect of MUS, a $GABA_A$ agonist, on the consolidation and reconsolidation phases. Based on findings from previous reports in other memory paradigms, an amnesic effect of this drug was expected. Consequently, we used a ST protocol (15 VDS-trials) to induce a perdurable CSM (Maldonado, 2002) in order to interfere with the consolidation or reconsolidation phases by increasing the GABAergic-tone. The drug's effect was expressed by a decrease of retention in the treated-drug pairs.

Experiment 1A was aimed to determine the effective dose of MUS when administered immediately after the CSM training. This experiment (Fig. 1A, upper panel) had an untrained control group (U) treated with saline solution (U-SAL) and three T: one trained group treated with saline (T-SAL) and the other two received doses of 1 μg/g (T-MUS1) or 1.5 μg/g (T-MUS1.5) respectively. The training session for the T consisted of a ST (15 trials). Fig. 1A (lower panel) presents results corresponding to the test trial for each group normalized with respect to the mean response of the U-SAL group (ANOVA $F_{(3,118)}=2.9$; $P<0.001$). Planned comparisons showed a significant difference for U-SAL vs. T-SAL ($P<0.01$) and U-SAL vs. T-MUS1 groups ($P<0.05$) outcomes that could be explained as memory retention. On the contrary, no significant differences were found between U-SAL-injected vs. a trained group that received 1.5 μg/g of muscimol (T-MUS1.5), a result that can be interpreted as memory impairment ($P=0.60$). Based on this finding, this dose seems to impair CSM consolidation. Therefore, the subsequent experiments explored the effect of this dose of MUS (1.5 μg/g) in the consolidation process.

Discarding unspecific drug effects, experiment 1B (Fig. 1B, upper panel) was planned to evaluate the action of this MUS dose on treating trained and untrained animals. The experimental design included two pairs of U–T, one pair was treated with SAL (U-SAL and T-SAL) and the other pair received 1.5 μg/g of MUS immediately after a ST on day 1. One day later, during the test session (ANOVA, $F_{(3,189)}=3.2$; $P<0.05$) the outcome of the planned comparisons was different for each pair of groups (Fig. 1B, lower panel). The comparison of the SAL-injected pair revealed a significant difference, indicative of memory retention ($P<0.01$). Additionally, there was no differences for the U comparison ($P=3.13$), indicating the lack of unspecific drug effects either on the animal's behavior or health, a result observed throughout all the experiments of these series. In contrast, the MUS1.5-injected pair showed no significant differences ($P=0.75$), which was explained as an amnesic effect.

In summary, it was concluded that the administration of MUS (1.5 μg/g) immediately after training impairs CSM consolidation.

To further confirm this conclusion, two more experiments we carried out. The first one was designed to determine the existence of an effective time window for MUS interference on consolidation as found with other drugs (Berón de Astrada and Maldonado, 1999; Pedreira et al., 2002; Troncoso and Maldonado, 2002), and the second was conducted to discard the presence of spontaneous recovery effect (Dudai and Eisenberg, 2004), which could reflect an effect on memory retrieval rather than on memory consolidation.

Experiment 1C had the same parametrical characteristic of experiment 1B (Fig. 1C, upper panel), except that in

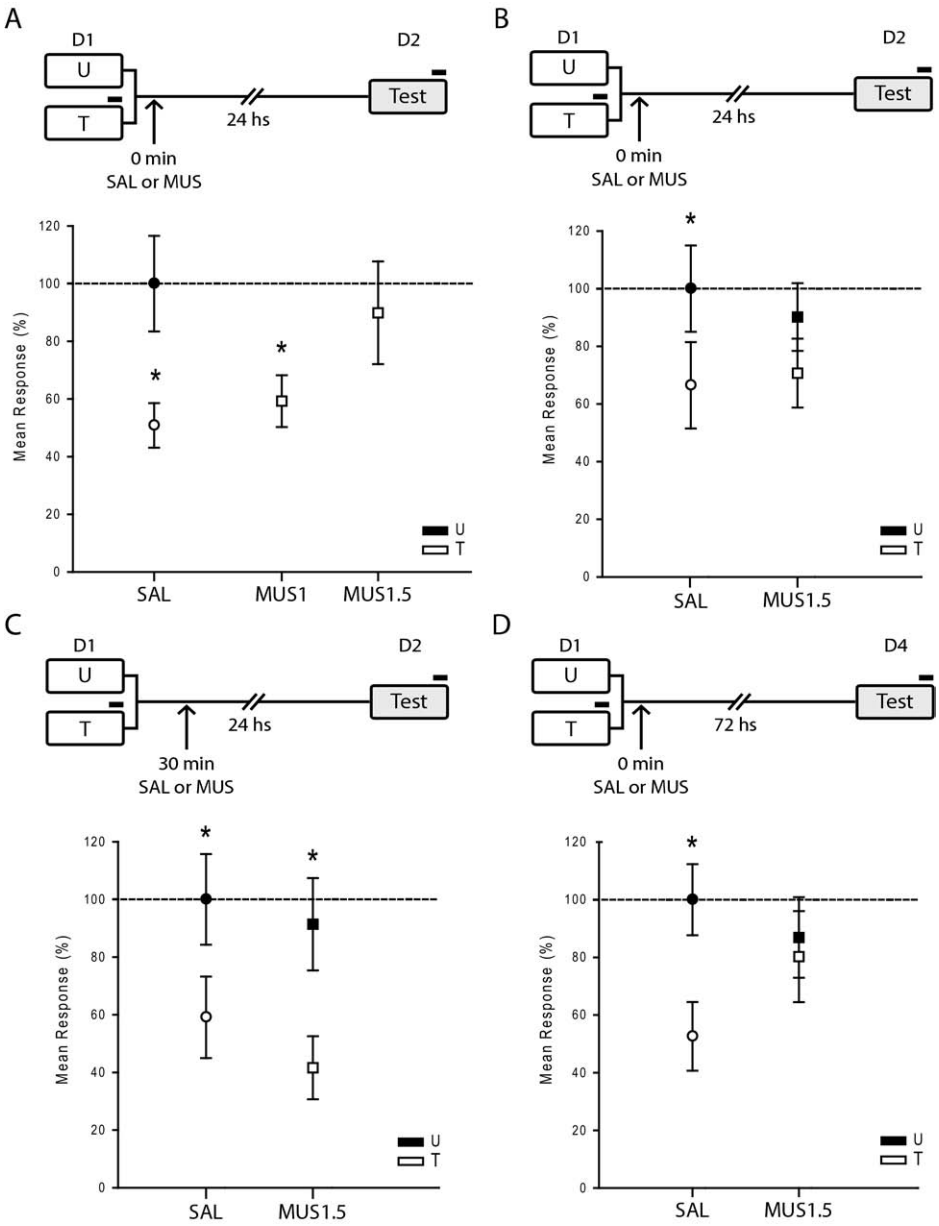

Fig. 1. MUS impair consolidation. (A) Experiment 1A: 1.5 μg/g of MUS impairs consolidation. Upper panel. Experimental protocol: White rectangles stand for training session, gray rectangle for testing session, and black hyphen for a VDS-trial. Arrow stands for the time of injection. Day 1 (D1): T. Animals received a ST (15 presentation of the VDS, intertrial interval of 3 min). U. Animals remained in the training context without being exposed to the VDS (45 min). Day 2 (D2): Test. One single presentation of the VDS was used to test the animals. Lower panel. Test session. Mean response to VDS presentation ± S.E. normalized with respect to the U of the SAL pair. Circles, stand for SAL groups; squares, MUS-treated groups. Two different doses of MUS were used: 1 μg/g (MUS1) and 1.5 μg/g (MUS1.5). Planned comparisons (LSD): * stands for $P<0.05$ (T<U, memory retention). (B) Experiment 1B: MUS impair consolidation process without an unspecific effect. Upper panel. Experimental protocol. Symbols as in A. Lower panel. Test session. Circles stand for SAL groups; squares, MUS groups. Graph ordinates, bars and symbols as in A. (C) Experiment 1C: MUS injected 30 min after training, no longer produced amnesic affect. Upper panel. Experimental protocol. Symbols as in A; the drug was administered 30 min after training ends (arrow). Lower panel. Test session. Graph ordinates, bars and symbols as in A. (D) Experiment 1D: The amnesic effect of MUS shows no spontaneous recovery. Upper panel. Experimental protocol. Symbols as in A, the intersession interval between training and testing lasted 3 days. Lower panel. Test session. Graph ordinates, bars and symbols as in A.

this case the injections were given 30 min after training. Fig. 1C (lower panel) shows the test session results (ANOVA, $F_{(3,156)}=3.9$; $P<0.01$). Planned comparisons revealed significant differences between the U-SAL vs. T-SAL groups ($P<0.01$) and U-MUS1.5 vs. T-MUS1.5 ($P<0.05$) groups. The closure of an effective time window supports the view that the effect observed is specifically exerted on the consolidation phase rather than due to unspecific effects on the animal response.

Experiment 1D was aimed to examine if the lack of CSM retention is still evident when the time between treatment and testing was augmented (Pedreira et al., 2002).

This type of manipulation allows us to discard that the impairment observed during testing, 24 h after the treatment, was a product of a retrieval deficit. The experimental design (Fig. 1D, upper panel) included the two pairs of groups, one treated with saline solution (U-SAL and T-SAL) and the other with MUS 1.5 μg/g (U-MUS1.5 and T-MUS1.5) supplied immediately after a ST. The animals were tested 72 h after training. The results are displayed in Fig. 1D (lower panel), (ANOVA $F_{(3,184)}=3.13$; $P<0.05$). Planned comparisons presented significant differences for the SAL pair ($P<0.05$), and no significant differences between the MUS1.5 pair ($P=0.67$) showing the maintenance of the CSM impairment when MUS was administered immediately after training.

Overall, this first series of experiments allows us to conclude that the activation of the GABAergic system through the systemic administration of a $GABA_A$ receptor agonist impairs CSM consolidation.

To further confirm that the effects reported selectively involved $GABA_A$ receptors, we administered the agonist together with a competitive $GABA_A$ antagonist BMI [i.e. a cocktail (COC) of an agonist and an antagonist, Dickinson-Anson and McGaugh, 1997]. To this end, we performed another experiment to establish the dose of BMI without an effect on memory and behavior. Experiment 2A comprised two pairs of groups, each pair was treated with SAL or BMI 1.54×10^{-3} μg/g (BMI1.5) after a ST (Fig. 2A, upper panel). The groups were tested 24 h after training (ANOVA, $F_{(3,217)}=10.3$; $P<0.001$). The outcome of planned comparisons (Fig. 2A, lower panel) was the same for both pairs of groups. Concerning the SAL and BMI1.5 pairs, the statistical analysis showed significant differences ($P<0.001$ and $P<0.05$, respectively). As a result, we used this dose of BMI (1.54×10^{-3} μg/g) for the COC experiment. Experiment 2B included three pairs of groups (Fig. 2B, upper panel): a U–T SAL pair, a U–T MUS1.5 pair and the U–T COC pair which received the co-administration of MUS (1.5 μg/g) and BMI (1.54×10^{-3} μg/g). The injections were given immediately after the 15 trial training and the groups were tested 24 h later. Fig. 2B (lower panel) exhibits the results obtained during testing (ANOVA, $F_{(5,264)}=3.12$; $P<0.01$). The outcome of the planned comparison revealed a significant differences for the SAL pair ($P<0.05$), indicative of memory retention. On the contrary, an impairment of CSM retention by the administration of 1.5 μg/g of MUS was disclosed ($P=0.39$); and finally, the recovery of the CSM deficit when MUS was co-administered with BMI ($P<0.01$), which demonstrated the action of both drugs on the same receptor. These results support the proposal that the modulation of the GABAergic system via the administration of a $GABA_A$-receptor agonist negatively affects the consolidation of the CSM.

Previous reports provided clear evidence that the robust CSM acquired by the crab became labile again after 5 min re-exposure to the learning context, proving to be vulnerable to diverse interfering agents (Pedreira et al., 2002; Merlo et al., 2005). Based on these studies, we performed the next series of experiments to analyze the functional role of the GABAergic system on the memory reconsolidation process. Each experiment included two pairs of U–T, one was SAL

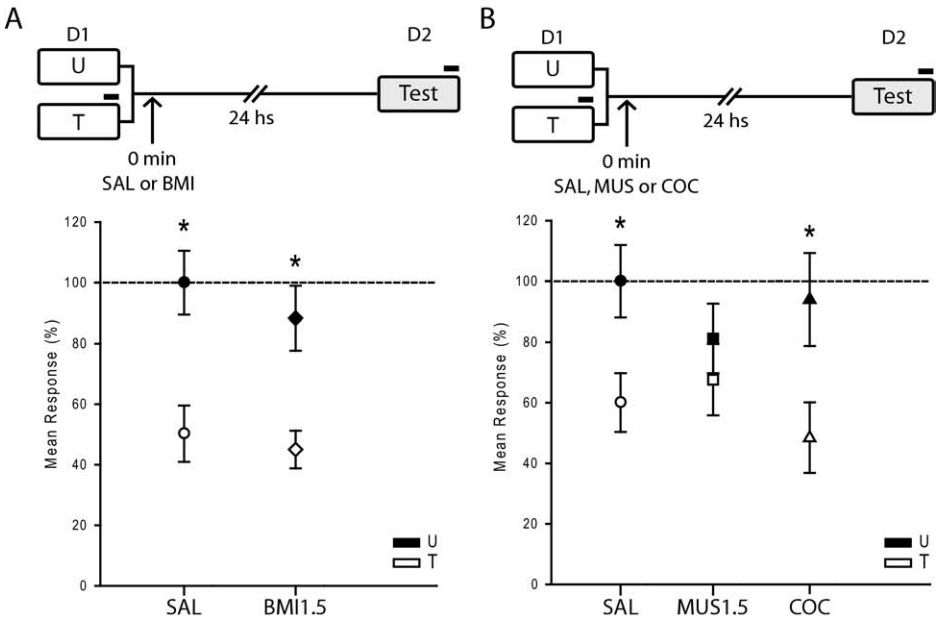

Fig. 2. BMI reverts the amnesic affect of MUS when co-administered. (A) Experiment 2A: 1.54×10^{-3} μg/g of BMI produce no effect on memory or behavior. Upper panel. Experimental protocol. Symbols as in Fig. 1A. Lower panel. Test session. Circles stand for SAL-treated groups and rhombus, BMI1.5-treated groups. Graph ordinates and symbols as in Fig. 1A. (B) Experiment 2B: BMI prevents the amnesic effect of MUS. Upper panel. Experimental protocol: Symbols as in Fig. 1A. Arrow shows the time of SAL, MUS (1.5 μg/g) or COC injection (MUS1.5 plus 1.54×10^{-3} μg/g of BMI). Lower panel. Test session. Circles, stand for SAL groups; squares, MUS groups and triangles, COC groups. Graph ordinates and symbols as in Fig. 1A.

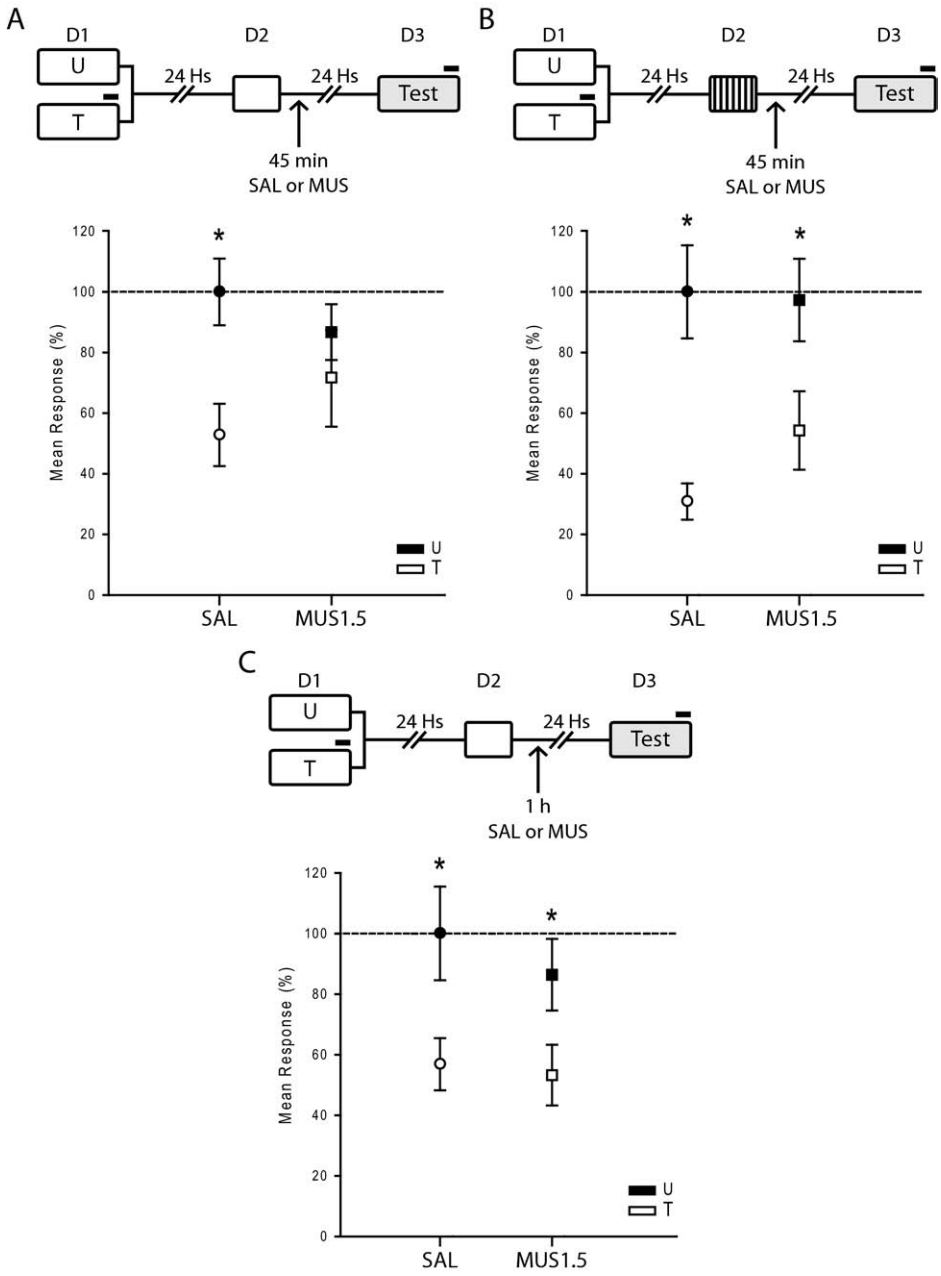

Fig. 3. MUS impairs reconsolidation. (A) Experiment 3A: 1.5 μg/g of MUS impairs reconsolidation process. Upper panel. Experimental protocol: Symbols as in Fig. 1A. Day1 (D1): T. Animals received a ST. U. Animals remained in the training context without being exposed to the VDS (45 min). Day 2 (D2): the animals were re-exposed to the training context for 5 min. Arrow stands for the time of injection. Day 3 (D3): Test. One single presentation of the VDS was used to test the animals. Lower panel. Test session. Circles stand for SAL groups; squares, MUS groups. Graph ordinates and symbols as in Fig. 1A. (B) Experiment 3B: The amnesic effect of MUS over reconsolidation depends on the reactivation-labilization process of the memory. Upper panel. Experimental protocol: Symbols as in A; a novel context is presented instead of training context on day 2. Lower panel. Test session. Bars as in A. Graph ordinates and symbols as in Fig. 1A. (C) Experiment 3C: MUS injected 1 h after re-exposure, no longer produced amnesic affect. Upper panel. Experimental protocol: Symbols as in A; the drug was administered 1 h after training ends (arrow). Lower panel. Test session. Bars as in A. Graph ordinates and symbols as in Fig. 1A.

injected and the other pair was MUS1.5 injected. The crabs received either SAL or MUS1.5 at diverse times relative to the 5 min of context exposure—the reminder—during day 2, 24 h after a ST. Fig. 3A (lower panel) shows the test results for experiment 3A (day 3)—normalized with respect to the U-SAL group—corresponding to the groups injected 45 min after a 5 min re-exposure period to the standard context on day 2 (ANOVA, $F_{(3,181)}=3.01$; $P<0.05$). Planned comparisons (Fig. 3A, upper panel) showed different outcomes for each pair. Indeed, the SAL-injected pair revealed a significant differences ($P<0.01$), which was considered as memory retention. In contrast, the MUS-injected pair exhibited no significant differences ($P=0.28$), which was interpreted as memory impairment.

A well-described control to show that the effect observed on the reconsolidation phase depends on memory-induced lability provoked by the presentation of the reminder is to expose the animals to a different context to that used during training. This procedure has not produced the retrieval-labilization process (Pedreira and Maldonado, 2003; Merlo et al., 2005). Consequently, the experiment implied a similar experimental design to the one previously used; however, in this case the animals were exposed to a different context for 5 min on day 2. As a consequence in experiment 3B we employed the same two pairs of U–T and we administered the drugs at the same time after the exposure to the new context (Fig. 3B, upper panel). Results obtained on day 3 are displayed in Fig. 3B lower panel (ANOVA, $F_{(3,115)}=6.7$; $P<0.001$). In contrast to the findings from the previous experiment, groups that were exposed to a different context presented significant differences for both pairs of groups (T-SAL<U-SAL; $P<0.01$; T-MUS1.5<U-MUS1.5; $P<0.05$), i.e. MUS failed to disrupt memory. This result is interpreted as a lack of memory reactivation due to the absence of the appropriate reminder, maintaining the memory in its stable state (Pedreira and Maldonado, 2003; Merlo et al., 2005).

Our next step was to investigate the drug effective time window. Thus, in experiment 3C the injections were given at distant time points after the 5 min re-exposure to the standard context on day 2. The design (Fig. 3C, upper panel) included two pairs of U–T treated with SAL or MUS1.5 1 h after the animals were confronted with the reminder. The results are shown in Fig. 3C (lower panel). Regarding the MUS-injected pair, when the drug was administered 1 h after the reminder it was no longer effective to impair CSM (ANOVA, $F_{(3,147)}=4.06$; $P<0.01$). In fact, planned comparisons showed significant differences for both the SAL- ($P<0.01$) and MUS-injected pair of groups ($P<0.05$). Therefore, the CSM acquired on day 1 and reactivated by a 5 min re-exposure to the training context on day 2 was blocked by MUS administered at 45 min but not after 1 h following reactivation. These results are indicative that a brief re-exposure to the original learning context turns the consolidated memory into a labile state, and once more, it can be disrupted by MUS administration.

BMI improves the consolidation and the reconsolidation processes

The goal of this series of experiments was to evaluate whether the administration of BMI could improve the CMS consolidation/reconsolidation. Since we expect a facilitating effect, a WT protocol (6 VDS-trials) was used to induce a weak CSM memory (Maldonado, 2002). This memory was only able to be expressed when different facilitatory treatments were applied with respect to acquisition, retrieval or labilization (Frenkel et al., 2005). Therefore, since the antagonism of the endogenous GABAergic system has been described to facilitate memory formation (Chapouthier and Venault, 2002), we expect an improvement of CSM retention as a result of BMI treatment.

Experiment 4A was aimed at determining the effective dose of BMI when it was administered immediately after a CSM WT. This experiment (Fig. 4A, upper panel) had a U-SAL and three T: one was T-SAL and the other two received bicuculline doses of 1.54×10^{-3} μg/g (T-BMI1.5) and 15.4×10^{-3} μg/g (T-BMI15). The training session for these T consisted of a WT (6 trials). Fig. 4A (lower panel) shows the level of response to the VDS, normalized with respect to the U-SAL group during the test session 24 h after training session (ANOVA, $F_{(3,156)}=9.77$; $P<0.05$). Planned comparisons showed no significant differences (memory deficit) for the U-SAL vs. T-SAL ($P=0.54$) and U-SAL vs. T-BMI1.5 groups ($P=0.54$). On the contrary, a significant difference (memory retention) for the U-SAL vs. T-BMI15 groups ($P<0.03$) was observed. In summary, these findings suggest that the dose of BMI 15.4×10^{-3} μg/g facilitates CSM consolidation.

To confirm this last inference, we performed additional experiments to explore the effect of this BMI dose (15.4×10^{-3} μg/g) on the consolidation and reconsolidation processes.

Discarding unspecific drug effects, experiment 4B (Fig. 1B, upper panel) was planned to evaluate the action of this BMI dose on treating trained and untrained animals. The experimental design (Fig. 4B, upper panel) included two pairs of U–T. One pair received injections of SAL (U-SAL and T-SAL) and the other pair received 15.4×10^{-3} μg/g of BMI immediately after a WT on day 1. One day later the test session was performed (ANOVA, $F_{(3,156)}=3.9$; $P<0.01$). The outcome of the planned comparisons (Fig. 4B, lower panel) regarding the SAL injected pair, revealed no significant differences, which was interpreted as memory retention deficit ($P=0.46$) as a consequence of the WT used. In addition, there were no differences for the U comparison ($P=0.60$), indicating the lack of drug's unspecific effect on the animal's behavior or health, a result observed throughout all this series of experiments. On the contrary, the BMI-injected pair showed a significant difference, which was explained as a retention enhancement of the CMS ($P<0.01$). Therefore, these data suggest that the administration of BMI (15.4×10^{-3} μg/g) immediately after the WT facilitates the consolidation of a CMS. To validate this inference, we performed an additional experiment designed to establish the limits of an effective time window (Berón de Astrada and Maldonado, 1999; Pedreira et al., 2002; Troncoso and Maldonado, 2002). Experiment 4C had the same parametrical characteristic of experiment 4B, but in this case the injections were administered 1 h after training (Fig. 4C, upper panel). Results are presented in Fig. 4C lower panel (ANOVA, $F_{(3,115)}=0.5$; $P=0.68$). Planned comparisons failed to show significant differences between the U-SAL vs. T-SAL groups and U-BMI15 vs. T-BMI15 groups ($P=0.56$ and $P=0.52$ respectively). Thus, the effective time window for the facilitating effect induced by BMI is between 0 and 60 min after a WT. Moreover, the fact of this time dependent effect supports the view that such an effect is specifically exerted on the consolidation phase.

As a whole, these experiments reveal that the reduction of the GABAergic tone induced by the systemic ad-

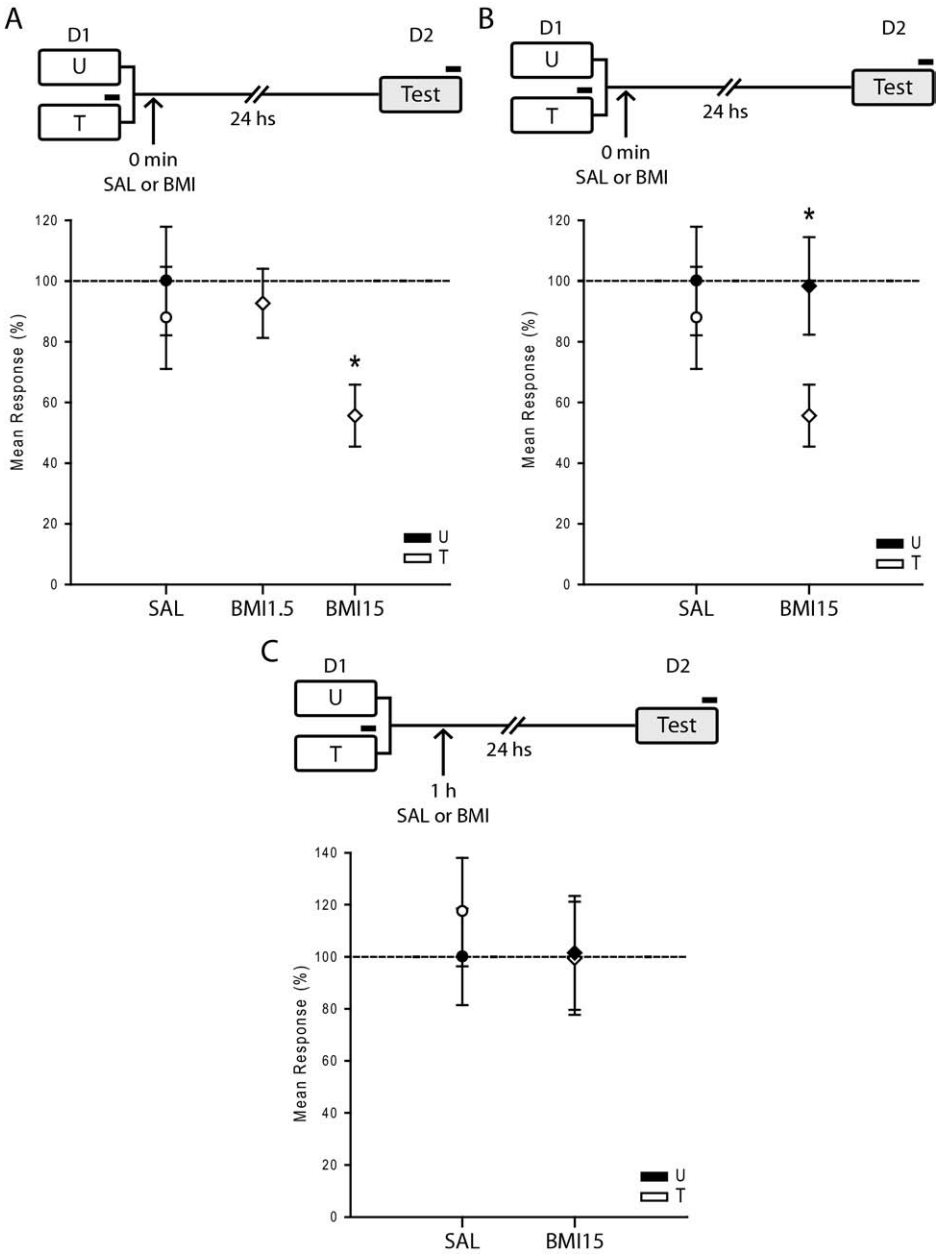

Fig. 4. BMI improves the consolidation process. (A) Experiment 4A: 15.4×10^{-3} μg/g of BMI improves consolidation. Upper panel. Experimental protocol: Symbols as in Fig. 1A. Day 1 (D1): T. Animals received a WT (six presentations of the VDS, intertrial interval of 3 min). U. Animals remained in the training context without being exposed to the VDS (15 min). Day 2 (D2): Test session. One single presentation of the VDS was used to test the animals. Lower panel. Test session. Circles, SAL groups; diamonds, BMI groups. Two different doses of BMI were tested: 1.54×10^{-3} μg/g (BMI1.5) and BMI15. Graph ordinates and symbols as in Fig. 1A. (B) Experiment 4B: BMI improves consolidation without any unspecific effect. Upper panel. Experimental protocol. Symbols as in Fig. 1A. Lower panel. Test session. Bars as in A. Graph ordinates and symbols as in Fig. 1A. (C) Experiment 4C: BMI injected 1 h after training, no longer improves consolidation. Upper panel. Experimental protocol. Symbols as in Fig. 1A, the drug was administered 1 h after training ended (arrow). Lower panel. Test session. Bars as in A. Graph ordinates and symbols as in Fig. 1A.

ministration of a $GABA_A$ receptor antagonist facilitates the formation of the CSM.

The next series of experiments examined a potential role of a GABAergic mechanism on the reconsolidation phase. As shown by Frenkel et al. (2005), a consolidated but unexpressed memory could be retrieved and reactivated by the presentation of a specific reminder, returning it to a labile state which is vulnerable to different facilitatory treatments. Therefore, we tested whether a consolidated but unexpressed memory could be retrieved and reactivated by the reminder (5 min of context re-exposure), returning this memory to a labile state that could be affected by the administration of a GABAergic antagonist. Each experiment included two pairs of U–T, one was SAL injected and the other pair was injected with 15.4×10^{-3} μg/g of bicuculline (BMI15). In this series of experiments,

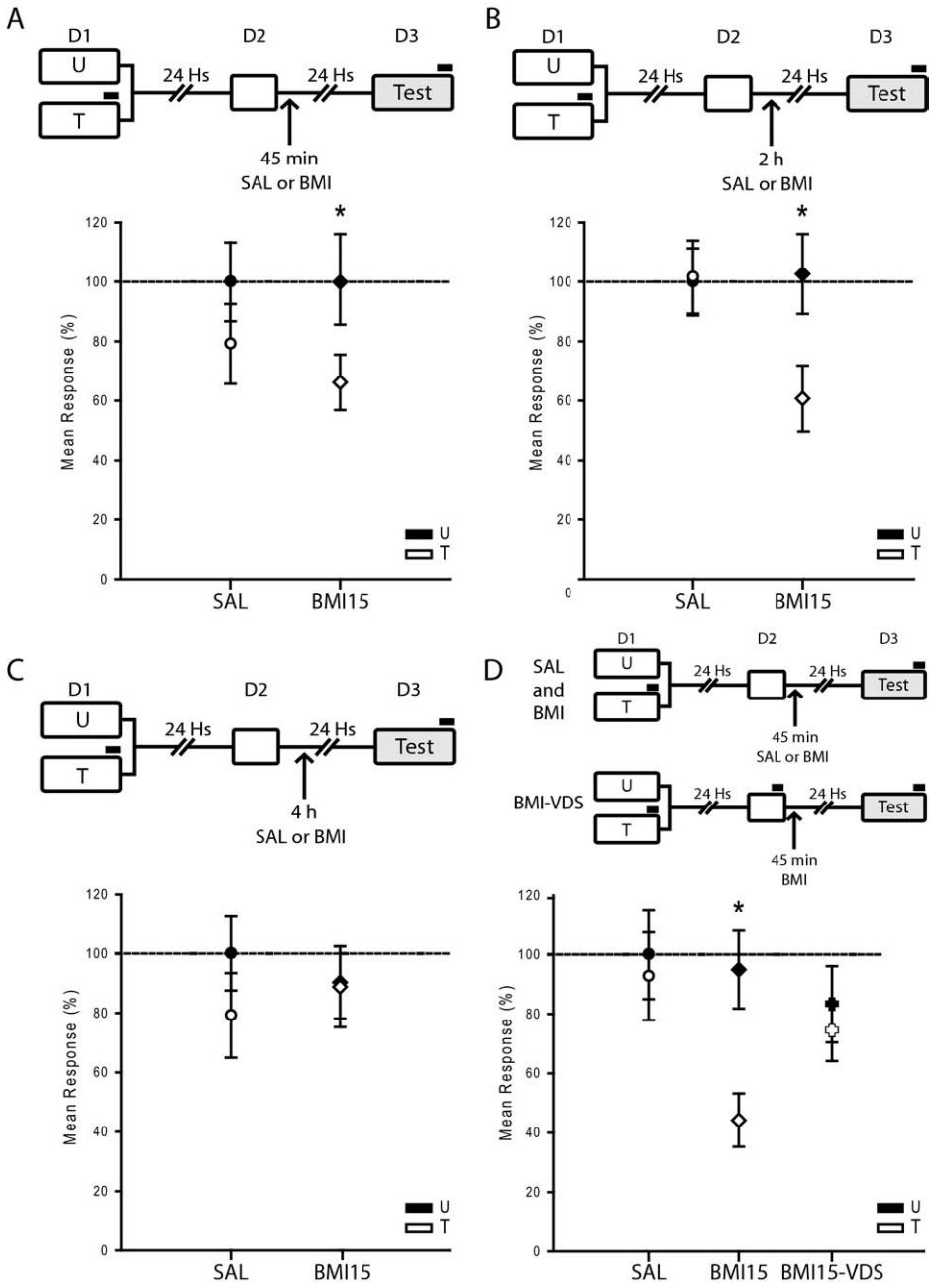

Fig. 5. BMI improves reconsolidation process. (A) Experiment 5A: BMI improves reconsolidation when administered after remainder presentation. Upper panel. Experimental protocol. Day 1 (D1): T. Animals received a WT protocol (WT, six presentations of the VDS, intertrial interval of 3 min). U. Animals remained in the training context without being exposed to the VDS (15 min). Day 2 (D2): the animals were re-exposed to the training context for 5 min. Arrow stands for the time of injection. Day 3 (D3): Test. One single presentation of the VDS was used to test the animals. Lower panel. Test session. Bars as in Fig. 4A. Graph ordinates and symbols as in Fig. 1A. (B) Experiment 5B: BMI injected 2 h after remainder presentation, still improves reconsolidation. Upper panel. Experimental protocol. Symbols as in A, the drug was administered 2 h after re-exposure ended (arrow). Lower panel. Test session. Bars as in Fig. 4A. Graph ordinates, and symbols as in Fig. 1A. (C) Experiment 5C: BMI injected 4 h after remainder presentation, no longer improves reconsolidation. Upper panel. Experimental protocol. Symbols as in A, the drug was administered 4 h after re-exposure ended (arrow). Lower panel. Test session. Bars as in Fig. 4A. Graph ordinates and symbols as in Fig. 1A. (D) Experiment 5D: When the reactivation-labilization process is canceled, BMI shows no improvement of reconsolidation. Upper panel. Experimental protocol. Symbols as in A, a pair of groups received a VDS at the end of the re-exposure (BMI-VDS). Lower panel. Test session. Circles, SAL group; diamonds, BMI15 group; crosses, BMI15-VDS group. Graph ordinates and symbols as in Fig. 1A.

crabs were treated 24 h after a WT, with either SAL or BMI (15.4×10^{-3} μg/g) at diverse time points relative to 5 min of context exposure on day 2. The experiment 5A design, included groups injected 45 min after the re-exposure to the standard context on day 2 (Fig. 5A, upper panel). These results are displayed in Fig. 5A lower panel (ANOVA,

$F_{(3,155)}=8.08$; $P<0.5$) corresponding to testing session on day 3. While planned comparisons showed no significant differences (memory retention deficit) for the SAL-injected pair ($P=0.56$), they showed a significant difference (memory retention) for the BMI-injected pair ($P<0.01$).

The following experiments investigated the time window for BMI's facilitating effect. For this purpose, experiments 5B and C were done and the injections were given at two different time points after the 5 min re-exposure to the standard context on day 2. Each experimental protocol included two pairs of U–T either treated with SAL or BMI (15.4×10^{-3} μg/g) 2 or 4 h after the reminder exposure (Fig. 5B–C, upper panel). The results of experiments 5B and C are shown in Fig. 5B and C lower panels respectively (ANOVA, $F_{(3,228)}=2.5$; $P<0.004$). When BMI was injected 2 h after re-exposure, the drug preserved its enhancing effect on memory reconsolidation, a conclusion that is expressed by the significant differences between the BMI groups ($P<0.01$) and the memory deficit for the SAL groups ($P=0.9$), which resulted from the WT received. In contrast, when BMI15 was administered 4 h after the reminder, the drug was no longer effective in enhancing CSM reconsolidation (ANOVA, $F_{(3,185)}=0.4$; $P=0.70$) . In fact, planned comparisons showed no significant differences for either SAL- ($P=0.89$) or BMI-injected pairs of groups ($P=0.93$). Thus, the weak CSM acquired on day 1 and reactivated by re-exposure to the training context on day 2 was facilitated by BMI injection administered 45 min or 2 h after but not 4 h after reactivation.

Finally, as previously proposed with our memory paradigm (Pedreira et al., 2004), another control group was necessary to show that the effect observed in the reconsolidation phase depends on memory-labilization. In experiment 5D we chose an experimental design similar to that used before, however, in this case we passed the VDS over the animals (reinforcement) in the last minute of the 5-min re-exposure to the standard context on day 2. Thus, in this experiment we utilized the same protocol for two pairs of groups included in the previous experiment plus a pair injected with 15.4×10^{-3} μg/g of bicuculline and confronted with the visual danger stimulus during the last minute (BMI15-VDS; Fig. 5D, upper panel). Results obtained on day 3 are displayed in Fig. 5D lower panel (ANOVA, $F_{(5,256)}=2.6$; $P<0.01$). In this experiment we confirmed previous outcomes obtained for the same groups (SAL and BMI15 pairs). In fact, planned comparisons revealed that BMI administration 45 min after the reminder improved memory reconsolidation, which was expressed as a significant difference between BMI15 groups ($P<0.01$) and the memory deficit for SAL groups ($P=0.67$). In contrast, no significant differences were found when animals received the VDS with the specific reminder (BMI15-VDS pair, $P=0.61$). These findings showed that BMI failed to improve memory retention when the reinforcement was administered during the re-exposure session. Therefore, the absence of the specific reminder (e.g. standard context for a brief time without reinforcement) results in no memory reactivation. Consequently, the memory state remained unchanged and immune to the facilitating BMI influence (Pedreira et al., 2004; Frenkel et al., 2005).

Altogether, these experiments further support the notion that stable and consolidated memories might become vulnerable to an agent such as BMI following a brief re-exposure to the original learning context. Moreover, these results represent a central topic of this work, taking into account the absence of antecedents on facilitating effects on memory reconsolidation by the administration of a $GABA_A$ antagonist.

DISCUSSION

The current research represents our initial contribution to establishing the role of the GABAergic system in the mnemonic processes in the crab *Chasmagnathus*. In order to achieve such a goal, we used a well-characterized experimental tool in our laboratory, the pharmacological-behavioral experiments, which included the administration of classic agonist and antagonist of the mammals $GABA_A$ receptors.

Results obtained with the $GABA_A$ receptor agonist, MUS (1.5 μg/g), administered immediately after a ST protocol provoked an amnesic effect (Fig. 1A–B). This effect could only be explained by the effect of changing the GABAergic functional state during consolidation but not by an unspecific effect on the animals' health (Fig. 1B).

The effective time window for MUS-induced interference was shorter than 30 min after training (Fig. 1C). In our memory paradigm, other drugs acting on other systems showed similar short effective periods (Kaczer, personal communication). Indeed, there is some evidence that other amnesic agents exerted their effects only when they were administered immediately after training (McGaugh, 1966, 2000). Finally, we designed an experiment to evaluate the presence of spontaneous recovery (Dudai and Eisenberg, 2004). Some earlier studies on memory consolidation indicated that memory disruption produced by diverse treatments was unlikely to be true amnesia, as the lost memories showed spontaneous recovery (Lewis et al., 1968; Miller et al., 1974; Banerjee and Das, 1977; Nader and Wang, 2006). Thus, the earlier impairment may be interpreted as a result of retrieval/performance deficits rather than a storage deficit. In the present report it is demonstrated that as training-to-test interval is increased, the treated animals with MUS maintained the impairment effect. This outcome does not definitively prove that it is true amnesia; however, it may be considered as strong support to the view that MUS impair CSM.

To further confirm that the effect of an agonist is due to its action on a specific receptor, this effect should be reverted by the co-administration of the antagonist of the same receptor (Brioni et al., 1989; Paredes and Agmo, 1992). In fact, the present study demonstrated that the co-administration of a $GABA_A$ receptor antagonist BMI reverts the amnesic effect of MUS (Fig. 2B), indicating that

the effect observed is due to the direct interaction of both drugs on same GABA receptor.

As in the consolidation phase, when the memory was labilized by the reminder presentation, the re-stabilization process was modulated by the GABAergic system. MUS injected 45 min after re-exposure to the standard context affected memory under re-stabilization 24 h after training (Fig. 3A). In effect, the memory was not affected by the drug when it was administered after the exposure to a novel context (Fig. 3B, i.e. in the absence of reactivation). In this case, the effective time window for MUS induced amnesia on reconsolidation lasted 1 h (Fig. 3C).

Results obtained with the $GABA_A$ receptor antagonist, BMI (15.4×10^{-3} μg/g), administered immediately after a WT improved memory retention 24 h after the administration (Fig. 4B). This effect would be explained by the effect of changing the GABAergic functional state during consolidation but not by unspecific effect on the animals' health (Fig. 4B–C).

The effective time window for the drug was around 1 h after training (Fig. 4C). This effective time window turns out to be similar to the ones obtained with other systems using the CMS (Berón de Astrada and Maldonado, 1999; Troncoso and Maldonado, 2002).

Similarly, BMI's facilitating effect (15.4×10^{-3} μg/g) on reconsolidation was observed using a WT protocol (Frenkel et al., 2005); moreover, this susceptible period lasted less than 4 h (Fig. 5C).

In a previous work we established that the absence of mismatch between what is expected and what actually occurs prevents the triggering of the memory labilization re-stabilization. This protocol implies the presentation of the VDS in the last minute of the 5 min re-exposure to the training context. In this study, through the incorporation of this technique the labilization process has been eliminated, causing the drug to be no longer effective in improving the reconsolidation process (Fig. 5D). To sum up, this result confirms previous ones obtained in our memory paradigm, which supports the view that the influence of any interfering agent on the reconsolidation phase depends on to the presence of this mismatching component (Pedreira et al., 2004).

In general, the effect produced by the agonist indicates that the system under study is susceptible to that agonist, since the drug would act on specific receptors. However, this does not necessary imply that these receptors are initially involved in the process. Nevertheless, the effect of a specific antagonist on these receptors reveals that these receptors do indeed play a physiological role on this mnemonic process.

It is necessary to analyze the difference between the time windows for each drug on each phase. Indeed, MUS presented effective time windows which were shorter than those determined for BMI and, in addition, the effective dose used for the agonist was higher. Therefore, the treatment with MUS would be interpreted as modulatory rather than an involvement of the GABAergic system on both phases. However, the results obtained with a lower dose of the antagonist reveal that the $GABA_A$ receptors play a role in the consolidation and reconsolidation processes. An additional explanation for the results with MUS would consider a possible low affinity of the crab's GABA receptors to this drug. Further studies with other agonists could highlight the role of the GABAergic tone activation on memory consolidation in our model.

As a whole, these results show that the systemic administration of MUS impairs the consolidation and reconsolidation processes. In contrast, the administration of BMI, improves both consolidation and reconsolidation processes. Furthermore, the co-administration of both drugs blocks the agonist amnesic effect on the consolidation phase.

It is important to note that the dose administered systemically is the effective dose acting on the nervous system. This conclusion arises from two conditions that may be considered to estimate the effective dose for each drug. First, systemic administration results in a concentration of roughly 0.45 μg/g for MUS and for 4.62×10^{-5} μg/g for BMI in hemolymph because its volume is estimated at 30% of body weight (Gleeson and Subkoff, 1977). Second, the lack of an endothelial blood–brain barrier in crabs along with the fact that hemolymph is distributed through an extensive capillary system in various areas of the brain (Abbot, 1970), determines that 1.2 mM for MUS and 10^{-6} M for BMI act on the different brain regions.

A great number of studies have shown the role of $GABA_A$ receptors on memory processes in vertebrates (Brioni, 1993; Salinas et al., 1993). More generally, when administered either shortly before or shortly after training in a variety of tasks, $GABA_A$ receptor ligands modulate post-training processes underlying memory consolidation (Breen and McGaugh, 1961; Brioni et al., 1989; Castellano and McGaugh, 1990; Ammassari-Teule et al., 1991). However, there are few studies which aim to establish the role of this system on memory processes in invertebrates (Oliver et al., 1970; Alkon et al., 1993; Crow, 2004; Liu et al., 2007).

The research of Bustos et al. (2006) examined the effect of a benzodiazepine ligand (midazolam a $GABA_A$ receptor positive modulator) on memory consolidation and reconsolidation using a contextual fear paradigm in rats. They showed that midazolam interferes with the re-stabilization of the reactivated memory. On the other hand, the drug has no effect on the consolidation process at the dose used. Thus, for this paradigm, the reconsolidation process has distinctive mechanisms that differ from those involved in the consolidation process (Taubenfeld et al., 2001). Conversely, our results substantially extend previous ones, showing similar requirements to consolidate a memory as well as to re-stabilize it after labilization (Pedreira et al., 2002; Frenkel et al., 2005; Merlo et al., 2005).

The use of antibodies against mammal $GABA_A$ receptors recognizes the presence of subunits across evolution from ciliated protozoa to mammals (Delmonte Corrado et al., 2002; Ramoino et al., 2004; Pirker et al., 2000; Feinstein et al., 2003). This ubiquity of the neurotransmitter and its receptors in the animal taxa allows us to use the classic agonist-and-antagonist administration procedure in *Chas-*

magnathus. Indeed, the antagonist reverts the agonist's amnesic effect, a result that strongly supports the suggestion that both drugs act on the same target. Thus, in view of the presence of this neurotransmitter in crabs, all the results reported in this paper can be judged as a result of the modulation exerted by the functional state of the GABAergic system in the CNS.

CONCLUSION

In conclusion, the results obtained with this invertebrate represent an additional evidence supporting the view that some molecular mechanisms subserving different memory phases could be the basic tools employed by phylogenetically disparate animals (Carew, 2000).

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