REACTIVATION CONDITIONS ARE CRITICAL TO PERMANENTLY REDUCE A FEAR MEMORY THROUGH THE REACTIVATION-EXTINCTION PROCEDURE

Piñeyro, M[1 2]; Alfei, J.M[1 2]; Bueno, A[1 2] & Ferrer, R[1 2]
1-Cátedra de Psicobiología Experimental, Facultad de Psicología, Universidad Nacional de Córdoba
2-Laboratorio de Psicología Experimental, Facultad de Psicología, Universidad Nacional de Córdoba

Monfils et al (2009) developed a drug-free behavioral approach to permanently modify fear memories: applying extinction training during the labile or destabilized phase of a reactivated memory. The procedure prevents spontaneous recovery, reinstatement or renewal. However, some researchers couldn't replicate these findings, but never proved to actually destabilize the memory. Given that not all reactivations destabilize a trace, we tried to find if this is critical to the reactivation-extinction procedure. With a contextual fear paradigm in rats, we determined reactivation conditions that could destabilize the trace. Using the amnestic benzodiazepine Midazolam (MDZ) (3 mg/kg), we found that 1 min. reactivation can't destabilize the memory. 3, 4 or 5 min actually destabilized the trace (MDZ reduced freezing compared to saline controls). Actually, 4 min. lead to the deepest MDZ effects. Then we tried to determine if extinction after 1 or 4 min. reactivations would produce differential effects. We trained two groups and applied extinction to only one. Extinction group expressed less fear than control in a 24 hs post extinction test but spontaneous recovery was observed one week later. A similar pattern was observed with a 1 min. reactivation prior to extinction. However, when a 4 min. reactivation preceded extinction, spontaneous recovery didn't occur. These results cannot be explained on the amount of extinction, since a total of 15 min. was always used (0-15, 1-14 and 4-11).

Contacto: r.ferrermonti@gmail.com

INTRODUCTION

In a recent review, Gisquet-Verrier y Riccio (2012) defined **reactivation** as a process thought which a memory is triggered from a latent state to another (active), in which it can be retrieved. This state involves the posibility of using the information represented by the memory, raising to the appearance of a behavioural output. **Labilization** is a process dependent on the degradation of the proteins involved in memory representation and through this process the mnesic trace becomes transitorily unstable, i.e, susceptible of modifications (Lee et al, 2008). It is important to emphazise that although the reactivation is a necessary condition for the recall of a memory, and so on for its labilitazion, reactivation is the previous step for labilization. Finally, the **reconsolidation** is understood as the re estabilization of the labilized memory, which requires the synthesis of new proteins (presumably compensating its labilization or destabilization). Throw reconsolidation, memory goes back to an inactive state which is insensitive to processes that could modify it (Finnie & Nader, 2012).

Considering that reconsolitation raises the possibility of modifying memories proviously consolidated, Monfils et al, (2009) developed a drug free paradigm in which an extinction procedure is applied after labilizing a fear conditioned memory. These results are promising becouse a fear memory is permanently modified by this procedure: it doesn´t present spontaneous recovery, renewal in a new context or reinstatement with unexpected presentations of the unconditioned stimulus.

These results are being reviewed by researchers who could manage to replicate them (Flavell, Barber y Lee, 2011; Roger, 2012) and who could not (Ishii, 2012; Kindt y Soeter, 2011; Costanzi, et al, 2011; Yee et al, 2010). One of the possible explanations for these results that cannot replicate Monfils et al (2009) is that the memories that are studied have not been labilized, idea that is also supported in the evidence that the reactivation of the memory is a necessary condition but is not enough fot its labilization (Bustos et al, 2009; Suzuki, 2004; Lee, 2008). We decided to prove the hypothesis that the success of the reactivation-extinction paradigm depends on the labilization of the memory through its reactivation before the application of the extinction training.

OBJECTIVE:

Determine if the permanent results of reactivation-extinction depends on the effective labilization of the trace, using different reactivation protocols.

MATERIALS AND METHODS:

ANIMALS: Male Rats (Wistar) weighing 280-320 gr., obtained from the Experimental Psychology Laboratory, Faculty of Psychology, National University of Cordoba. The animals were housed in standard cages, in groups of 3-4 animals at a constant temperature (21 ± 1 º C), in a 12 hours light-dark cycle (lights were on from 08:00 to 20:00h). The food and wáter were provided ad libitum. The research in this study was conducted according to the Guide for the Care and Use of Animals lined up by the National Research Council from the USA.

FEAR CONDITIONING: Experiments were carried out in a chamber in order to generate Contextual Fear Memories. The chamber was a rectangular box (24x22x22 cm) made of grey Plexiglas with a grid metal floor. The metalic bars (2mm.) had a 1 cm separation. The floor was connected to a control unit in which the intensity of the scrambled footshock could be set up (Ugo Basile, Comerio, Italia). All the experiments were carried out in an experimental room separated from the place where the animals were housed, in constant light, noise and temperature conditions. After each experimental trial, the cage was cleaned with water and dried with paper towels..

DRUG: Midazolam (MDZ) (Gobbizolam, Gobbi-Novag S.A., Argentina) was diluted in sterile isotonic saline to a concentration of 3mg/ml, administrated intraperitoneally. The total volumen of drug or an equivalent amount of SAL was 1.0ml/kg in all cases.

PROCEDURES

Rats were habituated to handling and injected with SAL 2 days prior to the start of each experiment.

CONTEXTUAL FEAR CONDITIONING: Training consisted in placing each rat in the chamber and allowing a 3 min aclimation period (pre-shock period). After this period, rats recieved 2 foot shocks (1mA, 3 s duration at inter-shock interval of 30 s; unconditioned stimuli). Lasted the second shock, animals were retired of the apparatus and returned to the colony room.

REACTIVATION: 72 h after training, animals were reexposed to the training context without foot shocks for different periods of time (0, 1, 3, 4 or 5 min) depending on the experimental protocol.

EXTINCTION: Animals were exposed to the training context for 15 min without foot-shocks. Depending on the experiment, the total duration of the trial could be distributed in 1 or 2 sessions.

TEST: 24 hs after the recall/extinction trials the animals were exposed to the training context 5 min without shocks.

RE-TEST: 7 days after the Test. Same conditions as the test.

EXPERIMENTAL DESINGS

Experiment 1: A factorial design of 5 (reactivation 0, 1, 3, 4 0r 5 min) x 2 (drug: MDZ or SAL) was used to determine which reactivation condition was necessary to labilize the trace (v. gr, MDZ had amnesic effects).

Experiment 2: Two groups were trained. One was submitted to a 15 min extinction (R0/E15), while the other was a Control group, without extinction (R0/E0). Both were evaluated during the Test and the Re-Test.

Experiment 3: Two groups were trained. One received a reactivation of 1 min and 30 min after, an extinction of 14 min (R1/E14). The other group was submitted to a 1 min reactivation (R1/E0). Both were evaluated in the Test and the Re-Test.

Experiment 4: Two groups were trained. One received a reactivation of 4 min and 30 min later, an extinction of 11 min (R4/E11). The other group (Control Group) only received one reactivation of 4 min. without extinction (R4/E0). Both were evaluated in the Test and the Re-Test

HYPOTHESIS

-MDZ will have amnesic effects only if the reactivation of the memory lasts 3, 4 or 5 min (the lack of reactivation or a short reactivation of 1 min is not enough to labilize the trace).

-If a memory is reactivated 1 min and is followed by an extinction procedure, the memory is modified temporarily. Along the time this memory will present spontaneous recovery as the extinction was applicated on a no-labilized memory.

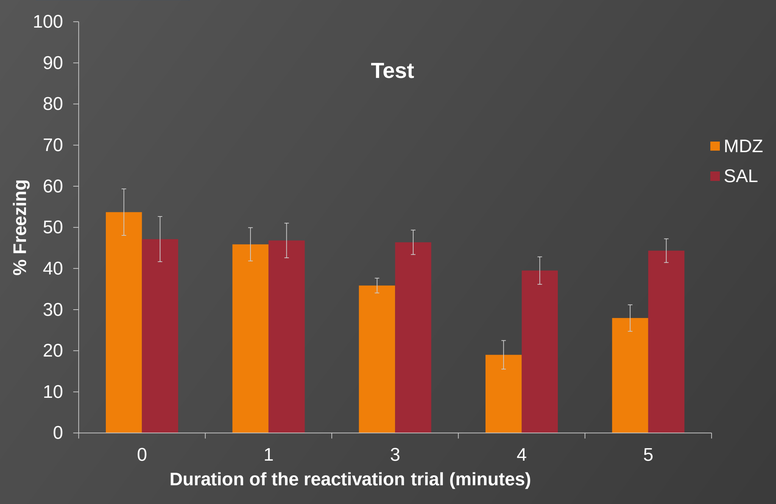
-If a memory is reactivated 4 min and is followed by an extinction procedure, the memory is modified permanently. Along the time it won´t present spontaneous recovery as the extinction procedure was applicated on a previously labilized memory.

DATA ANALYSIS

Results were expressed as mean ± SEM. Significant ANOVAs were followed by post hoc Turkey or Newman-Keuls analysis to enable specific group comparisons (α =0,05).

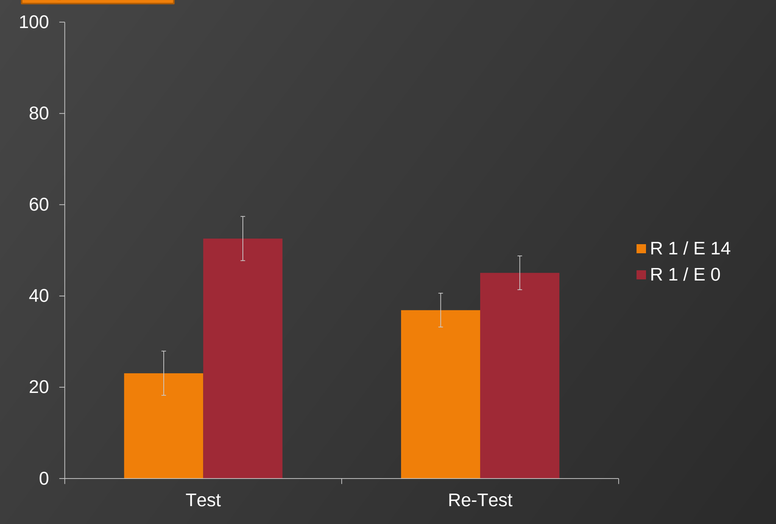
RESULTS

Experiment 1



The ANOVA reported that there was a significant effect of group [F(1, 14)=9,0362, p=,00944], there was no efect of evaluation trial [F(1, 14)=,48944, p=,49564] and there was a significant interaction group x evaluation [F(1, 14)=10,356, p=,00619]. A post hoc analysis revealed that the group R0/E15 showed less freexing during the test than the group R0/E0, what demonstrates that the fear response from the first trial was extinct. However, the groups did not differ during the Re-Test. Furthermore, the group R0/E15 increased the expression of the fear response, the typical pattern of "spontaneous recovery" by the simple passage of time. These results show that the exctinction alone cannot decrease permanently the fear response, as it is amply reported in the literature (Bouton, 2004).

Experiment 3



The ANOVA reported that there was a significant effect of group [F(1, 15)=35,595, p=,00003], there was no efect of evaluation trial [F(1, 15)=1,1884, p=,29285] and there was none significant interaction group x evaluation [F(1, 15)=1,1884, p=,29285]. A post hoc analysis revealed that the group R4/E11 showed less freexing during the test than the group R1/E0, what demonstrates that the fear response from the first trial was extinguished. This difference remained during the Re-Test, in contrast to the reported in previous experiments. This indicates that the reactivation of the memory 4 min before applicating the extinction trial avoids the spontaneous recovery of the extinct memory.

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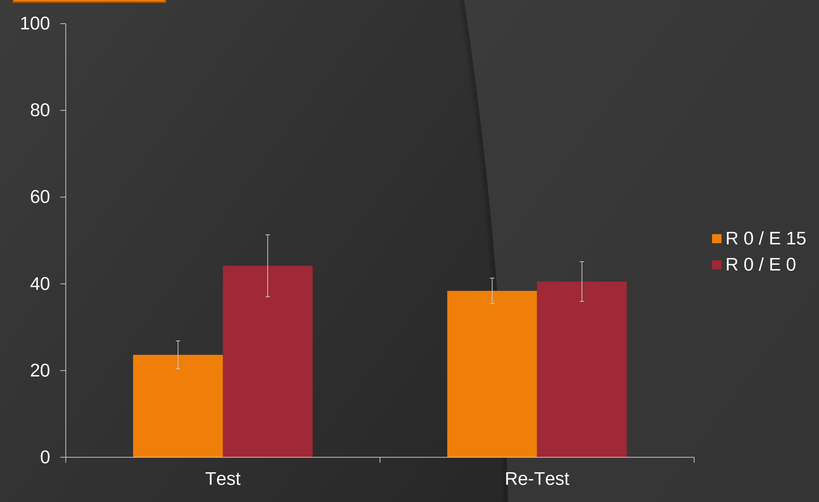
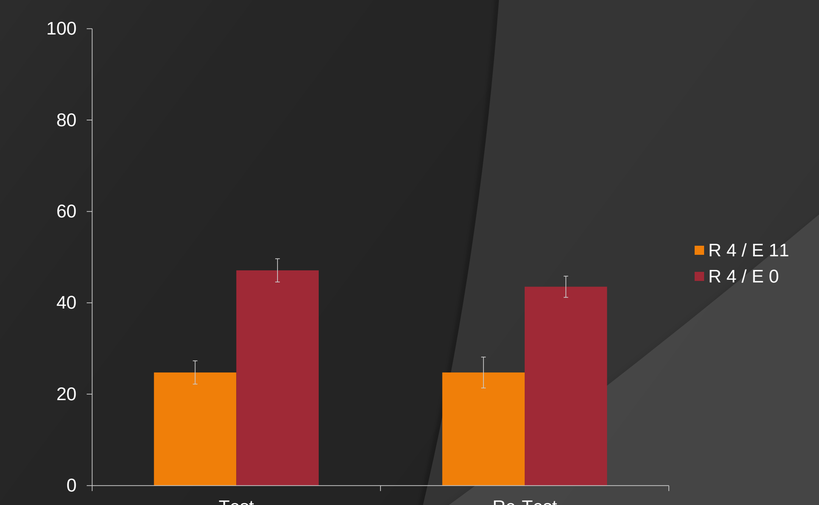
The ANOVA showed a significant effect of the drug [F(1, 63)=11,834, p=,00104], a sisgnificant effect of the reactivation [F(4, 63)=8,6270, p=,00001]and a significant interaction drug x reactivation [F(4, 63)=5,2433, p=,00104]. In order to analyze the cause of this interaction, one way ANOVAs were performed for SAL and MDZ separately to find the effects of each reactivation [F(4, 30)=,23648, p=,91554]. For SAL, there was not a significant effect of reactivation [F(4, 30)=,23648, p=,91554]. A post hoc analysis revealed that 4 min. enhable the highest amnesic effect of MDZ. According to data and as significant differences between 4 or 5 min were not found, in the next experiments we used 1 min reactivations (according to our results, this is not enough to labilize the trace because MDZ lacks of amnesic effect under these conditions) or 4 min (what may induce the highest labilization level).

Experiment 2



The ANOVA reported that there was a significant effect of group [F(1, 14)=17,496, p=,00092], there was no efect of evaluation trial [F(1, 14)=,45359, p=,51160] and there was a significant interaction group x evaluation [F(1, 14)=6,1212, p=,02677]. A post hoc analysis revealed that the group R1/E14 showed less freexing during the test than the group R1/E0, what demonstrates that the fear response from the first trial was extinct. However, the groups did not differ during the Re-Test. Moreover, the group R1/E14 increased the expression of the fear response, the same as the group R0/E15, demonstrating that a brief reactivation is not enough to labilize the trace so the extinction procedure can generate permanent effects.

Experiment 4



CONCLUSIONS

The results are in line with our hypothesis. In order to find permanent effects in a reactivation-extinction procedure it is critically relevant that the memory reactivation (previous to the extinction process) can destabilize the trace. We support our interpretations in the fact that the first experiment showed that 1 min. is not enough to make the memory sensitive to the amnesic agent, while 4 min. actually is. In the same way, when the pre-extinction reactivation lasted 1 min. there was spontaneous recovery, while in the 4 min. trials the recovery of the fear memory wasn´t founded. These findings lead us to conclude that the results found by Monfils et al (2009) are replicable and to do so, it is extremely important to know if the mnesic trace is being labilized through the reactivation procedure, before the application of the extinction learning.

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