**Universidade Federal do Rio Grande do Sul – UFRGS**

**Trabalho de Conclusão de Curso 2015/2 – Ciências Biológicas**

# Atividade do Nucleus Reuniens é necessária para a reconsolidação de uma memória de medo contextual.

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## Apresentação do Trabalho:

Reconsolidação da memória é um processo pelo qual uma memória consolidada é capaz de, após sua reativação com certo nível de diferença entre o evento no qual foi adquirida, entrar em um estado lábil e ativo a alterações podendo encorpar as novas informações relacionadas aos novos parâmetros da reativação através da plasticidade do conjunto de neurônios que a forma.

Desta forma, uma memória pode alterar seu conteúdo original. Esta característica torna o entendimento dos mecanismos biológicos por trás da importantes alvos de estudo na criação de novos implicações clínicas relacionadas a generalização patológica de memórias no Transtorno de Estresse Pós-Traumático.

Neste trabalho analisamos verificamos a influência do Núcleo Reuniens sobre a reconsolidação de uma memória aversiva. O Núcleo Reuniens é um dos núcleos do Tálamo Ventral Medial e funciona como uma comunicação indireta entre Córtex Pré-Frontal medial e Hipocampo, estruturas que já foram demonstradas importantes no processo de reconsolidação deste tipo de memória.

Utilizamos o paradigma de condicionamento aversivo ao contexto capaz de produzir memórias aversivas duradouras pareando um choque elétrico nas patas de ratos ao um contexto emocionalmente neutro.

Miramos o Núcleo Reuniens com cânulas de metal implantadas através de cirurgia estereotáxica e manipulamos sua atividade através de dois diferentes fármacos: Lidocaína, um inibidor da atividade dos canais de sódio e Ciclohexamida inibidor da fase translocacional do RNA transportador durante a tradução de proteínas e por consequência inibidor da síntese proteica.

Nossos resultados mostram que a atividade dos canais de sódio no Núcleo Reuniens durante a reativação de uma memória aversiva é determinante para a

reconsolidação da mesma, sendo que a ausência de sua atividade excitatória durante a reativação acaba diminuindo significativamente os níveis de congelamento do animal quando reexposto novamente ao contexto.

Não encontramos nenhum efeito da síntese de proteínas no Núcleo Reuniens sobre a reconsolidação, já que a infusão da ciclohexamida não interferiu nos níveis de congelamento entre os grupos.

Concluímos então que parece existir um efeito modulatório do Núcleo Reuniens sobre a reconsolidação de memórias aversivas, já que não é necessário que ocorra síntese de proteínas no mesmo, mas sua atividade excitatória é crítica para a correta reconsolidação.

Este trabalho esta escrito em inglês e em formato de artigo para submissão, conforme as regras de publicação do periódico Learning and Memory, no qual pretendemos publicá-lo. Futuramente ainda serão incluídos os resultados de mais dois experimentos com intuito de entendermos melhor a modulação excitatória da estrutura durante a reativação.

# Nucleus Reuniens activity is necessary for reconsolidation of contextual fear memories

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**Abstract: Memory reconsolidation is the process by which a consolidated memory can changed or disrupt by amnesic agents or behavioral interferences. This process require the activity of many neural structures conform the behavior task. In aversive memories pre-frontal cortex and hippocampus activity is critical to correct reconsolidation of the memory trace. Nucleus reuniens is the largest nucleus of midline thalamic nuclei and makes a bridge of communication between mPFC and HP. In this work we tested the effects of the excitatory activity and protein synthesis on NR during the reactivation of aversive memory on the reconsolidation process. We found a critic role of excitatory activity of NR in the reconsolidation of memory, but we not found any effect of protein synthesis of NR in the phenomena. We conclude that there is a modulator effect of NR during the reactivation that can affect the reconsolidation of the memory.**

## Introduction

Memory reconsolidation is a complex and dynamic process in which the content of a consolidated memory can be strengthened, updated or disrupted by amnesic agents (De Oliveira Alvares et al., 2013; Exton-McGuinness, Lee, & Reichelt, 2015). The process requires a labilization state dependent of protein degradation (Sol Fustiñana, de la Fuente, Federman, Freudenthal, & Romano, 2014), returning the memory an

active state unstable and susceptible a pharmacological and behavioral interference. Subsequently, protein synthesis is necessary in order to stabilize the memory trace (Nader, Schafe, & Le Doux, 2000).

There are boundary conditions to destabilize a memory trace as age, strength and retrieval conditions (Reichelt & Lee, 2012; Suzuki et al., 2004). The re-exposure to a conditioned stimulus (CS) associated with the original memory in absence of the unconditioned stimulus (US) is one of most common protocol in order to destabilize an associative learning during classical conditioning(De Oliveira Alvares et al., 2013; Fukushima, Zhang, Archbold, & Ishikawa, 2014; Lee, 2009).

Different reports have already showed the importance of medial PreFrontal Cortex (mPFC) and of the Hippocampus (HP) in the reconsolidation process. In fact, the pharmacological deactivation of PreLimbic region of mPFC as the deactivation of CA1 of dorsal HP impairs the reconsolidation of different kinds of memory such Contextual Fear Conditioning (CFC) and Passive Avoidance (PA) (Baldi & Bucherelli, 2015). Thus, to the correct reconsolidation of a trace could to be necessary an interaction between these structures.

The Nucleus Reuniens (NR) appears to be the main brain structure acting as an interaction pathway between PFC and HP. NR belongs to the midline thalamic nuclei and it is one of largest nucleus of this region. It is situated above the third ventricle and extends to the entire thalamus. NR projects excitatory axons to CA1 of the HP and subiculum, besides that NR also receives dense projections from the anterior cingulate cortex, the prelimbic region, the infralimbic region, and the medial agranular cortex, sub regions of the mPFC. The mPFC receives afferences from HP, but does not project to it. So this anatomical localization makes the NR might play the role of a hub relaying between both structures suggesting a possible function of the NR in the processes that

depend on this interaction(Cavdar et al., 2008; Varela, Kumar, Yang, & Wilson, 2014; Wouterlood, Saldana, & Witter, 1990).

Studies have already demonstrated the role of NR in different mnemonics processes, such as retrieval, consolidation and working memory. However, few works have ever evaluated the role of NR in aversive memories. In 2011 Davoodi et al shown that a reversible inactivation of NR in passive avoidance (PA) task does not impaired memory acquisition, but affected memory retention 24h after training. Besides that, inactivation of NR 5 min after training impaired consolidation, but not after 90 or 360 min. They also showed that the inactivation of the NR impaired memory retrieval in PA task (Davoodi, Motamedi, Akbari, Ghanbarian, & Jila, 2011). The participation of NR in the neural circuit of fear memory specificity and generalization was showed by Xu and Südhof, suggesting a model of how the patterns of NR activity control the details of an auditory aversive memory. (Xu & Südhof, 2014)

To date, was not verified the role of NR in the memory reconsolidation. In order to evaluate the role of NR during memory reconsolidation of aversive memories, we used local infusion of lidocaine or CHX before a brief memory reactivation of contextual fear conditioning.

We found that the excitatory activity of NR is critical to the reconsolidation of contextual fear memories. However, this modulation seems to be independent of protein synthesis in NR, suggesting that this structure is not involved in permanent storage of memory trace but its activity at the moment of reactivation is required to generate appropriate memory stabilization.

## Methods

**1. Animals.** Male Wistar rats weighting 250-350 g from our University breeding colony (CREAL/UFRGS) were used. Animals were housed in plastic cages, four to five in a cage, under a 12 h light/dark cycle and at constant temperature of 22°C, with water and

food *ad libitum*. All experiments were conducted in accordance to local animal care guidelines (Brazilian Federal Law 11,794/2008) and approved by the Ethics in the Use of Experimental Animals Committee of Federal University of Rio Grande do Sul.

**2. Behavioral procedure**. Each experiment consisted of three phases – conditioning, reactivation and test – as described below (see also the diagrams in figure). Memory was measured quantifying freezing behavior. *Freezing* is defined as the absence of all movements except those related to breathing, and expressed as percentage of the total session time.

**2.1. Conditioning chamber (context).** The conditioning chamber (context training) consisted of an illuminated Plexiglas box (20x25x22cm, with a grid of parallel 0.1 cm caliber stainless steel bars spaced 1.0 cm apart).

**2.2. Contextual Fear Conditioning (CFC): training session**. In the training session, rats were placed in the conditioning chamber to habituate for 3 min before receiving two 2-s, 0.7-mA footshocks separated by a 30-s interval - US, they were kept in the conditioning environment – CS for an additional minute before returning to their homecages.

**2.3. Reactivation session**. Two days after training, rats were either re-exposed to the training context (reactivation session), for 3 min. The US – footshock – was absent either during the reactivation session.

**2.4. Test session.** Test consisted of measuring animal´s freezing response to a 4 min exposition to the same training context on day 5 in absence of US.

**3. Stereotaxic surgery and cannulae placement.** Animals were anesthetized with a ketamine and xylazine association (75 and 10 mg/kg, respectively) infused intraperitoneally. A 22-gauge guide cannulae was implanted centrally at AP= -0,16 mm (from Bregma), LL= 0.0 mm, DV= -0,66 mm, positioned just 1.0 mm above the NR

(according to Paxinos and Watson, 1998). After a recovery from the surgery of at least 5 days, behavioral procedures were performed. After that, all animals were sacrificed, their brains dissected and fixed on 10% formaldehyde in order to verify the cannulae placement under low magnification. Animals with inaccurate cannulae placements were excluded from the statistical analysis.

**4. Drugs.** Lidocaine, an antagonist of sodium channels, was used to deactivate the structure impairing your activity during reactivation and Ciclohexamide was used to impair the protein synthesis on NR during the reactivation.

**5. Statistical analysis.** Statistical analysis was performed by using ANOVA for Repeated Measures followed by a Student Newman-Keuls post hoc test, when applicable. Significance was set at $P < 0.05$.

## Results

### Lidocaine impairs reconsolidation and disrupt the memory original trace:

In the first experiment we evaluated whether the deactivation of NR by Lidocaine was able to interfere in the reconsolidation process. Rats were trained in the contextual fear conditioning (CFC) and later re-exposed to the same context (reactivation session) in order to induce memory reconsolidation. Animals were tested two days after reactivation. ANOVA for Repeated Measures revealed a significant effect of group ($F(1,20) = 13,4750$, $P = 0,001517$) and group*session interaction ($F(1,20) = 4,36$, $P = 0,04$), but not of session ($F(1,20) = 0,0203$, $P = 0,88$). In the test session, Student Newman-Keuls post-hoc analysis has shown that the Lidocaine group expressed lower freezing levels compared to Control group ($P = 0,042$). In the reactivation session, however, there were no significant difference between the groups ($P = 0,42$ Newman-Keuls post-hoc analysis)( Figure 1).

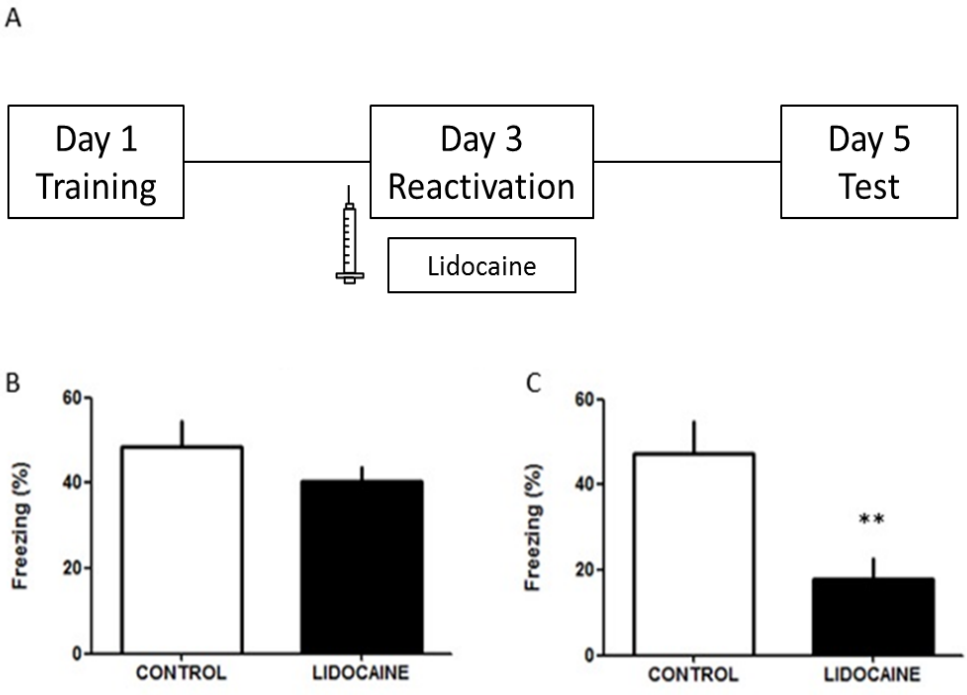


**Figure 1** – **A**. Experimental scheme used in the experiment. **B**. Comparison between control and lidocaine groups in the reactivation session. No significant differences were detected (SNK, $p < 0,05$, $n = 12$). **C**. Comparison between control and lidocaine group in the test session. There is a significant decrease of freezing levels in the lidocaine group when compared with control group (SNK, $p < 0,05$, $n = 12$). Figure 2

**Ciclohexamide in NR during the reactivation do not interfere in memory reconsolidation:**

In the next experiment we evaluate the effect of a protein synthesis inhibitor applied in NR during the reactivation. There is no significant effect using ANOVA for Repeated Measures between group ($F(1,8) = 0,04$, $P = 0,83$), session ($F(1,8) = 3,74$, $P = 0,08$) and group*session interaction($F(1,8) = 0,09$, $P = 0,76$) (Figure 2).

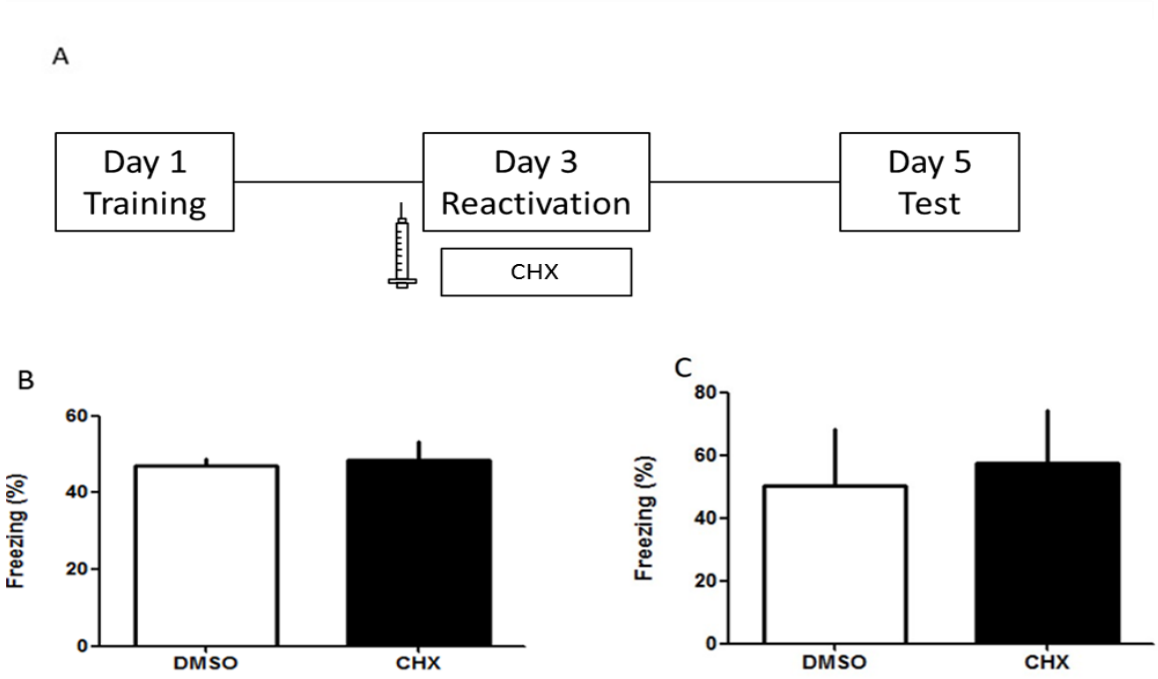


**Figure 2** – **A**. Experimental scheme used in the experiment. **B**. Comparison between DMSO and CHX groups in the reactivation session. No significant differences were detected(SNK, $p > 0,05$, $n = 6$). **C**. DMSO and CHX groups in the test session. No significant differences were detected (SNK, $p > 0,05$, $n = 6$).

## Discussion

Our results suggest that deactivation of NR during a brief reactivation of memory impair the reconsolidation process and disrupt the original memory trace. Currently, no works had evaluated the roles of NR in the CFC paradigm and it opens a series of question about the structures involved in the reconsolidation.

We not found decrease of freezing levels during the reactivation session. This finding is not the accordance with Davoodi reports that showed deficits in memory retrieval in the WM and IA tasks(Davoodi et al., 2011; Davoodi, Motamedi, Naghdi, & Akbari, 2009). It can be explained by the difference in the task used in our experiments. IA and WM tasks recruit decision making and navigation systems in order to promote appropriate memory retrieval turning more complex the reconstruction of memory trace.

NR sends information from mPFC to HP through excitatory connection(Cavdar et al., 2008). Thus, the activation of NR by the mPFC sends information to assemble in the connections of CA1 region of HP. This activity was demonstrated to participate in a neural circuit to generalization of fear memories(Xu & Südhof, 2014). We hypothesize that the deficit found in the reconsolidation may to be associated to with these findings. The precision of reactivation session could be decreased without NR activity and the memory reconsolidated in a weak form compared to original memory trace. However, this interpretation raises more questions than answers, for example, why retrieval is not disrupted during reactivation if memory precision decreases? Future experiments may be performed to elucidate the relation between adequate retrieval and memory precision.

Besides that, was suggested a critical role of NR in the working memory. Some authors suggest that some memory processes need attention systems to work(Exton-McGuinness et al., 2015; Lee, 2009; Pearce & Hall, n.d.). One recent example of this relation between attention and the reconsolidation was reported in our lab l. Crestani et

al., showed that a neutral distractor stimulus can alter the reconsolidation process and disrupt the memory trace if presented during the reactivation session in the CFC(Crestani et al., 2015). Working memory is accepted for many authors like one of cognitive processes involved in the attention system(Clark & Noudoost, 2014; Kiyonaga & Egner, 2013; Logue & Gould, 2014; Woodman, Carlisle, & Reinhart, 2013). This suggest that the recruitment of working memory system during the reactivation could drive the expression of defensive behaviours such as freezing response and would be necessary to engage the labile state during memory reconsolidation. In the absence of NR activity this processes can be inefficient in to destabilize the memory trace and can be responsible for an incorrect reconsolidation process, inducing the amnestic effect verified during memory test. To this point, however, this is all very conjectural and future investigations could identify the specific role of the NR in attention process and working memory performance during reconsolidation of contextual fear conditioning.

We also tested the necessity of protein synthesis in the NR during the reactivation to induce reconsolidation. Protein synthesis is not necessary in NR to reconsolidation of a CFC memory. Our result suggest that NR modulates memory reconsolidation by excitatory connections provides from NR to HP and not by protein synthesis.

## Conclusions

With the data presented in this work it is clear that the NR to participate reconsolidation of a contextual fear memory. Some questions still open about this finding. Now, we are working in identify the phase of memory reconsolidation (destabilization or restabilization) in which NR activity is necessary. Besides that, experiments with generalized memories are necessary to understand better how the NR mediate the interaction between mPFC and HP during the reconsolidation phenomena considering the precision and generalization of a memory trace.

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