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TRABALHO DE CONCLUSÃO DE CURSO
BIOMEDICINA

Aprendizados adicionais aceleram a reorganização de memórias aversivas

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Índice

1 Prefácio

A teoria da consolidação sistêmica é atualmente um dos temas de maior destaque e relevância na pesquisa da memória, pois busca responder uma questão fundamental: como o encéfalo consegue manter informações de maneira duradoura. Nas últimas décadas, os avanços obtidos nesta área da neurociência têm nos permitido vislumbrar o complicado e engenhoso caminho pelos quais as memórias passam do momento em que são formadas, até o momento em que são lembradas mesmo anos depois. Estudar a consolidação sistêmica é fascinante justamente por isso, nos aproxima do entendimento da memória não apenas nos rápidos fenômenos bioquímicos e moleculares em pequenos circuitos, mas também nas modificações graduais e difusas, envolvendo diversas estruturas, sem as quais provavelmente não lembraríamos de memórias remotas de nossas infâncias.

Os experimentos apresentados neste trabalho foram realizados durante o primeiro semestre deste ano, e foram motivados pelos resultados positivos obtidos em experimentos realizados em 2009 (Anexo 2). Aqui buscamos, em uma tarefa comportamental distinta, descobrir se o fenômeno que observamos é uma característica específica daquele modelo comportamental ou se é mais abrangente, influenciando também outros tipos de memória.

2 Resumo

Introdução e objetivo: Após os períodos iniciais de aquisição e consolidação, as memórias passam por um lento processo de reorganização conhecido como consolidação sistêmica. O hipocampo é apenas necessário para a evocação da memória da tarefa de Esquiva Inibitória (EI) até cerca de 30 dias pós-treino - após isso a evocação se torna independente do hipocampo e dependente de estruturas corticais. O objetivo deste estudo foi avaliar se aprendizados adicionais aceleram o processo de reorganização da memória e a sua independência do hipocampo.

Métodos: 50 ratos Wistar machos foram treinados na tarefa de EI (2 choques de 0,7 mA/2s), e divididos em dois grupos, Multitarefas (MT) e Sem Multitarefas (SMT). A canulação estereotáxica bilateral no hipocampo foi feita 5 dias antes do teste, que ocorreu 20 dias após o treino. Durante o intervalo entre o treino e o teste da EI, apenas os animais do grupo MT foram submetidos às tarefas adicionais de Reconhecimento de Objetos e Labirinto Aquático de Morris. Em ambos os grupos, os animais foram infundidos com TFS (tampão fosfato-salina) ou muscimol 1ug/lado 20 min. pré-teste.

Resultados: Os tempos de latência (medida de memória) dos ratos do grupo SMT tratados com TFS foram significativamente superiores no teste em relação ao treino (N = 10; p = 0,01 - Teste de Wilcoxon), indicando que os ratos aprenderam com sucesso a tarefa, mas nos animais do grupo SMT tratados com muscimol não houve diferença entre treino e teste (N = 7; p = 0,8 - Teste de Wilcoxon), indicando um efeito amnésico da droga. Já no grupo MT, os ratos tratados tanto com veículo quanto com Muscimol apresentaram tempo de latência no teste significativamente superior ao treino (N = 25 e N = 17; p = 0,001 e p = 0,002, respectivamente – Teste

de Wilcoxon). Análise das latências do teste por Kruskal-Wallis revelou diferença significativa entre grupos (p=0,02). Comparações individuais por Mann-Whitney entre todos os grupos revelaram diferença significativa entre o grupo SMT tratado com muscimol com os grupos SMT tratado com TFS (p=0,001), MT tratado com TFS (p=0,002) e MT tratado com muscimol (p=0,003).

Conclusão: O muscimol intrahipocampal foi amnésico na evocação da memória de EI 20 dias pós-treino apenas nos animais que não passaram pelo protocolo MT, o que sugere que os aprendizados adicionais de alguma forma aceleraram o processo de reorganização da memória, fazendo com que seu traço original se torne independente do hipocampo mais rapidamente.

3 Introdução

Uma das capacidades mais notáveis dos animais é a de modificar seu comportamento através do aprendizado. Há mais de 100 anos, tenta-se entender quais modificações ocorrem no encéfalo durante o aprendizado, onde as memórias são armazenadas e uma vez armazenadas, como são retidas.

Após um aprendizado, a memória não se estabelece de maneira instantânea. Müller e Pilzecker, em trabalhos do final do século 19, observaram que memórias recentemente aprendidas eram prejudicadas por um novo aprendizado induzido logo após o primeiro, sugerindo que após um aprendizado ocorre a formação de um traço de memória que inicialmente é frágil e lábil, levando tempo para se consolidar e se tornar estável. Estudos mais recentes com cobaias mostram que nos primeiros minutos após a aquisição, as memórias são suscetíveis a interferências de drogas, lesões, novas experiências e outros tratamentos como hipotermia e eletrochoque convulsivo. Com o passar do tempo, porém, as mesmas interferências não mais são efetivas (Alberini et al., 2006).

Pode-se dividir a memória em quatro fases distintas: a aquisição, que é o período em que o animal está sendo exposto à experiência; a consolidação, que é o período onde o traço da memória ainda é instável e suscetível a interferências; o armazenamento, onde ocorre a internalização e estabilização do traço de memória e a evocação, onde a memória do passado é lembrada e expressada na forma de uma mudança comportamental. Sabe-se que durante a evocação, a memória torna-se novamente lábil e suscetível a interferências, podendo passar por dois processos distintos: a reconsolidação, onde a memória original é fortalecida e novas informações são a ela adicionadas ou a extinção, onde é formada uma nova memória

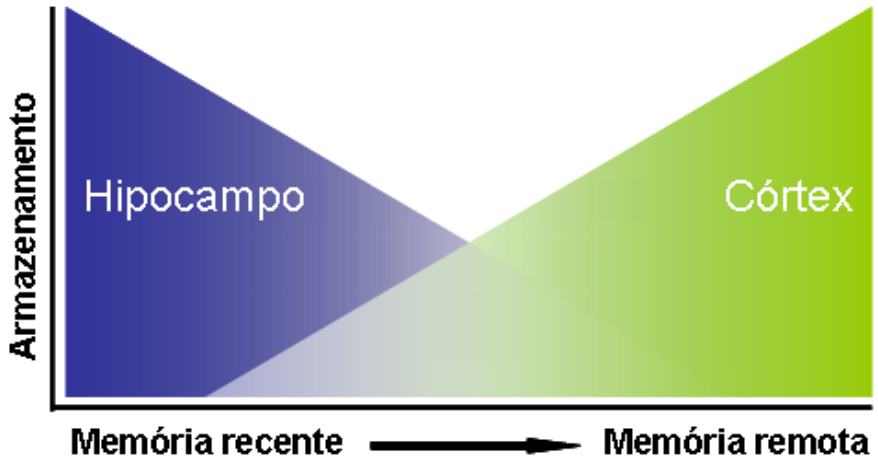
com significado diferente, que irá competir com a memória original (Izquierdo, 2002).

O breve processo de consolidação descrito anteriormente é concluído no intervalo de algumas horas após a aquisição e reflete mudanças em vias intracelulares de sinalização, síntese protéica e expressão gênica, que levam a modificações sinápticas e estruturais nos neurônios envolvidos com o traço de memória formado após o aprendizado. No entanto sabe-se, a partir da observação de pacientes com lesões seletivas em certas estruturas encefálicas, que um outro tipo de consolidação ocorre numa escala de tempo mais ampla. Esse tipo de consolidação, cunhado de consolidação sistêmica, pode levar semanas, meses e até anos para ser concluído. Em humanos, danos no hipocampo produzem amnésia retrógrada temporalmente gradual, onde as memórias remotas são preservadas e as memórias recentes são afetadas, e esta constatação é o pilar central da idéia de que à medida que as memórias amadurecem, elas se reorganizam em diferentes estruturas encefálicas de maneira tempo-dependente (Frankland & Bontempi, 2005).

No final do século 19, o psicólogo francês Theodule Ribot descreveu como a amnésia após danos encefálicos era geralmente relacionada com a idade da memória, mostrando que a dissolução da memória é inversamente proporcional à sua idade (Ribot, 1882). Essa dissociação ficou conhecida como Gradiente de Ribot. Foi apenas na metade do século passado, porém, que a relação entre a localização do dano encefálico e o gradiente foi estabelecida quando Henry Molaison, conhecido como paciente H.M., foi tratado para sua epilepsia com a remoção bilateral do lobo temporal medial, incluindo o hipocampo. Apesar do sucesso da cirurgia no tratamento da epilepsia, H.M. passou a exibir incapacidade de adquirir novas

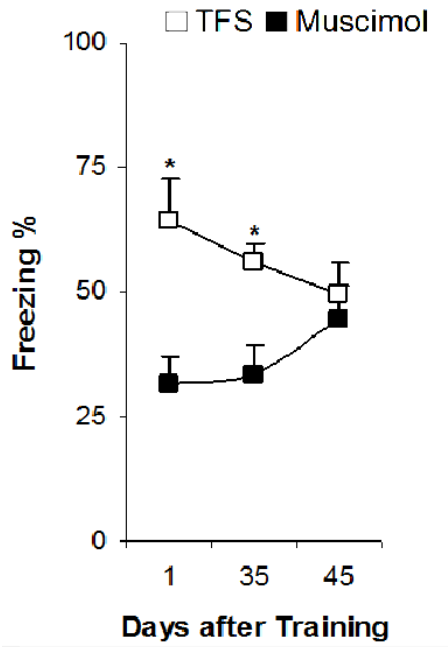
memórias de pessoas, eventos e lugares, e amnésia retrógrada. Sua amnésia retrógrada, porém, era incompleta: ele perdeu suas memórias de eventos que ocorreram de meses a alguns anos antes da cirurgia, mas lembrava bem de eventos que ocorreram há aproximadamente mais de 11 anos antes da cirurgia (Scoville & Milner, 1957; Sagar et al., 1985). Estas foram a primeira evidência que o lobo temporal medial está envolvido na consolidação, e por um tempo no armazenamento, de memórias de longa duração.

Estudos posteriores com pacientes com danos mais seletivos em diferentes regiões do lobo temporal medial demonstraram que danos no hipocampo tipicamente levam à amnésia retrógrada com gradiente temporal (Rempel-Clower et al., 1996), e que a extensão da amnésia retrógrada é proporcional à extensão do dano nesta estrutura e em estruturas adjacentes (Squire & Alvarez, 1995). Por outro lado, danos que vão além do lobo temporal medial levam a amnésia retrógrada não graduada temporalmente que afeta até mesmo memórias de infância, mas poupa memórias mais recentes, possivelmente por afetar regiões de armazenamento permanente de informações (Squire & Alvarez, 1995). Estas observações evidenciaram que o hipocampo tem um papel temporalmente limitado no armazenamento das memórias, e que o armazenamento permanente dependeria de redes corticais difusas.

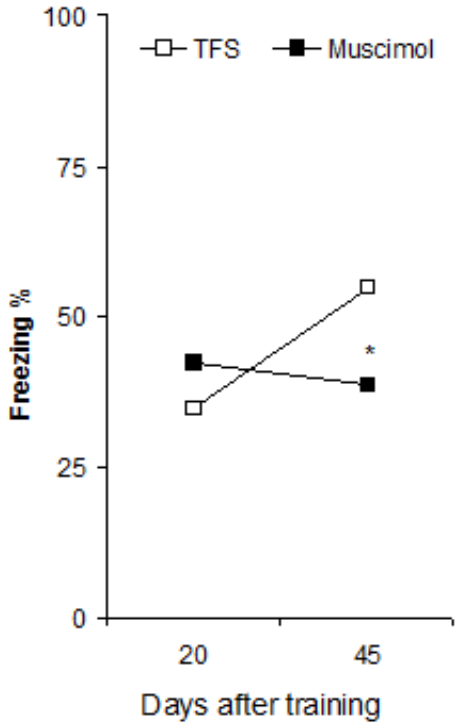


Diversos estudos, utilizando várias espécies, modelos comportamentais e inativações do hipocampo (incluindo inativações farmacológicas, por lesão e manipulações genéticas) são congruentes com os achados clínicos, mostrando que prejuízos na função hipocampal afetam preferencialmente memórias recentes, e não remotas (Kim & Fanselow, 1992; Squire, 1992; Quillfeldt et al., 1996; Shimizu et al., 2000). A extensão do gradiente varia entre os estudos, indo de alguns dias a várias semanas, sendo que provavelmente esta diferença temporal se deve a fatores como espécie, tipo de tarefa comportamental e tamanho da lesão ou inativação do hipocampo.

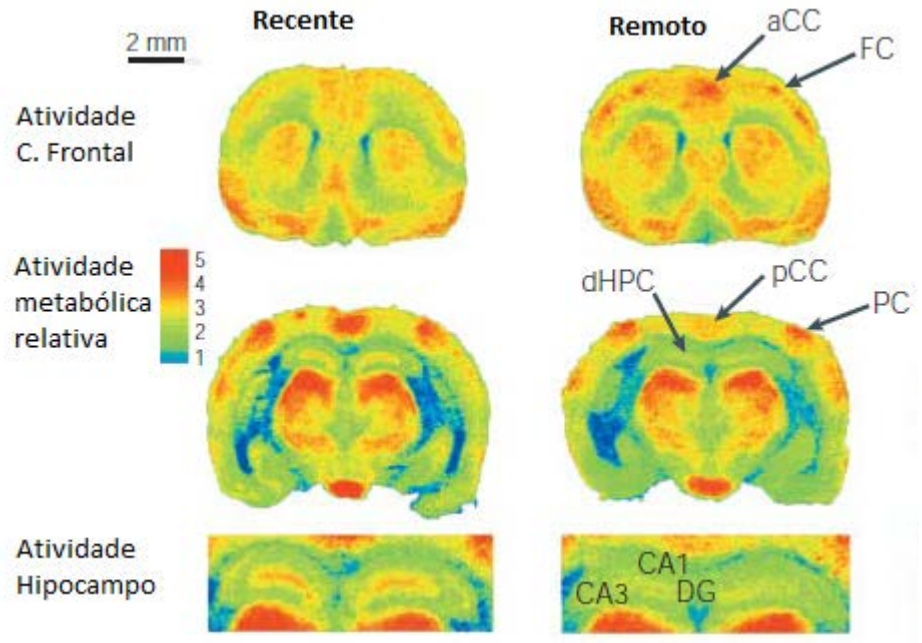
Dados preliminares do nosso laboratório (não publicados) mostram que na tarefa de Condicionamento Aversivo ao Contexto, a inativação farmacológica do hipocampo provocada pela infusão localizada do agonista gabaérgico muscimol é amnésica quando feita 1 e 35 dias após o treino, mas não quando feita 45 dias após o treino.



De maneira oposta, também vimos que a infusão de muscimol no córtex cingulado anterior provoca amnésia quando feita 45, mas não 20 dias após o treino.



Além disso, Bontempi et. al, 1999, treinaram ratos em uma tarefa espacial discriminatória e observaram a atividade metabólica de diferentes regiões encefálicas na evocação da memória 5 dias ou 25 dias após o treino. A evocação de memórias recentes foi associada com ativação do hipocampo e córtex entorrinal, e a evocação de memórias remotas com ativação de regiões corticais como o córtex pré-frontal, frontal, cingulado anterior e temporal.

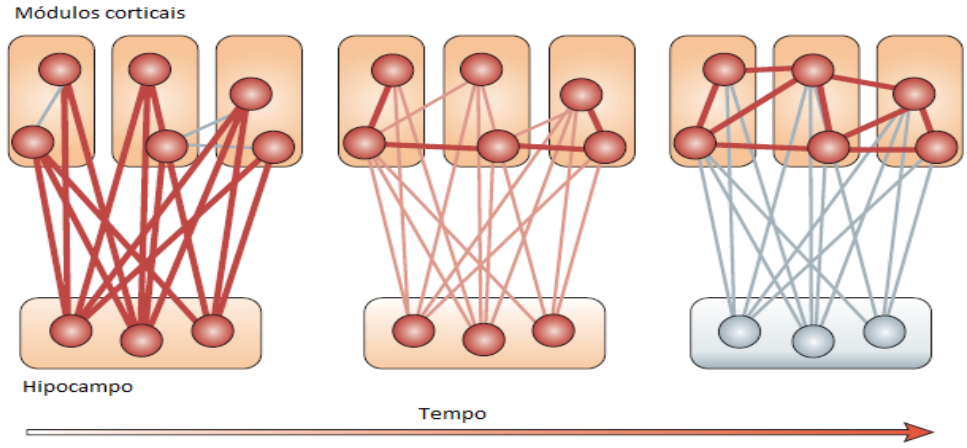


(adaptado de Bontempi et al., 1999)

Juntos, estes trabalhos clínicos e experimentais sugerem que as memórias são gradualmente reorganizadas com o passar do tempo, com a transferência de memórias recentes para áreas corticais para armazenamento permanente. Desta forma, a contribuição de diferentes estruturas varia com o tempo. Inicialmente, algumas estruturas como o hipocampo contribuem de maneira importante para a evocação das memórias, mas essa contribuição diminui com o passar do tempo e a memória passa a ser independente delas. De maneira oposta, outras estruturas inicialmente não são essenciais para a evocação, mas em tempos remotos elas passam a ter um papel preponderante.

Os principais mecanismos envolvidos na formação de novas memórias dependentes do hipocampo são hoje razoavelmente bem compreendidos, mas muito pouco se sabe sobre como estas memórias persistem por longos períodos, às vezes mesmo por toda a vida de um organismo.

David Marr foi o primeiro a formular um modelo explicando a consolidação sistêmica, propondo que o hipocampo rapidamente armazenaria memórias de eventos antes que estas se transferissem para o córtex. Segundo ele, esta transferência de informações dependeria de reativações ou replays de padrões neurais, talvez durante o sono. Desta forma, inicialmente haveria um processamento paralelo das memórias no hipocampo e no córtex. Sucessivas reativações das redes hipocampais ativariam redes corticais, levando a um fortalecimento gradual das conexões cortico-corticais, que eventualmente se tornariam maduras o suficiente para permitir que as memórias se tornassem independentes do hipocampo (Marr, 1970; Marr, 1971).



(adaptado de Frankland & Bontempi, 2005)

A fim de avaliar a importância da comunicação entre o hipocampo e o córtex na formação de memórias remotas, Remondes et al., 2004, lesionaram projeções do córtex entorrinal para o hipocampo, mantendo o hipocampo funcional mas impedindo interações cortico-hipocampais. Após a lesão, as cobaias foram treinadas na tarefa de Labirinto Aquático de Morris e submetidas a um teste 24 horas ou 28 dias depois. Os ratos lesionados tiveram um aprendizado normal e nenhum prejuízo no teste realizado 24 horas após o treino, mas 28 dias após a memória foi

prejudicada, em concordância com a necessidade de interações cortico-hipocampais para a formação de memórias remotas teorizada por Marr.

Artigos recentes mostram que a consolidação sistêmica não é um processo passivo e pré-determinado. Treinos intensivos produzem memórias que se reorganizam mais rapidamente do que treinos fracos (Winocur et al., 2005; Lehman et al., 2009). Além disso, após a lenta e gradual reorganização de uma memória no córtex, novas memórias similares podem rapidamente serem incorporadas nos esquemas corticais pré-existentes e serem evocadas na ausência de um hipocampo funcional (Tse et al., 2007).

Este trabalho partiu da hipótese de que (i) o hipocampo tem uma capacidade limitada de armazenamento de informações (Willshaw & Buckingham, 1990) e (ii) atua no sentido de codificar e armazenar informações recentes de maneira rápida, mas apenas enquanto estas informações ainda não estão estabilizadas no córtex. Não sendo o hipocampo apto a armazenar uma grande quantidade de informações ao mesmo tempo, o período que uma dada memória levaria para se reorganizar e se tornar independente desta estrutura seria influenciado pela quantidade de novas informações processadas e armazenadas. Assim, em uma situação com intensos aprendizados e formações de novas memórias, as memórias mais recentes competiriam com as mais antigas pelo armazenamento hipocampal, acelerando a transferência das mais maduras ao córtex.

4 Objetivos

Nosso objetivo geral foi investigar a duração da dependência hipocampal de uma memória aversiva de Esquiva Inibitória em ratos (por quanto tempo ela fica sensível à infusão de muscimol no hipocampo), comparando animais que foram treinados na tarefa aversiva e apenas testados vinte dias depois, com animais que, além disso, realizaram diferentes e sucessivos aprendizados adicionais durante esse intervalo de tempo.

Objetivos específicos:

● Verificar o efeito da droga muscimol, infundida 20 minutos pré-teste no hipocampo dorsal, vinte dias pós-treino em animais que apenas aprenderam a tarefa de *esquiva inibitória* (protocolo “sem multitarefas”);

● Verificar o efeito da droga muscimol, infundida 20 minutos pré-teste no hipocampo dorsal, vinte dias pós-treino em animais que passaram por tarefas adicionais interpostas entre o treino e o teste em *esquiva inibitória* (protocolo “multitarefas”);

● Verificar se também houve aprendizado adequado nas tarefas adicionais interpostas entre o treino e o teste em *esquiva inibitória*, isto é, no *Reconhecimento de Objetos* e no *Labirinto Aquático de Morris* (protocolo “multitarefas”);

● Verificar se há diferença na atividade motora e no comportamento exploratório na tarefa de *Campo Aberto* entre os animais que passaram e os que não passaram pelo protocolo “multitarefas”;

● Verificar se há diferença nos níveis de ansiedade na tarefa de *Labirinto em Cruz Elevado* entre os animais que passaram e não passaram pelo protocolo de multitarefas.

5 Artigo Científico

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Abbreviated title: New learning accelerate systems consolidation

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Abstract

After initial encoding, memory undergoes a time-dependent reorganization process, whereby its retrieval becomes independent of hippocampus and sustained by cortical regions. Despite the great number of evidences showing the time-limited role in memory storage and retrieval of hippocampus, it is still not fully understood how does it happens, why does it happens and which parameters influence it. In this work, we investigated the influence of new learning experiences upon the system consolidation of a Step-down inhibitory avoidance task (IA). The experiments showed that retrieval of the IA memory is still dependent of hippocampus even twenty days after the acquisition, as we can find in literature. However, when the animals were exposed to additional learning after IA training, the retrieval of this memory was already independent of hippocampus. The newly acquired information probably accelerated the IA system consolidation, making it reorganizes and store in extra-hippocampal regions earlier. Our data indicate that reorganization of memory and the process by which its retrieval becomes independent of hippocampus is dynamic and can be influenced by other non-related learning episodes.

Introduction

The idea that older memories are more resistant and recent memories are more vulnerable to injuries and interferences have been hypothesized since the 19th century with Ribot's observations of patients with brain damages and temporally graded amnesia (Ribot, 1882). Since then, clinical and experimental studies have been corroborating with this view.

Clinical studies show that patients with localized CA1 lesion usually displays 1 to 2 years retrograde amnesia, while perirhinal, enthorinal e parahippocampal lesions lead to retrograde amnesia with an extension of decades. Some patients with damages to frontal, parietal, temporal and occipital cortex had impairments to recall autobiographical episodes even for their early life (Bayley, 2003).

Using several animal´s behavioral models, previous studies showed that lesions or pharmacological inactivation of hippocampus made at short delays after training hindered memory expression, but not at longer delays (Clark et al., 2002; Izquierdo et al., 1997; Kim and Fanselow, 1992; Kim et al., 1995; Quillfeldt et al., 1996; Winocur, McDonald and Moscovitch, 2001). Genetic approaches had similar results (Shimizu et al., 2000; Yasuda and Mayford, 2006). Also, neuroimaging studies of Bontempi et al. (1999) shown that retrieval of a spatial discrimination task 5 days after training causes an increased hippocampal metabolic activity, while at 25 days after training, the elevated metabolic activity is found in anterior cingulated, prefrontal, medial and temporal cortices. Taken together, these studies turned clear that memory, in a time-dependent manner, reorganizes itself, becoming independent

of hippocampus and dependent of cortical regions, a process that have been called systems consolidation.

During consolidation, the strengthening of cortico-cortical connections appears to be crucial for memories become independent of hippocampus, since the inhibition of cortical plasticity hinders the expression of remote, but not recent memories (Frankland et al., 2001). The hippocampus would have a key role in this process, as successive rounds of reactivation of hippocampal-cortical connections would precede the cortico-cortical stabile connections. Theoretical models propose that cortical consolidation must be slow, otherwise new information would destabilize others already stored (Marr, 1970, 1971).

Recent works brought interesting evidences indicating that system consolidation of memory is not static, but a dynamic process. Extensive experience in contexts or tasks enables fast cortex consolidation and memory survival after hippocampal lesion (Lehman et al., 2009; Winocur et al., 2004). Also, once memories are cortically established, neuronal circuits can create mental schemas that make possible to new similar memories rapidly become independent of the hippocampus (Tse et al., 2007).

The memory reallocation process, proposed as a modulatory mechanism whereby memories would be stored in specific neuronal networks (Han et al, 2007), may also contribute to systems consolidation. It is possible that this mechanism operates regulating storage of old memories in the long term reorganization process of systems consolidation. Thus, new memories may force older ones to be stored and integrated in neocortical networks sooner (Won & Silva, 2008).

Since the hippocampus is a necessary structure for initial memory encoding, but only essential in retrieval for a limited time, it is valid to ask why memory becomes hippocampus-independent. One possibility may be that the hippocampus, despite its central role in memory encoding, may have a limited storage capacity. We hypothesize that newly acquired information would compete with an older one for hippocampus housing, making it reorganizes and store in extra-hippocampal regions earlier. To address this issue, we investigated if aversive memory's reorganization and its hippocampus-independency are influenced by additional non-related learning.

Material and Methods

Male Wistar rats were trained in a Step-down inhibitory avoidance task (IA) and tested for retention 20 days later. Rats were placed on a 2.5 cm high, 8.0x25.0cm platform facing a 42.0x25.0cm grid of parallel 0.1cm-caliber stainless steel bars spaced 1.0cm apart. Latency to step down placing their four paws on the grid was measured. In training sessions, immediately after stepping down, animals received a 0.7mA, 2sec scrambled footshock. In test sessions no footshock was given. In both training and test, step down latency was cut off at 180 seconds.

In order to investigate whether the hippocampus was necessary or not for the retrieval of the IA memory, rats were submitted to stereotaxic surgery and 15 min before the test session a classical amnesic drug or its vehicle was infused into the dorsal hippocampus. Surgeries took place five days before the IA test, when all animals were bilaterally implanted with cannulae aimed at the CA1 area of the dorsal hippocampus, according to Paxinos and Watson (1986). Animals that have not correct cannulae placements were not included in statistical analysis. The drug used

in the infusions was muscimol, a selective GABAa agonist (1 ul/side; Tocris Cookson Inc., Ellisville, MO, USA), known amnesic in retrieval of hippocampus dependent memories (Holt and Maren, 1999), or its vehicle, TFS (phosphate-buffered saline).

Animals were divided into two experimental groups: Single-task (ST) and Multiple-tasks (MT) (Fig. 1). In the meanwhile of training and test of IA, the ST group has not gone through any different experience. At the same period, however, the MT group was trained into two additional tasks: Object Recognition (OR) and Morris Water Maze (WM). If an increase in mnemonic activity of hippocampus can really accelerate the corticalization of a previous memory, we expect that in the MT group the IA memory will be independent of hippocampus more quickly.

The OR task was conducted in a transparent acrylic open-field arena (60 × 40 × 50 cm). Before training, animals were habituated to the arena by allowing them to freely explore it 30 min per day for 2 days in the absence of objects. Objects were behaviorally irrelevant and equally conspicuous for the rats. On training day, rats were placed in the open field containing two objects and left to freely explore them for 5 min. Test session, lasting 5 minutes, was performed 24h later, when one of the objects was randomly exchanged for a novel one. Exploration was defined as sniffing or touching the stimulus object with the nose and/or forepaws. Data were expressed as percentage of the total exploration time in seconds.

The WM was a circular divided in four equal imaginary quadrants. Two centimeters beneath the water surface and hidden from the rats' view, there was a platform 12 cm

in diameter The WM was located in a room with several distal visual stimuli on the walls to provide spatial cues. The WM task was carried out during five consecutive days. In each training day/session, rats were submitted to four consecutive training trials, while the hidden platform was kept in a constant position. A different starting location was used for each trial, which consisted of swimming followed by a 20s sitting on the platform. Rats that did not find the platform within 60 s were guided to platform. Memory retention was evaluated during a 60s probe trial carried out at the last day of this task, in the absence of the escape platform

To ensure that the elevated number of activities and the major manipulation of animals on the MT group did not affect the motility and anxiety behavior, we performed the Open Field (OF) and Elevated plus-maze (EPM) tasks one day after the end of all experiments.

Results

We first accessed if there was significant latency difference between training and test in each individual group. In ST group, rats treated with TFS had significantly higher latency in test compared to training, showing successful retrieval of the IA task (N=10; p=0,01, Wilcoxon test). Rats in ST groups treated with muscimol, in the other hand, had no difference between training and test, showing a amnesic effect of the drug (N=7; Wilcoxon test). In MT group, rats treated with TFS had significant difference between training and test latency (N=25; Wilcoxon test) as well as rats treated with muscimol (N=17; p=0,002, Wilcoxon test), indicating a lack of effect of this drug.

Test session step-down latency differences among groups were evaluated by one-way Kruskal-Wallis analysis of variance, showing significant difference among groups (p=0,02). Comparisons between test session values of all groups were done by individual Mann-Whitney U-test, showing significant differences only between ST group treated with muscimol and ST group treated with TFS and MT group treated with both TFS and muscimol (p=0,001, p=0,002 and p=0,003, respectively). Thus, in the group exposed to additional tasks, muscimol administered into hippocampus had not any amnesic effect (Fig. 2). This experiment shows that retrieval of the IA memory is still dependent of hippocampus twenty days after training session, but only in animals that were not exposed to additional learning after the IA training.

In MT group, animals also effectively learned all the additional tasks that they were submitted. In the OR test, that was significant difference between novel and familiar objects exploration (p= 0,000; Paired Samples Test), as shown on Fig. 3a. In addition, there was no preference for objects on the training day. The time taken to find the platform in the last day of WM training was significantly lower compared to the first one (p=0,000; Paired Samples Test), as we can see on Fig. 3b. On the WM test, the animals significantly explored more the Target Quadrant than the Opposite one (p= 0,001; Paired Samples Test) (Fig. 3c). This results show that the great number of activities in this group, and possible stress, did not affect the cognitive capacity of the subjects.

The number of crossings and rearings on the OF was used to analyze the exploratory behavior (Fig. 4a and 4b). Independent T Test showed no difference between groups

in the number of crossings (p=0,7) as well in the number of rearings (p=0,9). The time spent in the open arms on the EPM was used to evaluate anxiety-like behavior. Independent T Test showed no differences in the time spent in the open arms between groups (p=0,9) as displayed in Fig. 4c. At that time, the raised high activities and the elevated manipulation of the MT animals did not affect their exploratory activity neither their anxiety state.

Discussion

In this work we tested if new leanings during the system consolidation of a contextual fear memory could interfere with this process. The main task used to investigate the hippocampal participation in retrieval was the step-down inhibitory avoidance. We choose this task because it is a hippocampus dependent and easily learned in a single training session. Moreover, hippocampus dependency of Step-down inhibitory avoidance task across time has been well studied (Quillfeldt et al., 1996; Izquierdo et al., 1997). To propitiate new experiences WM and OR tasks were chosen, for three major reasons: (i) Both are hippocampus dependent (Redish & Touretzky, 1998; Kelly et al. 2003); (ii) their acquisition require many sessions, maximizing the requirement of hippocampus during the period of IA systems consolidation; and (iii) this tasks do not involve shock neither food restriction, diminishing the stress of animals. Although there are some discussion if OR is hippocampus-dependent or not (Langston & Wood, 2009), we were based on protocols that have demonstrated the requirement of hippocampus activity and protein synthesis (Rossato et al., 2007; Langston & Wood, 2009). Also, context habituations included on OR protocol classically requires hippocampus (Izquierdo & Medina, 1995; Squire, 1992). These characteristics allowed us to test our hypothesis.

During training and test sessions we tried to minimize stress at maximum. Stress and fatigue was a constant concern, as our goal was to verify only the mnemonic effect of the additional tasks, which could be disguised by higher anxiety and lower mobility. The multi-task protocol, however, did not interfere in EPM and OF performance.

In accordance with other works (Bianchin et al., 1993; Quillfeldt et al., 1996), we found that pre-test administration of muscimol induces significant retrograde amnesia, even 20 days after training. However, with additional learning the Step-down inhibitory avoidance memory was spared. As memory maintenance is dependent of different structures over time, with hippocampus playing a major role in early stages but not in remote points (Frankland and Bontempi, 2005; Frankland et al., 2007; Wiltgen et al., 2004), the preserved fear memory of MT group after muscimol treatment suggests that this memory has already become independent of hippocampus and is probably been sustained by parahippocampal or cortical structures. The additional learning, which occurred after the training session of the Step-down inhibitory avoidance, somehow accelerated memories reorganization process. This influence was purely mnemonic since exploratory and anxiety-like behaviors were unaffected.

Recent works have also demonstrated some circumstances that can influence the system consolidation. Tse et al., (2007) reported that this process can occur extremely fast if an associative “schema”, into which new information is incorporated, has previously been created. Winocur et. al., (2005) demonstrated that extensive and long experience in a same environment leads to spatial representations

that are independent of hippocampus. All these works, different of ours, influenced the corticalization rate of memories by reinforcing the same learning or environment, and acting before the acquisition of the studied memory. Here, we reported for the first time that new learning after a fear memory acquisition can influence its reorganization rate.

Since the amount of post-training experience was the differential factor between the groups in this study, and that these experiences lead to learning and memory storage, our major conclusion is that the amount of encoded information somehow influenced and accelerated memory reorganization from hippocampus to other structures. We showed that one memory system consolidation is affected by the consolidation of other dissimilar memories.

Possibly, the additional learning lead to a higher hippocampal activity and a faster clearance of the IA memory, with a parallel faster consolidation in cortex, avoiding hippocampus overloading and making possible to new memories be acquired and consolidated. Faster hippocampal clearance might happen due increased neurogenesis (Tsient et al., 2001), since neurogenesis appears to be regulated by experience (Gould et al., 1999; Greenough et al., 1999)

During memory consolidation, the hippocampus activates neurons in cortical areas (Mcnaughton, 1997), which could allow to different cortical modules to be reactivated together, strengthening connections between them. This is a proposed mechanism, called synaptic reentry reinforcement, by which memories would turn hippocampus independent through the strengthening of cortico-cortical connections

(Shimizu et al., 2000; Wang et al., 2006). Hippocampal elevated activity may also lead to enhanced synaptic reentry reinforcement, accelerating cortical consolidation and hippocampus-independency observed in our results.

Accordingly, hippocampus main function might be, besides encoding, to storage memories fast, but just while they are not stabilized in cortex. After this, previously occupied hippocampal networks would be disengaged from the preceding memory (partially or completely) and would be ready again for new memories. Thus, as more the hippocampus is required, more rapidly memories stored in this structure would be disengaged from it.

Some theoretical models suppose that the hippocampus serves as an "index" or "pointer" to cortically encoded information, guiding the slow systems-level consolidation process that is thought to involve hippocampal-neocortical interactions over time (Taylor and DiScenna, 1986; Siapas and Wilson, 1998). So, another possible reason for the system consolidation acceleration by new learning is that it happend as a simple consequence of cortico-hippocampal interaction enhancement caused by increased activity of hippocampus. These explanations are not exclusive, and could act together to reach the same result, allowing the faster memory disengagement of hippocampus.

In a great example of how the synaptic consolidation of a memory trace can influence another, Ballarini et al. (2009) submitted animals to two different tasks, one that can induce Long Term Memory and another that just induced Short Term Memory. When these ones were paired, at specific time window, the weak

experience used the proteins synthesized for the strong one by memory tags (Frey & Morris, 1997), and produced effectively Long Term Memory. Those mechanisms that enabled this phenomenon could be occurring in a major level, as the system consolidation of memory. If it is true, we can suppose that similar mechanisms could be contributing to the additional tasks interference on the systems consolidation of IA memory in our experiment.

In conclusion, we found for the fist time that new learning experience can accelerate the systems consolidation of a step-down inhibitory avoidance task, making it hippocampus-independent earlier. It is possible that this observed phenomenon occurs in order to disengage this memory faster and thus "release space" on hippocampus to the newly acquired information. Thinking in that way, the amount of hippocampus requirement can influence the reorganization rate of a contextual memory and the consequent storage on neocortical structures. These findings have implications for the neurobiology of learning and memory, indicating that the reorganization of memory and the process by which its retrieval becomes independent of hippocampus is dynamic and can be influenced by other non-related learning episodes. In consequence, the time course for systems consolidation of a given memory can be different depending of the circumstances on which it occurs.

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Figures and Legends

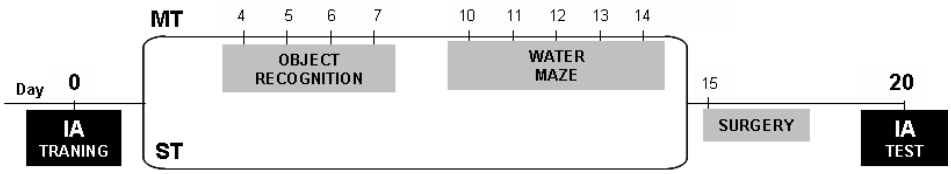

Figure 1 – Experimental design. On day 0, all animals were trained on Step-down inhibitory avoidance task (IA) and after divided in two groups: Single-task (ST) and Multiple-tasks (MT). Animals of MT group, on day 4 and 5, were habituated to the experimental arena of OR for 30 min. On day 6, rats were placed in the arena containing two objects and left to freely explore them for 5 min. At the day 7, the test was performed, where one of the objects was exchanged for a novel. On days 10 to 14 animals were submitted to the WM task. Rats were subjected to four training sessions, each session consisting of four trials, and to a probe trial in the 14th day. At the day 15, surgeries were made in all groups for bilaterally implantation of cannulae in hippocampus. Finally, on day 20, all groups received intrahippocampal infusion of Muscimol or its Vehicle, and were submitted to IA test 15min later.

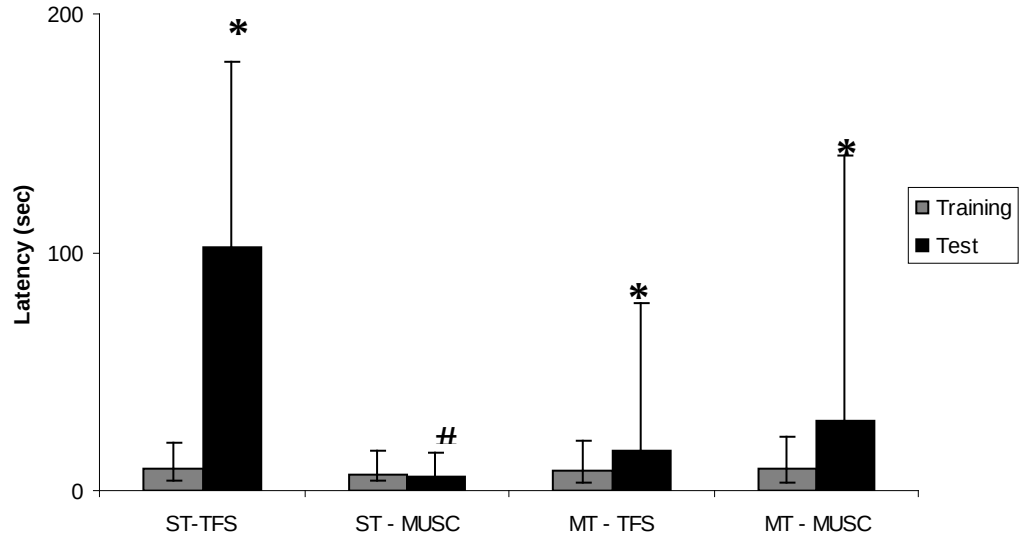

Figure 2 - Effect of intrahippocampal infusion of Muscimol (1ug/side) upon Step-down inhibitory avoidance test session performed 20 days after training. Data expressed as Median $\pm$ ii. of stepping down latency. Significant difference in test session step-down latency among groups was explicited by Kruskal-Wallis analysis of variance (p=0,02). * Significant difference between training and test (ST-TFS: P=0,01, N=10; MT-TFS: P=0,001, N=25; MT-MUSC: P=0,001, N=17. Wilcoxon test). There were no significant difference between training and test on ST-MUSC group (P=0.2, N=7; Wilcoxon test). # Significant differences between ST-MUSC and ST-TFS, MT-TFS and MT-MUSC (p=0,001, p=0,002 and p=0,003; Mann-Whitney U-test).

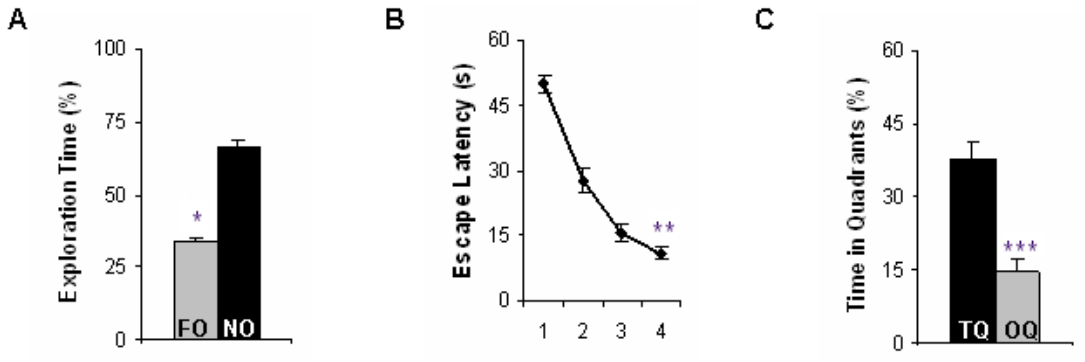

Figure 3 - Performance of MT group in the additional tasks. Data expressed as Mean ± S.E.M. N=23. **A**. RO test session, showing percentage of time exploring a particular object over the total time of object exploration. **B**. Mean escape latency (s) during WM training sessions. **C**. WM test, showing the time (s) spent in target (TQ) and opposite (OQ) quadrants during the 60 seconds of exposure. * Significant difference between novel (NO) and familiar (FO) objects in the RO test session (p= 0,000; Paired Samples Test). ** Significant difference between the escape latency time of the first and the fourth day of training (Paired Samples Test). *** Significant difference between quadrants (p= 0,001; Paired Samples Test).

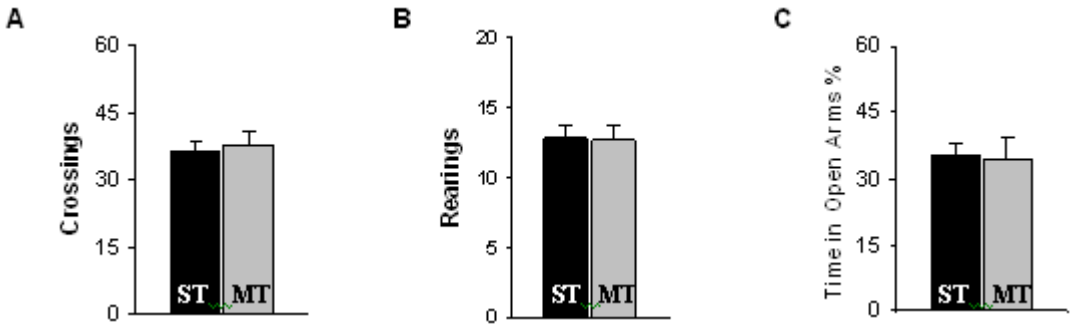

Figure 4 – Performance of animals on motility and anxiety tests. Data expressed as Mean ± S.E.M. N=20-23 per group. **A and B.** Number of crossings and rearings in Open Field during 3min of exploration. **C.** Percentual of time spent in the open arms during the 3-min exposure to the elevated Plus Maze. There were no significant difference between groups in all tests (Independent T Test, p>0,05).

6 Conclusões

Com os experimentos realizados neste trabalho vimos que:

● A infusão de muscimol intrahipocampal foi amnésica sobre a evocação da memória da EI vinte dias após o treino no grupo “sem multitarefas”;

● A infusão de muscimol intrahipocampal não foi amnésica sobre a evocação da memória da EI vinte dias após o treino no grupo “multitarefas”;

● Os animais do grupo “multitarefas” aprenderam adequadamente as tarefas interpostas entre o treino e o teste da EI, tanto no Reconhecimento de Objetos, quanto no Labirinto Aquático de Morris;

● Não houve diferença na atividade motora na tarefa Campo Aberto entre os grupos “multitarefas”e “sem multitarefas”;

● Não houve diferença de ansiedade na tarefa de Labirinto em Cruz Elevado entre os grupos “multitarefas”e “sem multitarefas”.

Nossos dados sugerem que aprendizados adicionais e a conseqüente formação de novas memórias aceleraram o processo de reorganização da memória inicial de EI.

Como o protocolo “multitarefas” parece não ter afetado a motilidade e os níveis de ansiedade dos animais a ele submetidos, podemos interpretar seus efeitos como estando, em princípio, relacionados apenas a processos puramente mnemônicos.

Uma interpretação para o fenômeno observado é que, em função do aumento na quantidade de informações sendo processadas pelo hipocampo, este pode ter acelerado a reorganização das memórias mais antigas em circuitos neocorticais (a chamada “consolidação sistêmica”), liberando, assim, mais espaço no hipocampo para poder registrar novas memórias. A consolidação sistêmica, portanto, seria influenciada e regulada pela quantidade de novas informações processadas, de modo

que quanto maior a quantidade de novas informações, mais rápida a reorganização e migração das memórias em direção aos seus sítios de armazenamento permanente.

7 Perspectivas

Neste trabalho, conseguimos replicar, em um modelo comportamental distinto, resultados similares que obtivemos na tarefa de Condicionamento Aversivo ao Contexto em experimentos anteriores (Anexo 2). Nossos próximos objetivos são

● Investigar se há variação nos níveis de corticosterona nos ratos submetidos ao protocolo de multitarefas, afim de melhor garantir a ausência de um componente emocional nos nossos resultados;

● Verificar se o processo de reorganização acelerado induzido pelas multitarefas, que fez com que a memória se tornasse independente do hipocampo mais cedo, leva paralelamente a uma precoce dependência de estruturas corticais como o córtex cingulado anterior;

● Observar a participação e não-participação do hipocampo e do córtex após aprendizados adicionais, em diferentes tempos pós-treino, por imunohistoquímica e western blot, investigando a atividade de genes de ação imediata relacionados com a memória como c-fos e zif268.

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9 Anexos

9.1 Anexo 1: Normas da revista Journal of Neuroscience para publicação de uma Brief Communication

Organization of the Manuscript

Manuscripts must be written in English. Multiple-part papers are discouraged. Although it is recognized that this arrangement is sometimes necessary, authors will often be asked to collapse multiple papers into a single manuscript.

Manuscripts must include the sections listed below in the order they are presented. All word limits include citations. The entire text should be double-spaced. Submitting an incomplete manuscript or a manuscript that does not adhere to the word limits will cause a delay in review.

Brief Communications are subject to the same word restrictions, with the additional requirement that the abstract, introduction, materials and methods, results, discussion references and figure legends cannot exceed 4500 words.

Title Page

The first page of the manuscript should be a title page with the following:

- Journal Section (Cellular & Molecular, Neurobiology of Disease, Behavioral/Systems/Cognitive, or Development/Plasticity/Repair)
- Title
- Abbreviated title
- Authors and author addresses
- Corresponding author with complete address, including an email address
- Number of figures and tables
- Contents of supplemental material (if applicable)
- Number of pages
- Number of words for Abstract, Introduction, and Discussion (separately)
- (For Brief Communications) Total number of words (Abstract, Introduction, Materials & Methods, Results, Discussion, References and Figure Legends)
- Six keywords

- Acknowledgements

Authors who normally write their names in non-Latin characters may include their names in their native writing system in parentheses immediately following a transliterated version, for example, Jingbing Xue (薛晶冰). Any non-Latin languages that can be represented in Unicode characters will be accepted. This second rendering is allowed only for the original written form of a transliterated name, and may not be used to include nicknames, degrees, ranks, titles, etc.

Acknowledgements should be used to identify all funding sources. Acknowledgements may also be used to note intellectual, technical or other assistance that does not warrant authorship. Individuals should be informed before the publication of any such acknowledgements and given the opportunity to decline the recognition. Promotional statements are not permitted.

The Journal generally does not allow dedications. The only exception we allow is dedications to recently deceased neuroscientists who made a specific scientific contribution to the work described in the article. We do not allow dedications to living people.

Abstract (250 words maximum, including citations)

The abstract should be clearly written and readily comprehensible to the broad readership of the Journal. It should provide a concise summary of the objectives, methodology (including the species studied), key results, and major conclusions of the study. It should be written in complete sentences, without subheadings.

Introduction (500 words maximum, including citations)

The Introduction should briefly indicate the objectives of the study and provide enough background information to clarify why the study was undertaken and what hypotheses were tested.

Materials and Methods

The materials and methods section should be brief but sufficient to allow other investigators to repeat the research (see also Policy Concerning Availability of Materials). Reference should be made to published procedures wherever possible; this applies to the original description and pertinent published modifications. The sex of subjects should be stated. All companies from which materials were obtained should be listed. If materials were obtained from an individual, an affiliation for that individual should be listed.

None of the Materials and Methods may be placed in Supplemental Materials.

Results

This section should present clearly but succinctly the experimental findings. Only results essential to establish the main points of the work should be included. Numerical data should be analyzed using appropriate statistical tests.

For guidelines on how to report statistical results, see Bailar, JC, Mosteller, F (1988) Guidelines for statistical reporting in articles for medical journals. Ann Intern Med, 108:266–273; Curran-Everitt, D, Benos DJ, (2004) Guidelines for reporting statistics in journals published by the American Physiological Society. J Neurophysiol, 92:669-671; Lang, TA, Secic, M (2006) How to report statistics in medicine: annotated guidelines for authors, editors and reviewers, 2nd edition, Philadelphia, PA, ACP Press; Sarter M, Fritschy JM (2008) Eur J Neurosci 28:2363-2364.

Discussion (1500 words maximum, including citations)

The discussion section should be as concise as possible and should include a brief statement of the principal findings, a discussion of the validity of the observations, a discussion of the findings in light of other published work dealing with the same or closely related subjects, and a statement of the possible significance of the work. Extensive discussion of the literature is discouraged.

References

Only published and "in press" (i.e., accepted for publication in a specific journal or book) references should appear in the reference list at the end of the paper. The latest

information on "in press" references should be provided. Any "in press" references that are relevant for reviewers to see in order to make a well-informed evaluation should be included as a separate document text file along with the submitted manuscript. "Submitted" references should be cited only in text and in the following form: (A. B. Smith, C. D. Johnson, and E. Greene, unpublished observations). The form for personal communications is similar: (F. G. Jackson, personal communication). Authors are responsible for all personal communications and must obtain written approval from persons cited before submitting the paper to the Journal. Proof of such approval may be requested by the Journal.

References should be cited in the text as follows: "The procedure used has been described elsewhere (Green, 1978),"or "Our observations are in agreement with those of Brown and Black (1979) and of White et al. (1980),"or with multiple references, in chronological order: "Earlier reports (Brown and Black, 1979, 1981; White et al., 1980; Smith, 1982, 1984).... " In the list of references (to be typed double-spaced), papers should be given in alphabetical order according to the surname of the first author. In two-author papers with the same first author, the order is alphabetical by the second author's name. In three-or-more-author papers with the same first author, the order is chronological. The name of the author(s) should be followed by the date in parentheses, the full title of the paper as it appeared in the original together with the source of the reference, the volume number, and the first and last pages. Do not number or bullet the references. If the author list for a paper in the references exceeds 20, the paper should be cited as Author A et al. The following illustrate the format to be used:

Journal article

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth F (1981) Improved patch-clamp techniques for high-resolution current recordings from cells and cell free membrane patches. Pflugers Arch 391:85-100.

Hodgkin AL, Huxley AF (1952a) The components of membrane conductance in the giant axon of Loligo. J Physiol (Lond) 116:473-496.

Hodgkin AL, Huxley AF (1952b) The dual effect of membrane potential on sodium

conductance in the giant axon of Loligo. J Physiol (Lond) 116:497-506.

Book

Hille B (1984) Ionic channels of excitable membranes. Sunderland, MA: Sinauer.

Chapter in a book

Stent GS (1981) Strength and weakness of the genetic approach to the development of the nervous system. In: Studies in developmental neurobiology: essays in honor of Viktor Hamburger (Cowan WM, ed), pp288-321. New York: Oxford UP.

Abbreviations of journal titles should follow those listed in the Index Medicus. Responsibility for the correctness of the references lies with the author(s). After manuscript revisions, authors should double-check that all in-text citations are in the reference list and that all references on the reference list have at least one corresponding in-text citation. Failure to do so will result in the delay of proof generation and possibly publication. Please make sure that the References are double-spaced and no bullets, numbers, or other listing formats are used.

Figure Legend

Manuscripts that include figures must include figure legends as part of the main manuscript text. A legend must be supplied for each figure. It is helpful to reviewers if the figure legend is also included with each figure when it is uploaded to the online submission site.

Illustrations

All figures must be cited in the text and numbered consecutively (Fig. 1, Fig. 2, etc.).

All illustrations must be submitted at the size they are to appear in The Journal of Neuroscience. Illustrations should be the smallest size that will convey the essential scientific information, and sized to 1 column (8.5 cm), 1.5 columns (11.6 cm) or 2 columns (17.6 cm). Vertical dimensions cannot exceed 22 cm.

Figures should be appropriately lettered and labeled with characters that will be 2-6 mm high in the final reproduction. Graphs and histograms may not include top and right borderlines and panels may not be boxed in by borderlines.

To ensure that your figures will appear at the highest quality, please review the detailed instructions for figure preparation at http://cpc.cadmus.com/da/index.jsp. To better assist our authors with digital art preparation, the Journal has made available Cadmus', RapidInspector application, which alerts users when their files do not meet acceptable specifications and provides instructions on how to reformat their files to meet those specifications. Sign up at the RapidInspector web site: http://rapidInspector.cadmus.com/RapidInspector/zo5/index.jsp to download the application. If you have problems downloading the application please see the FAQs at the Cadmus site.

Technical guidelines for preparing images

•TIFF and EPS are the only acceptable formats for figure files for publication. For the initial submission of a manuscript, however, figures may be included in a single PDF file that contains the manuscript and all tables and figures.

•Color figures should be saved in RGB format. For information on converting files to RGB format please see: http://www.jneurosci.org/misc/ifa_rgbworkflow.dtl.

•Grayscale or color images must be supplied at a minimum of 300 dpi. Halftone images must be supplied at a minimum of 600 dpi. Monochrome images must be supplied at 1200 dpi.

•For figures supplied in vector-based format, all fonts should be converted to outlines and saved as EPS (Encapsulated PostScript). If fonts are not converted to paths or outlines, there is a possibility of character substitutions or that your graphic may have to be converted to a bitmap, which reduces resolution and quality of the images. For information on converting to outlines, please see: http://www.jneurosci.org/misc/ifa_rgbworkflow.dtl.

Figures and Tables

Figures should be submitted as separate files in TIFF or EPS format.

All figures and tables must be cited at the relevant point in the manuscript text (e.g., Figure 1). Do not duplicate data by presenting it both in the text and in a table or figure.

A legend for each figure or table must be included in the manuscript document following the reference list. The legend should include sufficient experimental detail so as to be intelligible without reference to the text. Legends must define all labels and symbols used in the figure art and provide other essential information such as scale bar dimension. Rather than stating "See text," the legend should be more specific; for example, "See Results."

For figures, a title should be part of the legend and not lettered onto the figure itself.

For tables, a title should appear above the table. Each table should be double-spaced and include only essential data.

Abbreviations

Use abbreviations only if a term appears three or more times. Spell out the term at first occurrence, and introduce the abbreviation by placing it in parentheses after the term. Units should conform to the International System of Units (SI) (see Handbook of Chemistry and Physics), except for temperatures, which should be expressed in degrees Celsius.

BRIEF COMMUNICATIONS

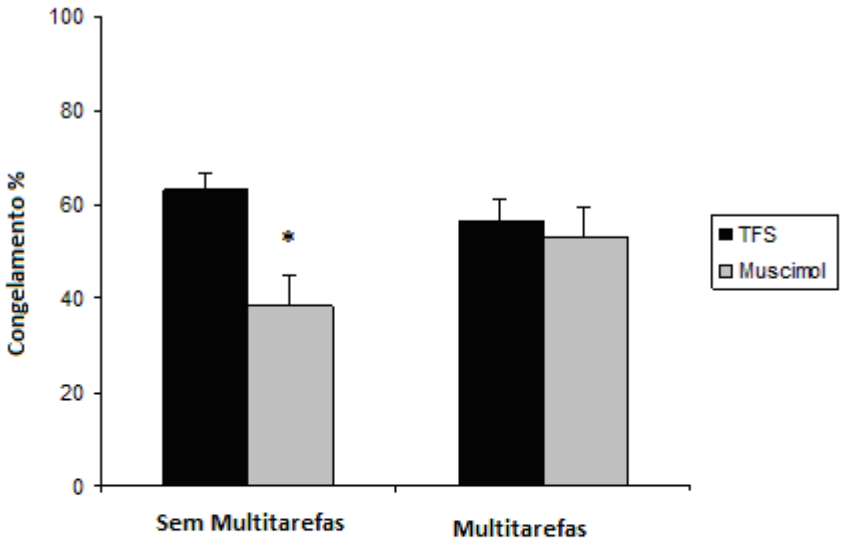
Overview

Brief Communications are short research articles intended to present exciting findings that will have a major impact in neuroscience. Brief Communications are limited to 4,500 words, including the abstract, introduction, materials and methods, results, discussion, references and figure legends. The total word count must be listed on the title page. Additionally, Brief Communications may include no more than 4 figures and/or tables, which together may occupy no more than one full page. It is

not acceptable to move materials and methods or essential figures into supplemental material in order to adhere to these limits.

Manuscripts should be organized as described for Regular Manuscripts and the abstract, introduction, and discussion must adhere to the word limits listed for Regular Manuscripts. Authors will be contacted if their manuscript does not conform to these guidelines, and will be asked to reduce the content or reclassify the paper as a Regular Manuscript.

9.2 Anexo 2: Resultados anteriores na tarefa de Condicionamento Aversivo ao Contexto



Efeito da infusão de muscimol (1ug/lado) intrahipocampal na evocação da tarefa de Condicionamento Aversivo ao Contexto 20 dias após o treino.

* Diferença significativa entre TFS e muscimol no grupo que não passou por multitarefas (N=10 e 12, respectivamente; P=0,02; Tukey's multiple range test). Não houve diferença entre os ratos tratados com TFS e muscimol no grupo multitarefas (N=11 e 12, respectivamente; P=0.97, Tukey's multiple range test), assim como entre os grupos TFS sem multitarefas e TFS sem multitarefas (P=0,84; Tukey's multiple range test)