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MODIFICAÇÕES NA PLASTICIDADE HIPOCAMPAL RELACIONADAS COM O PROCESSAMENTO DA MEMÓRIA PARA A TAREFA DE RECONHECIMENTO DE OBJETOS

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Nós, homens do conhecimento, não nos conhecemos;

de nós mesmos somos desconhecidos.

Friedrich Nietzsche (1844-1900)

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A todos vocês, muito obrigada

Julia Helena Rosauro Clarke

Lista de Abreviações

AMPA = receptores glutamatérgicos tipo AMPA (α-amino-3-hidroxi-5-metil-4-isoxipropronato)

CA1 = sub-região hipocampal, Corno de Amon 1

CA2 = sub-região hipocampal, Corno de Amon 2

CA3 = sub-região hipocampal, Corno de Amon 3

CREB = do inglês, *cAMP-responsive element binding protein*

DG = sub-região hipocampal chamada giro denteado

LTP = potenciação de longa duração. Do inglês: *long-term potentiation*

MAPK = do inglês, *mitogen activated protein kinase*

NMDA = receptores glutamatérgicos tipo NMDA (N-metil-D-aspartato)

RO = Reconhecimento de Objetos

SNC = Sistema Nervoso Central

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Resumo

O fenômeno da potenciação de longa duração (LTP) é amplamente aceito como correlato celular da formação de memórias. A tarefa de Reconhecimento de Objetos (RO) é particularmente útil para estudar as memórias declarativas em roedores, porque se baseia em sua preferência inata por objetos novos sobre objetos familiares. Neste estudo, camundongos com eletrodos implantados nas vias colaterais de Schaffer hipocampais foram treinados na tarefa de RO. Registros de campo de potenciais excitatórios pós-sinápticos evocados na sinapse CA3-CA1 foram obtidos durante a sessão de treino ou a diferentes momentos após o seu término. Um fenômeno de potenciação sináptica "LTP-like" foi observado 6 horas após o treino. Uma sessão de teste foi conduzida 24 horas após o treino, na presença de um objeto novo e de um objeto familiar. Uma facilitação sináptica foi observada enquanto os animais exploravam os objetos, independentemente do tipo de objeto (novo ou familiar). Um curto período de depotenciação foi observado logo após o teste, seguido de uma fase tardia de potenciação sináptica. Demonstrou-se, portanto, que a consolidação da memória de RO é acompanhada por uma potenciação sináptica transitória na sinapse CA3-CA1, enquanto que a reconsolidação desta memória desencadeia uma fase curta de depotenciação - que poderia ser a responsável pela vulnerabilidade característica da memória – que é seguida por uma fase tardia de potenciação sináptica.

1. Introdução e Revisão da Literatura

O aprendizado é o processo por meio do qual nós, humanos, e outros animais adquirimos conhecimento sobre o mundo. No curso da evolução da vida na Terra, o surgimento da capacidade de armazenar informações permitiu que os seres vivos se beneficiassem de experiências passadas para resolver problemas apresentados pelo meio ambiente, oferecendo aos animais uma maior adaptabilidade. Coerentemente, verifica-se que os grupos taxonomicamente mais antigos, como os invertebrados, já apresentam alguma capacidade mnemônica. No caso específico dos seres humanos, a memória exerce um papel ainda mais nobre. Funcionando como um arcabouço que armazena nossa história pessoal, torna possível que cresçamos e mudemos ao longo da vida (Kandel *et al.*, 2000).

Para que uma memória se forme, informações originárias de fontes externas (experiências sensoriais oriundas da interação com o ambiente) ou internas (cognição, emoção) devem ser adquiridas. A experiência que envolve a aquisição de informações corresponde à aprendizagem, e imediatamente após este evento inicia-se a retenção da informação. A retenção de curta duração (minutos, horas) pode ser convertida em memória de longa duração (dias, semanas, anos), pelo processo denominado consolidação (McGaugh, 2000). Enquanto estiver retida, a informação pode ser recuperada, e esta etapa é também chamada de evocação, sinônimo de lembrança. Por fim, com o passar do tempo, mesmo as informações mais consolidadas podem desaparecer: trata-se do esquecimento.

De todas as informações processadas pelo sistema nervoso, apenas algumas são de fato retidas por longos períodos. A maioria nem sequer é adquirida, sendo filtrada devido a variações nos níveis de atenção e emoção do indivíduo. Dentre aquelas que são adquiridas, apenas algumas são consolidadas como memórias de longa duração, e mesmo dentre estas, muitas são esquecidas. Apenas as informações

mais relevantes para a cognição, mais marcantes emocionalmente, mais focalizadas pela atenção ou mais fortes sensorialmente perduram por um longo tempo. Vale aqui frisar que o esquecimento, muito longe de ser reduzido a apenas um vilão ou uma anormalidade das funções mnemônicas, na verdade desempenha um papel muito importante como mecanismo de prevenção de sobrecarga nos sistemas cerebrais dedicados à memorização. Sem esquecer, torna-se impossível ignorar detalhes para generalizar alguma coisa, deste modo limitando o pensamento e o raciocínio.

As memórias podem ser classificadas de acordo com diversos critérios, como função, conteúdo e tempo de duração. Quanto ao tempo que permanecem armazenadas, as memórias são ditas de curta duração, com duração de poucas horas; de longa duração, com duração de horas ou dias; ou ainda memórias remotas, que são as memórias de longa duração que persistem por muitos meses ou anos. Quanto ao seu conteúdo, as memórias são chamadas declarativas se forem referentes a fatos, eventos ou conhecimentos que possam ser contados ou relatados por nós; e procedimentais, quando forem referentes a capacidades ou habilidades motoras ou sensoriais difíceis de serem declaradas ou descritas. Andar de bicicleta, dirigir um automóvel e digitar são exemplos de memórias procedimentais. As memórias declarativas podem ser subdivididas em episódicas ou semânticas. As episódicas são aquelas referentes a eventos aos quais assistimos ou participamos, ou seja, são as memórias autobiográficas. As memórias semânticas carregam informações que são de conhecimento geral, como nosso conhecimento em medicina, língua portuguesa ou história.

Atualmente, há extensas evidências de que todas essas funções envolvendo o armazenamento de informações, bem como outras atividades mentais, emergem como resultado do funcionamento do sistema nervoso central. A correlação entre a participação de certas estruturas cerebrais na formação de memórias, que constitui a

ciência chamada neuropsicologia cognitiva, originou-se a partir da observação dos sintomas de pacientes com lesões acidentais em áreas específicas do Sistema Nervoso Central (SNC), sendo o mais famoso deles o paciente H.M., que teve removidos em uma cirurgia para correção da epilepsia uma parte do hipocampo, a amígdala e o giro parahipocampal; estruturas formadoras do sistema límbico importantíssimas para as capacidades mnemônicas. Após o evento cirúrgico, o paciente seguiu capaz de aprender tarefas motoras e reter informações por algum tempo, porém era incapaz de transferir outros tipos de informação como, por exemplo, sobre localização espacial ou reconhecimento de faces para bancos permanentes de memória (Scoville e Milner, 1957). A partir desta observação, o crescente interesse pela participação das estruturas do sistema límbico na formação de memórias, com destaque à elucidação do papel do hipocampo, acrescentou muito ao nosso conhecimento a respeito do processamento mnemônico.

O hipocampo é uma estrutura subcortical bilateral do lobo temporal que compõe o sistema límbico. Seu nome deriva de seu formato curvado apresentado em secções coronais do cérebro humano, se assemelhando a um cavalo marinho (Grego: *hippos* = cavalo, *kampi* = curva). O hipocampo é formado por duas regiões principais: o giro denteado e o Corno de Amon, que por sua vez é subdividido nas regiões CA1 (Corno de Amon 1), CA2 (Corno de Amon 2) e CA3 (Corno de Amon 3; Figura 1).

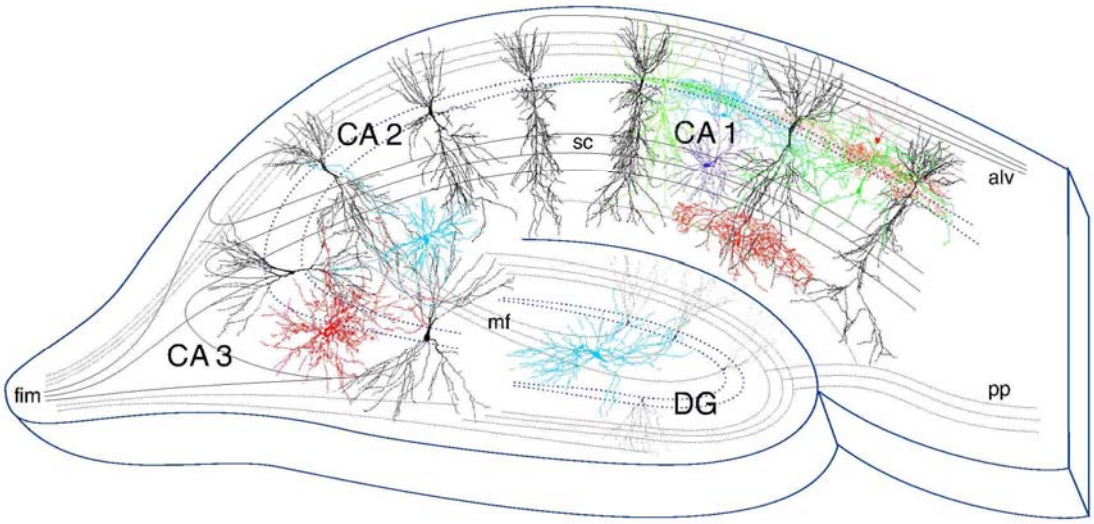

Figura 1. Representação de uma secção coronal do hipocampo de rato, com indicação da localização e integração entre as distintas sub-regiões. DG = giro denteado; CA1 = Corno de Amon 1; CA2 = Corno de Amon 2; CA3 = Corno de Amon 3; pp = via perforante; mf = fibras musgosas; fim = fímbria; sc = via colateral de Schaffer (Reproduzido de Pittson et al., 2005).

Como mostrado na figura 1, o arranjo anatômico das conexões sinápticas no hipocampo rende uma boa estrutura para experimentos. Três classes distintas de conexões sinápticas do hipocampo tem sido estudadas: a via perforante, que consiste em axônios do córtex entorrinal fazendo sinapse com células granulosas do giro dentado; as fibras musgosas, que são células do giro dentado fazendo sinapse com células piramidais da região CA3, e a via colateral de Schaffer que consiste em sinapses entre as células piramidais de CA3 com neurônios de CA1 (Kandel, 2000).

O hipocampo desempenha um papel fundamental na formação de memórias de curto e longo prazo (Izquierdo *et al.*, 1998a), e diferentes áreas corticais aferentes e eferentes interagem com o hipocampo para regular a aquisição e o armazenamento de nova informação (Izquierdo *et al*., 1998b). A formação de uma memória de longa duração envolve uma série de processos metabólicos no hipocampo e em outras estruturas cerebrais que compreendem diversas fases e que requerem entre três e oito

horas. Enquanto esses processos não estiverem concluídos, as memórias de longa duração encontram-se lábeis. O conjunto desses processos e o seu resultado final são denominados consolidação. Os mecanismos neurofisiológicos e neuroquímicos que subjazem os processos mnemônicos são um dos temas mais fascinantes das neurociências e objeto de estudo de pesquisadores há muito tempo.

Acredita-se, ainda, que o aprendizado culmine em alterações na representação neural, através de eventos plásticos que modificam a comunicação entre os neurônios (Dudai *et al.*, 1989). Estes eventos plásticos podem incluir alterações na estrutura, na distribuição e no número de sinapses e também alterações morfológicas (Rusakov *et al.*, 1997; Woolf, 1998; Geinisman, 2000), sendo que no caso específico do aprendizado pode significar apenas alterações estruturais e funcionais de sinapses, de modo a possibilitar a codificação dessas memórias. Uma das primeiras propostas de explicação dos mecanismos celulares envolvidos na memória data de 1911, quando o grande neuroanatomista espanhol Santiago Ramón y Cajal sugeriu que novas memórias eram originadas por modificações precisas nas intensidades das conexões neuronais existentes, e não por um aumento no número de células nervosas. Grande parte do que hoje se conhece a respeito dos mecanismos celulares e moleculares envolvidos nos fenômenos de plasticidade neuronal deve-se à descrição detalhada de um processo de plasticidade descrito em 1973 pelos noruegueses Bliss e Lømo. A teoria deste fenômeno já havia sido proposta em 1949 por Donal Hebb (Hebb, 1949), e foi nomeada Ponteciação de Longa Duração (LTP, do inglês *long-term potentiation*). A LTP é um dos eventos plásticos mais conhecidos e estudados. Definida como um aumento da eficiência sináptica dependente de atividade neuronal, a LTP é atualmente aceita como o correlato celular geral para a plasticidade sináptica induzida pelo aprendizado, no hipocampo (Bliss e Lømo, 1973). Os eventos bioquímicos envolvidos na formação da memória e da LTP incluem, inicialmente, a ativação de receptores glutamatérgicos do

tipo AMPA (α-amino-3-hidroxi-5-metil-4-isoxipropronato) seguida pela ativação dos receptores glutamatérgicos NMDA (N-metil-D-aspartato) e dos receptores glutamatérgicos metabotrópicos (Izquierdo e Medina, 1995; 1997). O sinal inicial para a indução de LTP é um aumento das concentrações intracelulares de íons cálcio, que geralmente é desencadeado pela ação prolongada do glutamato, liberado por estimulação tetânica de um terminal pré-sináptico sobre a pós-sinapse.

Nas últimas décadas, diversos estudos buscaram esclarecer a relação de equivalência entre LTP e memória. Estes estudos ajudaram no entendimento de ambos os processos separadamente e mostraram que eles possuem muitas similaridades (Bliss e Collingridge, 1993; Izquierdo *et al.*, 2006) principalmente no que concerne à seqüência temporal dos eventos bioquímicos necessários para o seu estabelecimento. No entanto, poucos foram capazes de esclarecer de forma definitiva se estes dois fenômenos são dependes e obrigatoriamente co-existentes, ou seja, se a indução de LTP é indispensável para que haja a formação de uma dada memória. Através de registros eletrofisiológicos *in vivo*, demonstrou-se recentemente que o aprendizado em certas tarefas de condicionamento é acompanhado por um aumento duradouro da eficiência sináptica nas vias colaterais de Schaffer hipocampais (que conectam as sub-regiões CA3 e CA1) de roedores (Withlock *et al.*, 2006; Gruart *et al.*, 2006). Interessantemente, estes dois trabalhos estudaram o envolvimento da LTP na formação de memórias do tipo aversiva, as quais, do ponto de vista biológico e evolutivo são dignas de serem armazenadas por um longo período de tempo por sua utilidade em experiências futuras.

No entanto, nem todas as memórias de longa duração têm necessariamente este destino. Uma vez consolidada, a memória tem dois destinos possíveis: ela pode permanecer inalterada ao longo de todo o período pelo qual fica armazenada, independentemente de quantas vezes for evocada; ou ela pode sofrer alterações

conforme for necessária a incorporação de novas informações àquelas previamente armazenadas. Este processo de revisão e modificação de uma memória consolidada chama-se reconsolidação (Sara, 2000; Nader, 2003; Eisenberg e Dudai, 2004; Lee *et al.*, 2004), e acontece, por exemplo, cada vez que uma memória é evocada na presença de alguma novidade. Ainda que inicialmente haja sido encontrada para a memória de medo condicionado em ratos, a existência do processo de reconsolidação foi recentemente constatada em outros paradigmas de condicionamento em distintas espécies animais (Debiec *et al.*, 2002; Kida *et al.*, 2002; Sangha *et al.*, 2003; Eisenberg *et al.*, 2003; Stollhoff *et al.*, 2005; Inda *et al.*, 2005; Gainutdinova *et al.*, 2005; Merlo *et al.*, 2005). Esta universalidade sugere, segundo vários autores, que a reconsolidação desempenha um papel central no processamento de informações (Dudai, 2006; Tronson e Taylor, 2007; Lee 2009), com evidente valor na adaptação dos indivíduos ao ambiente em que se encontram inseridos.

Ao contrário do que seu nome propõe, o processo de reconsolidação não se trata de uma nova etapa de consolidação, nem de um reforço na informação armazenada. Do ponto de vista bioquímico, muitos eventos em comum foram descritos para ambos os processos, entre eles a expressão de genes de expressão imediata tais como c-fos (Tronel e Sara, 2002) e zif268 (Bozon *et al.*, 2003), a ativação de MAPK (do inglês, *mitogen activated protein kinase*) e outras proteínas quinase hipocampais (Kelly *et al.*, 2003; Bonini *et al.*, 2007) e a ativação do fator de transcrição CREB (do inglês, *cAMP-responsive element binding protein*). As diferenças principais entre os dois processos incluem o requerimento de síntese protéica apenas sob determinadas circunstâncias (presença de novidade), no caso da reconsolidação (Rossato *et al.*, 2007), contra a indispensabilidade de síntese de proteínas durante a consolidação, e diferenças nas janelas temporais de envolvimento de diversos efetores moleculares (Alberini, 2005). Interessantemente, Lee e colaboradores (2008) demonstraram

recentemente que para que haja reconsolidação deve haver um curto período de degradação protéica no hipocampo, com aproximadamente 2 horas de duração, para que posteriormente ocorra a reorganização da informação armazenada. Podemos destacar este achado como uma das principais diferenças entre os processos de consolidação e reconsolidação.

A elucidação do papel funcional da reconsolidação constitui hoje um dos principais tópicos de estudo da neurobiologia da memória. Porém, a maioria dos estudos abordando este fenômeno emprega tarefas comportamentais baseadas no estabelecimento de memórias implícitas e, portanto, pouco se conhece acerca do efeito da evocação sobre a estabilidade das memórias declarativas. Com relação às memórias declarativas, sabe-se com base em avaliações neurofisiológicas de pacientes amnésicos e experimentos com animais de laboratório que a integridade do lobo temporal, que inclui a formação hipocampal, é essencial para os processos de consolidação e reconsolidação de memórias espaciais e de reconhecimento (Ennaceur e Delacour, 1988; Riesenhuber e Poggio, 2002; Crane e Milner, 2005).

Com o intuito de estabelecer um paradigma que possibilitasse o estudo de memórias declarativas em roedores, Ennaceur e Delacour, em 1988, propuseram o paradigma de Reconhecimento de Objetos (RO), embasado na observação de um comportamento exploratório inato destes animais. Esta tarefa baseia-se no fato de que os roedores sempre exploram durante um maior período de tempo aqueles objetos que desconhecem, na eventualidade de que estes sejam apresentados simultaneamente a objetos familiares. Além da vantagem de envolver uma memória correspondente à memória declarativa em humanos, este paradigma apresenta a interessante vantagem de não requerer um treinamento preliminar extenso, já que o aprendizado se dá após uma única sessão. Ainda, o paradigma não expõe o animal a estímulos aversivos (como choque), não requer restrição a alimento ou água e já foi replicado em muitos

laboratórios, utilizando uma grande variedade de aparatos e objetos, tanto com ratos como com camundongos. Devido a todas estas vantagens, a tarefa de RO torna-se uma ferramenta útil para o estudo dos processos neurais e comportamentais envolvidos na formação ou evocação de memórias sem interferir no comportamento natural do animal, e por este motivo foi eleita como paradigma a ser utilizado neste estudo. A reconsolidação da memória também pode ser facilmente estudada por este paradigma, pois cada vez que um objeto novo é apresentado simultaneamente a um objeto familiar, as informações a respeito do objeto novo são adicionadas às informações já armazenadas, referentes ao objeto familiar, constituindo assim o fenômeno da reconsolidação.

As evidências de que existem numerosos processos bioquímicos hipocampais envolvidos no processamento da memória de RO (Rossato *et al.*, 2007; Myskiw *et al.*, 2008; Furini *et al.*, 2010), nos levam a questionar se o alvo final destes processos poderia ser a indução de eventos plásticos como a LTP, que já foi comprovadamente associada à formação de outros tipos de memória. Desta maneira, buscando responder a esta pergunta e levando em consideração: 1) o comprovado envolvimento do hipocampo no processamento de memórias declarativas, entre elas a memória de reconhecimento de objetos; 2) a necessidade de se estabelecer se a LTP poderia ser o mecanismo formador de memórias declarativas e de reconhecimento, como é o caso da memória de RO em roedores e 3) a ausência de estudos visando elucidar se ocorrem modificações na plasticidade sináptica hipocampal como conseqüência da reconsolidação da memória de RO; este estudo teve como objetivos aqueles especificados na seguinte seção.

2. Objetivos

2.1 Objetivo Geral

Avaliar se ocorrem modificações da plasticidade sináptica no hipocampo de camundongos 0, como conseqüência do processamento da memória de reconhecimento de objetos.

2.2 Objetivos Específicos

- Verificar se durante a aquisição, ou em diferentes tempos após o treino na tarefa de RO, ocorrem alterações na eficiência sináptica na via colateral de Schaffer hipocampal.
- Estudar se as alterações acima mencionadas, se efetivamente observadas, não são um efeito inespecífico desencadeado pela simples exposição do animal à caixa de RO.
- Investigar se há interdependência entre a formação da memória de RO e as alterações na plasticidade sináptica nas vias colaterais de Schaffer hipocampais, através da administração de pelo menos um fármaco que bloqueie ambos os processos.
- Examinar se durante a evocação, ou em diferentes tempos após o teste com objetos iguais para a memória de RO, ocorrem alterações na eficiência sináptica na via colateral de Schaffer hipocampal.
- Verificar se durante o evento de reconsolidação, desencadeado pelo teste em presença de um objeto novo e um objeto familiar, ou em diferentes tempos após esta sessão, ocorrem alterações na eficiência sináptica na via colateral de Schaffer hipocampal.
- Averiguar se as eventuais alterações na plasticidade sináptica hipocampal registrados durante a sessão de teste se devem a um efeito tardio desencadeado pelo treino no RO, e não a um efeito específico desencadeado pela reconsolidação da memória de RO em si.

- Investigar se as alterações na plasticidade sináptica hipocampal que ocorrem como consequencia da reconsolidação da memória de RO, se observadas, são específicos ao evento de evocação da memória em presença de novidade ou se ocorrem pela simples re-exposição do animal a um contexto idêntico ao do momento do treinamento.
- Verificar se a indução de LTP por estímulo tetânico na via colateral de Schaffer, capaz de induzir a saturação desta via, é capaz de inibir o aprendizado na tarefa de RO. No caso de que o aprendizado realmente seja impedido, avaliar se este efeito é reversível ou irreversível.
- Avaliar se as modificações na plasticidade da via colateral de Schaffer conforme ocorre o processamento da memória para o RO, são na verdade causadas por alterações no ritmo teta hipocampal.

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4. Artigo Científico

Os resultados obtidos ao final desta Tese de Doutorado foram publicados em forma de artigo científico no periódico *Proceedings of the National Academy of Sciences* em 9 de Fevereiro de 2010 (Volume 107, Número 6). O artigo encontra-se reproduzido a partir da próxima página.

Plastic modifications induced by object recognition memory processing

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Abstract

Long-term potentiation (LTP) phenomenon is widely accepted as a cellular model of memory consolidation, although its participation in memory formation has been shown only for aversive memories. Object recognition (OR) is a particularly useful way of studying declarative memory in rodents because it makes use of their innate preference for novel over familiar objects. In this study, mice had electrodes implanted in the hippocampal Schaffer collaterals-pyramidal CA1 pathway, and were trained for OR. Field EPSPs (fEPSPs) evoked at the CA3-CA1 synapse were recorded at the moment of training and at different times thereafter. LTP-like synaptic enhancement was found 6 h post-training. A testing session was conducted 24 h after training, in the presence of one familiar and one novel object. Hippocampal synaptic facilitation was observed during exploration of familiar and novel objects. A short depotentiation period was observed early after the test and was followed by a later phase of synaptic enhancement. Here we show that OR memory consolidation is accompanied by transient potentiation in the hippocampal CA3-CA1 synapses, while reconsolidation of this memory requires a short-lasting phase of depotentiation which could account for its well-described vulnerability. The late synaptic enhancement phase, on the other hand, would be a consequence of memory re-stabilization.

\body **Introduction**

Long-term potentiation (LTP) is defined as an activity-dependent enhancement of synaptic strength (1), and is the general cellular model of learning-induced plasticity in the hippocampus. Recent remarkable findings show that the acquisition of conditioned fear responses is accompanied by a long-lasting enhancement in synaptic strength (2, 3). In the CA1 region of the hippocampus, the posttraining consolidation period involves, and requires, biochemical changes identical to those that have been described for LTP (4).

Information about spatial and contextual characteristics of previously encountered items is an important element of most declarative memories. In fact, impaired recognition of familiar objects and the associated difficulty in distinguishing them from novel ones is one of the early traits of cognitive decline observed in Alzheimer's patients (5). Evidence suggests that the hippocampus is essential for memory processing during OR tests (6). In particular, lesion and pharmacological studies indicate that the hippocampal formation is required for acquisition and storage of the contextual details and temporal order of previous experiences (7). However, the question remains as to whether acquisition and consolidation of OR long-term memory (LTM) can induce in the hippocampus the same associative synaptic plasticity mechanisms believed to be necessary for the lasting storage of other memory types (4). It also remains unanswered whether other phases of memory processing can induce changes in synaptic efficacy. When a given memory is retrieved in the presence of novelty, it is thought to be set into a labile phase which is then subjected to stabilization (8-10). This new period in mnemonic processing is called reconsolidation and can be observed, in the case of the object recognition paradigm, every time a new object is simultaneously presented with a familiar one, thus requiring information concerning the new object to be added to the previously stored memory. Many biochemical similarities have been found between consolidation and reconsolidation phases, including protein synthesis requirement (11, 12), and the activation of certain transcription factors (13, 14), protein kinases (15), and immediate early genes (16). In the present study we investigated whether OR memory processing leads to changes in hippocampal synaptic efficacy.

Results

We examined whether training in the OR paradigm is capable of inducing changes in synaptic strength in the hippocampal CA3-CA1 synapse. C57BL/6 mice had stimulation and recording electrodes stereotaxically implanted in the CA3 and CA1 regions respectively (Fig. 1). When recovered from surgery, mice were trained (Fig. 2*A*, left panel; Tr) in the OR paradigm using two different objects, denoted by A and B. During the training session, evoked fEPSPs were recorded at the moment that animals explored each object. No changes in the amplitude of recorded fEPSPs were observed during exploration of the two objects used for training (Fig. 2*B*; 107.3 ± 4.7% and 99.33 ± 7.4% for A and B respectively, n = 12). Animals were removed from

their home cages and taken to the recording room at different times after training (1.5, 3, or 6 h). At each of these events, mice were submitted to a 5 minute-long recording session, where fEPSPs evoked at the CA3-CA1 synapse were again recorded. These recording sessions were meant to detect any late training-induced change in synaptic strength. LTP-like enhancements in synaptic efficacy were detected 6 h post-training (110.4 ± 4.4%, $p < 0.05$, as compared with 100, n = 13; Fig. 2*C*) lasting less than 24 h (see Supplementary Fig. 1*B* online, left panel). To find out whether these observed changes were really a consequence of OR training, and not to human handling or to exposure to the OR box alone, a group of electrode-implanted mice were submitted to the same protocol, with one difference: no objects were presented at the training session — i.e., one extra habituation session was performed instead. Stimuli were presented at 0, 1.5, 3, 6, and 24 h for 5 min (every 20 ± 5 s). Quantitative analysis showed that the percentage of variation of fEPSP amplitudes across this time, as compared with the mean value (100%) computed during the baseline (fourth day of habituation) period, was ≤ 11.2%, with no statistically significant tendency towards a decrease or increase ($p = 0.67$; Supplementary Fig. 2 online).

In addition, we investigated whether a pharmacological treatment capable of hindering OR LTM formation was also capable of preventing training-related synaptic plasticity events in the hippocampus. Pre-training systemic treatment with the NMDA-receptor antagonist MK-801 hindered OR LTM formation and concomitantly blocked the late training-induced changes in CA3-CA1 fEPSP enhancements recorded in vehicle-treated animals ($p > 0.05$; Supplementary Fig. 3 online).

OR memory retention was assessed in a test session performed 24 h after training (Fig. 2*A*, right panel; Test) in which a familiar and a novel object were used (denoted by A and C respectively). Successful learning was obtained when animals explored the novel object for a significantly longer time than the familiar one, and only data from these animals were analyzed. Approximately 10% of all animals assigned to the experiment were excluded for not having learned the task effectively (no significant changes in fEPSP amplitudes were observed in those animals, $p > 0.05$). In contrast to the training session, exploratory behavior towards both familiar and novel objects employed in the test session was accompanied by significant increases in CA3-CA1 synaptic efficacy (139.4 ± 17.6% and 133.6 ± 12.8% respectively, $p < 0.05$ compared with baseline values collected during the last habituation session, n = 12; Fig. 2*D*). Interestingly, there were no changes in the hippocampal fEPSPs in animals submitted to retraining instead of a test session (Supplementary Fig. 4 online). In that retraining session, there was no novel feature relating to training, since the same environment and objects were used in both sessions.

For those animals submitted to regular test sessions (a novel and a familiar object), 5 minute-long recording sessions were carried out at different times post-test in order to evaluate test-induced late changes in fEPSP (Fig. 2*A*, right panel). We found that a perceptible, although not statistically significant, depotentiation occurs at 1.5 h post-test, followed by an even later

potentiation phenomenon (6 h; Fig. 2*C*, right panel). No such modifications were observed when the test session was omitted (Supplementary Fig. 1*B*, right panel). Importantly, none of the recorded changes in fEPSP was accompanied by a significant alteration in the hippocampal theta or gamma rhythms (Supplementary Fig. 5 online), indicating that all data were collected from similar alertness states.

In a second set of experiments, we investigated whether saturation of the CA3-CA1 synapse by experimental high-frequency stimulation (HFS) hindered OR-evoked changes in synaptic strength. On their last day of habituation to the open field, one group of mice received a HFS capable of causing a 5-day persistent LTP (197.3 ± 19.1% at 20 min after HFS, $p < 0.05$ compared with baseline; Fig. 3*B*; filled circles; see ref. 17 for details). Twenty-four hours after HFS presentation, mice were trained for OR. For as long as they expressed HFS-evoked potentiation of hippocampal CA3-CA1 synapses (Fig. 3*D*, filled circles), mice spent approximately the same amount of time exploring both novel and familiar objects in a test session conduced 24 h after training (Fig. 3*E*, right panel; LTP), suggesting that saturation of that pathway by external HFS makes animals incapable of acquiring OR memory. However, when the same group of mice was trained and tested two weeks after HFS — a situation in which no significant increase in fEPSP could be distinguished (Fig. 3*F*, filled circles) — they were able to acquire OR LTM normally (Fig. 3*G*, right panel; LTP). The control group exhibited fEPSPs similar to baseline in all recording sessions (Fig. 3*C*, *D*, *F*, open circles) and learned normally (Fig. 3*E*, *G*, right panel; Contr).

Discussion

It is currently believed that experience-dependent changes in synaptic strength are the underlying physiological mechanism of mnemonic processes. In the hippocampus, the two known phenomena of synaptic plasticity modification — LTP and LTD — have been correlated with different types of memory formation (18-20). LTP, in particular, was shown to accompany associative aversive learning in the hippocampus (2-4).

When considering OR memory, hippocampal functionality is necessary for the acquisition of a temporal sequence of events (21), as well as for the distinction of spatial information about objects (22, 23). Although it may not play a direct role in distinguishing the different features of each object, it is fundamental as a novelty detector because of its role in comparing previously stored information with new incoming aspects of one particular situation. The hippocampus receives inputs from the perirhinal cortex, which is itself the site of entrance for visual, olfactory, and somato-sensory information, all of which are relevant for object recognition. As happens when other behavioral paradigms are employed, OR also originates strong long-lasting mnemonic traces that can be accessed for over 24 h after the acquisition phase (24). It would be expected, therefore, that this type of memory would also induce biochemical and

electrophysiological changes in specific structures, including the hippocampus. Many biochemical aspects of OR memory have been described by our group (24-27), and we now provide the first evidence that OR LTM formation induces synaptic efficacy changes comparable to those that underlie the lasting storage of other memory types.

Here we show the induction of object-dependent LTP-like synaptic changes, given that simple exposure to the environment does not induce similar modifications. This endogenous phenomenon reaches its peak at 6 h after the acquisition phase (training). These results are at variance with those of previous studies in which acquisition of information about novel objects (28, 29), or object configurations (18), facilitates LTD expression, while information about novel environments favors LTP. Interestingly, our results show that training-induced hippocampal LTP lasts less than 24 h, although animals do still express OR memory for 24 h or more (24). This observation suggests that while memory consolidation is hippocampus-dependent, this may not be true for memory persistence, which has been studied in other paradigms (30, 31) but not in the OR task. One possible explanation is that information about spatial and contextual characteristics of objects could end up being relocated to other parts of the brain once memory is consolidated (32-34).

When a given memory is retrieved in the presence of novelty, it is set into a labile phase and requires stabilization in order to persist (35). This phase of memory processing is called reconsolidation. It is considered an active phase that takes place in order to allow reorganization of the already formed memories, allowing incorporation of new information (36, 37).

From this point of view, and considering the OR paradigm (see Methods), the test phase could itself act as a trigger for a reconsolidation-like labile phase of memory, since it necessarily involves a novel object being presented simultaneously to a familiar one (26). Our results constitute the first evidence that reactivation of a consolidated memory induces changes in synaptic efficacy. Here, we show that reactivation of OR memory induces novelty-dependent synaptic modifications in the hippocampus as a consequence of memory reactivation, suggesting that similarly to memory consolidation, the reconsolidation phenomenon is also capable of modifying hippocampal plasticity. In some way, reactivation-induced changes are similar to those observed after a training session (6 h post-training), but occur only after a brief period of depotentiation observed at 1.5 h after reactivation. A possible explanation for this rapid LTD phase is that memory updating after reactivation requires a transient protein degradation period. In fact, this theory has already been discussed in a recently published paper showing that the process of adding new information to a preexisting memory requires a protein degradation phase that takes place approximately 2 h after retrieval (38). In that study, basal protein levels were reestablished at 6 h after retrieval, a fact that could account for the depotentiation/potentiation pattern observed in our study.

If we assume that OR memory formation is totally reliant on CA3-CA1 synaptic functionality, then any experimental procedure capable of disturbing hippocampal patterns of synaptic strengths would be enough to prevent memory consolidation. The assumption is that the huge wave of plasticity generated by experimentally evoked LTP produces retrograde amnesia by interfering with the activation of hippocampal memory networks (39). This was proved correct for other types of memory (2, 19, 40), and is considered a relevant argument to support the theory that memory acquisition is dependent on hippocampal LTP. In our experiments, the application of an external HFS in a certain time window before OR training reversibly hindered OR LTM formation — i.e., animals were incapable of learning while CA3-CA1 synapses were potentiated by the HFS-evoked LTP. Once fEPSP values had returned to basal level and animals were retrained in the OR task, they showed normal acquisition.

Taken together, our results indicate that the OR test, as well as the acquisition of conditioned fear responses is accompanied by an enhancement in synaptic strength (2, 3, 43) and that NMDA receptors are involved in this adaptive process (2, 44). The fact that saturating LTP is able to occlude the learning-induced synaptic modifications further reinforces the hypothesis that LTP-like processes are involved in actual learning (44, 45). Moreover, our results suggest that OR LTM formation is dependent on hippocampal integrity and is capable of inducing, in the CA3-CA1 synapses, long-lasting plastic changes that play a role in memory codification for the first few hours. Reconsolidation of OR memory also leads to important synaptic modifications, although with a different pattern.

Methods

Animals. Experiments were performed using C57BL/6 adult mice (3-5 months old, 25-35 g) obtained from an official supplier (University of Granada Animal House, Granada, Spain). Before surgery, animals were housed in separate cages (n = 10 per cage), but they were switched to individual cages after electrode implantation. Mice were kept on a 12 h light/dark cycle with constant ambient temperature (21.5 ± 1.5 ^{o}C) and humidity (55 ± 8%). Food and water were available *ad libitum*. All experiments were conducted in accordance with the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1996) and with the Guidelines of the European Union (2003/65/CE) for the use of laboratory animals in chronic experiments. Every effort was made to reduce the number of animals used and to minimize their suffering.

Surgery. Animals were anesthetized with 0.8-1.5% isoflurane, at a flow rate of 1-4 L/min oxygen (17), and were implanted with bipolar stimulating electrodes in the right Schaffer collateral pathway of the dorsal hippocampus (2 mm lateral and 1.5 mm posterior to bregma, and 1-1.5 mm from the brain surface; see ref. 41) and with a recording electrode aimed at the right CA1 *stratum radiatum* (1.2 mm lateral and 2.2 mm posterior to bregma and 1-1.5 mm from the brain surface).

Hippocampal electrodes were made of 50 μm Teflon-coated tungsten wire (Advent Research Materials, Eynsham, UK). The final location of the recording electrodes in the CA1 area was determined following the field potential depth profile evoked by paired (10-500 ms interval) pulses presented to the Schaffer collateral. A bare silver wire was fixed to the skull as ground. The four wires were soldered to a four-pin socket (RS Amidata, Madrid, Spain) which was then fixed to the skull using dental cement (2, 17). Animals were allowed to recover from surgery for at least four days before behavioral experiments began. Only data from animals with correct electrode placement were analyzed.

Object recognition protocol. OR experiments were conducted in an open-field arena (30 x 25 x 20 cm) built of polyvinyl chloride plastic, plywood, and transparent acrylic as described elsewhere (25, 27). The outside walls of the box were covered with metal plates, connected to ground, and the box was placed inside the set-up rack. During habituation, training, and test sessions, animals had their pin-sockets connected to a wire suspended above the open-field arena which allowed us to stimulate and record the CA3-CA1 synapses while animals were performing the task — i.e., while exploring either of the objects (minimum stimulation interval: 20 sec).

Stimulus objects were made of plastic. There were several copies of each object, which were used interchangeably. The role (familiar or novel), as well as the relative position of the 2 stimulus objects, were counterbalanced and randomly permuted for each experimental animal. The open-field arena and the stimulus objects were cleaned thoroughly between trials to ensure the absence of olfactory cues. Exploration was defined as sniffing or touching the stimulus object with the nose and/or forepaws. Sitting on or going around the objects was not considered exploratory behavior. A video camera was positioned over the arena and the behavior of the mice was recorded using a video tracking and analysis system. The experiments were performed by an observer blind to the treatment condition of the animals. During all behavioral sessions, lights were kept dim (30-40 lux).

Electrode-implanted animals were habituated to the open-field arena by allowing them to explore it freely for 20 min per day for 4 days in the absence of any other behaviorally relevant stimulus. On the fifth day of experiment, OR training occurred: mice were placed in the open-field arena containing two different objects (denoted by A and B) and left to explore them freely for 5 min. A 5 minute-long test session was performed 24 h later for evaluation of LTM retention (42). For this purpose, one of the objects used at training was randomly replaced by a novel one (denoted by C), and mice exploratory behavior towards familiar and novel objects was quantified. Only data from animals that learned successfully — i.e., explored the new object for significantly longer than the familiar one — (approximately 90% of subjects) were analyzed. In both training and test sessions, home-made equipment was used to trigger hippocampal stimulation every time mice approached one object or the other with exploratory intentions.

Recording and stimulation procedures. Before the experiment was started, synaptic field potentials in the CA1 were evoked by paired (40 ms interval) 100 μs, square, biphasic (negative-positive) pulses applied to the right Schaffer collaterals. Stimulus intensity ranged from 50 to 350 μA. For each animal, the stimulus intensity was set at 30-40% of the intensity necessary to evoke a maximum fEPSP response (i.e., well below the threshold for evoking a population spike) (2, 17), and remained unchanged until the end of the experiment.

For the first set of experiments, baseline was obtained by recordings held on the last day of habituation to the open field. Stimuli were applied every 20 s for the first 5 min of the session (20 min) in order to evoke synaptic field potentials in the CA1. The average of these values was considered baseline for data recorded 24 h and 48 h thereafter: training and test session respectively. In addition, simple 5 minute-long recording sessions were conducted 1.5, 3 and 6h after habituation session 4, and were used as baseline values for data collected following training or test in the same number of hours. Therefore, each animal served as its own control. All data were analyzed offline.

In the externally induced LTP experiments, fEPSP baseline values were recorded for 15 min before LTP induction. For LTP induction, each animal was presented with an HFS protocol consisting of five 200 Hz, 100 ms trains of pulses at a rate of one per s. This protocol was presented six times, at intervals of 1 min. After HFS presentation, recordings of double-pulse stimulation at Schaffer collaterals were conducted for another 30 min to evaluate whether the LTP protocol was effective. On subsequent days, shortly before behavioral procedure, recordings were made for 15 min to assess the persistence of LTP response. According to previous studies from our group (2, 17) this HFS was enough to evoke a saturating LTP response, lasting around 5 d, without the appearance of abnormal spikes in EEG recordings and/or any noticeable epileptic seizure.

Pharmacological treatment. The NMDA-receptor antagonist MK-801 was acquired from Sigma–Aldrich (St Louis, MO, USA) and was dissolved in DMSO 0.1% up to 0.01 mg/mL. The dose used was defined by pilot experiments in which a dose with no effects on exploratory and locomotor activities was sought. The drug at the dose of 0.01 mg/kg of body weight or its vehicle alone were administered i.p. 15 min before training in the OR paradigm.

Data collection and analysis. All data were stored digitally on a computer through an analog/digital converter (1401 Plus; CED, Cambridge, England), at a sampling frequency of 11-22 kHz and with an amplitude resolution of 12 bits. Behavioral and electrophysiological data were analyzed offline for quantification of exploratory behavior towards each of the objects and of evoked fEPSPs amplitudes, with the help of commercial representation programs (Spike 2,

Microsoft Excel and Graphpad Prism 5). In simple recording sessions and in recordings made during the performance of the task, electrophysiological data from the whole session (5 min) were averaged for each animal after having been normalized with respect to baseline, which was determined on the last day of habituation. The amplitude of averaged fEPSPs was quantified and stored for later statistical analysis. In the case of LTP experiments, data are expressed as average of amplitude of fEPSP for every 2 min of recording.

Behavioral data were expressed as percentage of total exploration time for each object, and analyzed using a one-sample Student's *t* test, by comparing the group's means with the fixed value of 50%, which represents no differentiation between objects.

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Figures

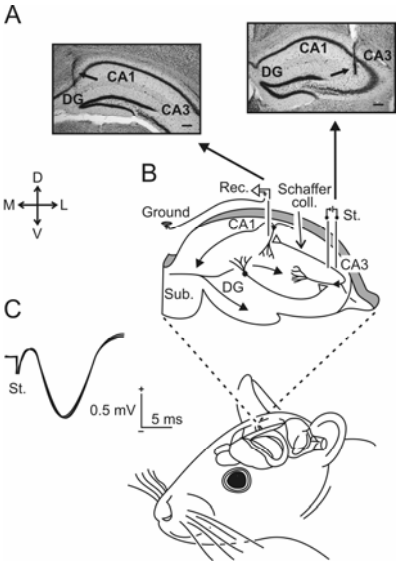


Figure 1. Experimental design. (A) Photomicrographs illustrating the location of stimulating (left) and recording (right) sites (arrows). Calibration bar is 200 µm. (*B*) As shown at the top diagram, animals were implanted with stimulating and recording electrodes aimed to activate CA3-CA1 synapses of the right hippocampus. (C) The three superimposed records illustrate the extracellular synaptic field potential recorded at the stratum radiatum of the CA1 area following electrical stimulation (St.) of Schaffer collaterals. Calibrations as indicated. Abbreviations: D, L, M, V, dorsal, lateral, medial, ventral; DG, dentate gyrus; Sub, subiculum.

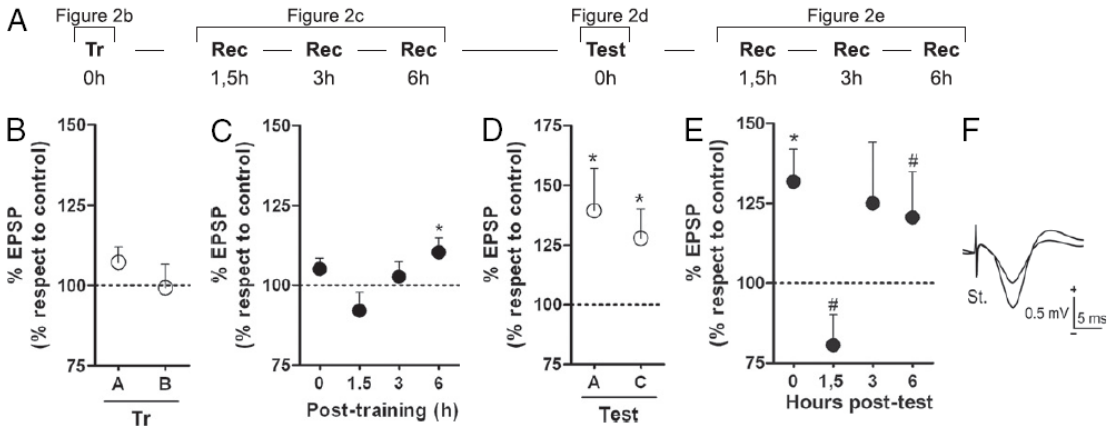


Figure 2. Object recognition training and testing increases the strength of the CA3-CA1 synapse. (*A*) Schematic representation of the entire experiment. Electrode-implanted mice were trained in the OR paradigm using two different objects (*A*, left panel, Tr 0 h), denoted by A and B. (*B*) To find out whether simple exposure to the new objects was capable of modifying synaptic plasticity, fEPSPs were recorded following stimulation of Schaffer collaterals every time animals approached either of the objects during the training session. No synaptic potentiation was observed during OR training (107.3 ± 4.7% and 99.33 ± 7.4% for A and B, respectively). Three 5 minute-long recording sessions were carried out at different times thereafter (*C*), to accompany training-induced modifications of fEPSP. LTP-like potentiation was found late after training (6 h). Twenty-four hours after training, OR memory retention was assessed in a test session (*A*, right

panel, Test 0 h), using a familiar and a novel object (A and C, respectively), and, once again, fEPSPs were recorded when animals explored either object (*D*). Enhanced fEPSPs were observed in both cases (139.4 ± 17.6% for the familiar object and 133.6 ± 12.8% for the novel object). (*E*) Three 5 minute-long recording sessions were carried out at different times post-test to accompany test-induced fEPSP modifications. Late LTP was also observed (6 h post-training) following a brief period of depotentiation occurring at around 1.5 h post-test. In all cases, data are presented as mean ± S.E.M. of the CA3-CA1 fEPSP amplitude normalized relative to the last day of habituation to the OR box, which was taken as baseline. Typical examples of mean recorded fEPSP for a representative mouse during training and test sessions are shown in (*F*). Asterisks and # indicate statistically significant differences ($p < 0.05$; $p < 0.10$) from the fixed value of 100%, in a Student's *t* test (n = 8 to 12 per group).

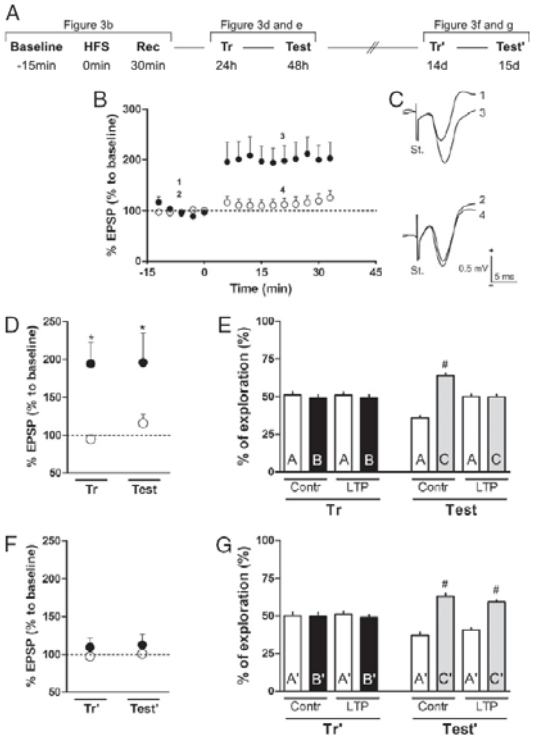
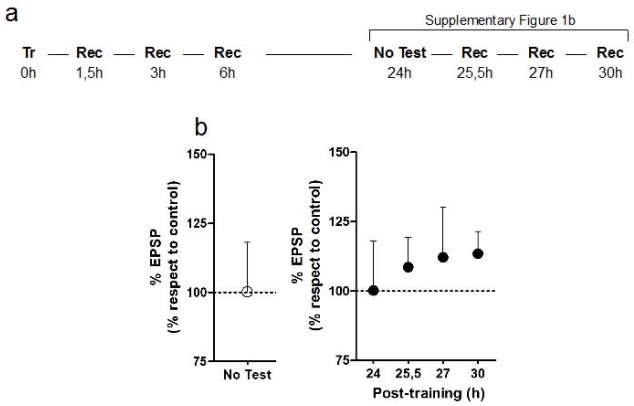


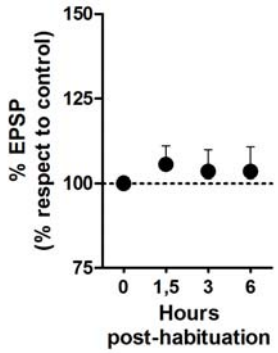
Figure 3. Experimental saturation of CA3-CA1 synapses reversibly hinders OR LTM formation. Electrode-implanted mice were divided into two groups: the first group was submitted to an HFS protocol capable of inducing a 5-day-long LTP (filled circles and LTP group); the second group was submitted to the same experimental protocol, but did not receive the HFS protocol (open circles and Contr group). In both groups, fEPSPs were recorded following stimulation of Schaffer collaterals every 20 s for 15 min to obtain a baseline. After HFS was applied to the LTP group, LTP induction was confirmed by 30 min of fEPSP recording, in which stimulation was given at the same initial intensity and at the same rate (*A*, left panel). LTP induction was significant (197.3 ± 19.1%, $p < 0.05$ compared to baseline) in the group that received HFS (*B*). Typical examples of fEPSPs recorded from the two groups are shown in (*C*). Both groups were normally trained in the OR task 1 d and 14 d thereafter (*A*, right panels). The non-HFS group exhibited fEPSP values similar to baseline throughout the experiment (*D* and *F*, open circles), and acquired OR LTM normaly in both assessment phases (*E* and *G*, Contr). The HFS group was amnesic (*E*, LTP), while fEPSP was still enhanced due to external HFS (*D*, filled circles). When the effects of HFS on recorded fEPSP could no longer be observed (*F*, filled circles), the LTP group had normal OR LTM formation (*G,* LTP). In *B*, *D*, and *F*, electrophysiological data are expressed as mean fEPSP normalized to baseline mean amplitude, and * indicates a statistically significant difference ($p < 0.05$) from the fixed value of 100%, in a

Student *t* test. In *E* and *G*, behavioral data are expressed as percentage of total exploration time, and # indicates a statistically significant difference ($p < 0.05$) from the fixed value of 50% (n = 10 per group).

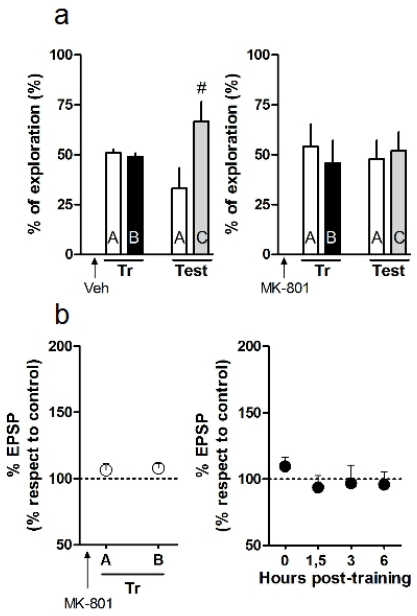
Suplementary Figures



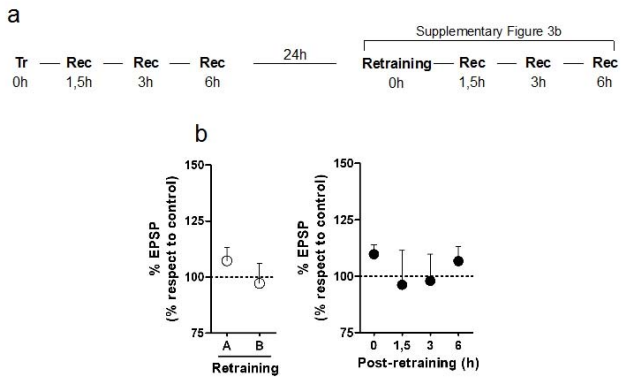
Supplementary Figure 1. LTP-like phenomena seen during and after the OR test session are not a late consequence of OR training. Electrode-implanted mice were trained (**a**, left panel, Tr) in the OR paradigm using two different objects, denoted by A and B. Stimuli at Schaffer collaterals were triggered at training and at different times thereafter as described in Fig. 2. Twenty-four hours after training, animals were submitted to an ordinary recording session instead of being tested for LTM (**a**, right panel, *No Test*). Three 5-minute-long recording sessions were carried out at different times after it (**a**, right panel, Rec). None of the alterations seen during exploration testing of objects and at different times after the test were reproduced (**b**). Data are presented as mean ± S.E.M. of the CA3-CA1 fEPSP amplitude normalized relative to habituation 4 (n = 7).



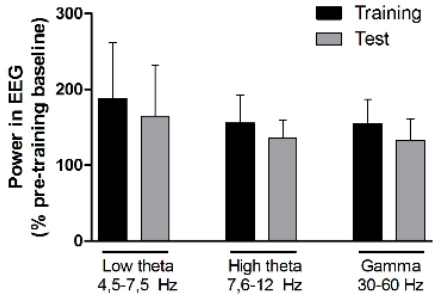
Supplementary Figure 2. LTP-like phenomenon seen after OR training session is not a consequence of exposure to the OR box alone. Electrode-implanted mice were habituated to the OR box, and on the 5th day of the experiment, an extra habituation session was carried out instead of training. None of the observed changes in fEPSP seen after the training session were observed at any of the assessed times thereafter. Data are presented as mean ± S.E.M. of the CA3-CA1 fEPSP amplitude normalized relative to habituation 4 (n = 6).



Supplementary Figure 3. Pre-training systemic treatment with MK-801 hinders OR LTM formation and blocks late training-induced hippocampal fEPSP modification. Electrode-implanted mice received an i.p. injection of the NMDA-receptor antagonist MK-801 (0.01 mg/kg of body weight) or its vehicle 15 min before a training session in the OR paradigm using two different objects, denoted by A and B. Twenty-four hours after training, OR memory retention was assessed in a test session using a familiar and a novel object (A and C, respectively). Vehicle-treated animals explored the novel object significantly longer, while MK801-treated mice did not recognize the familiar object used for training, and spent approximately the same amount of time exploring the objects (**a**). Stimuli at Schaffer collaterals were triggered at training and at different times thereafter as described in Figs. 1 and 2, and three five minute-long recording sessions were carried out at different times thereafter to accompany training-induced modifications of fEPSP in drug-treated animals (**b**). Note how the MK801-treated group does not express training-induced changes in hippocampal CA3-CA1 synaptic efficacy, while vehicle infused animals show synaptic potentiation equivalent to that shown in Fig. 2 (data not shown). In (**a**), behavioral data are expressed as percentage of total exploration time, and # indicates a statistically significant difference ($p < 0.05$) from the fixed value of 50%. In (**b**), data are presented as mean ± S.E.M. of the CA3-CA1 fEPSP amplitude normalized relative to the last day of habituation to the OR box, which was taken as baseline (n = 5 per group).



Supplementary Figure 4. LTP-like phenomena seen during and after OR test session are novelty-dependent. Electrode-implanted mice were trained (**a**, left panel, Tr) in the OR paradigm using two different objects, denoted by A and B. Stimuli at Schaffer collaterals were triggered at training and at different times thereafter as described in Figs. 1 and 2. Twenty-four hours after training, animals were submitted to a second training session (**a**, right panel, Retraining) — i.e., with no novel object. This session was called Retraining, and three 5-minute-long recording sessions were carried out at different times after it (**a**, right panel, Rec). None of the alterations seen during exploration of testing objects (**b**, left panel) and at different times after the test (**b**, right panel) were reproduced. Data are presented as mean ± S.E.M. of the CA3-CA1 fEPSP amplitude normalized relative to habituation 4 (n = 8).



Supplementary Figure 5. Synaptic changes induced by OR memory processing are not accompanied by changes in either low- or high-frequency theta rhythm. Power spectral analysis conducted at training (black bars) and test (gray bars) sessions did not reveal systematic variations in the spontaneous EEG recorded in electrodes showing fEPSP enhancements.

5. Considerações Finais

Os resultados obtidos nesta Tese de Doutorado nos permitem concluir que:

- Não ocorrem alterações na plasticidade sináptica da via colateral de Schaffer durante o treinamento da tarefa de RO. No entanto, observamos um fenômeno de aumento da eficiência destas sinapses 6 horas após o aprendizado, mas não 1,5 horas ou 3 horas depois.

- Tal aumento na eficiência sináptica não se deve exclusivamente à exposição à caixa de RO, já que a substituição da sessão de treino por uma sessão extra de habituação não desencadeou os mesmos efeitos sobre a eficiência da sinapse CA3-CA1 hipocampal.

- Existe interdependência entre os fenômenos de formação da memória de RO e o aumento na eficiência sináptica observado pós-treino, visto que a administração intra-peritoneal de MK-801, um antagonista dos receptores NMDA, minutos antes do treino, é capaz de bloquear tanto a retenção da memória de longa duração para o RO como o aumento da eficiência sináptica que ocorre como conseqüência do treino nesta tarefa.

- A sinapse CA3-CA1 encontra-se potenciada durante uma sessão de teste equivalente a uma sessão de reconsolidação, ou seja, uma sessão em que se apresenta um objeto novo e um objeto familiar simultaneamente ao animal. Vimos, no entanto, que não há diferença significativa entre as eficiências sinápticas observadas no momento da exploração do objeto novo ou do objeto familiar, dentro da mesma sessão.

- Em diferentes momentos após a sessão de teste, observam-se processos distintos de eficiência sináptica hipocampal: redução da eficiência sináptica 1,5 horas após o teste e aumento de eficiência sináptica 6 horas pós-teste.

- Tais alterações na eficiência sináptica pós-teste ocorrem exclusivamente quando um objeto novo é inserido ao contexto, e não quando a sessão de teste é realizada utilizando-se objetos e contexto idênticos ao da sessão de treino.

- As alterações observadas durante a sessão de teste e em diferentes tempos depois desta sessão não podem ser atribuídas a um efeito tardio iniciado pela sessão de treino.
- A saturação das vias colaterais de Schaffer induzidas por um estímulo tetânico externo pré-treino, impede a consolidação da memória de RO. Tal efeito caracteriza-se por ser reversível, uma vez que quando a eficiência desta sinapse retorna aos níveis basais, os animais podem aprender a tarefa normalmente.
- As alterações na plasticidade sináptica nas vias colaterais de Schaffer registradas não se devem a modificações no poder espectral hipocampal.