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Papel do Sistema Adrenérgico na Formação da Memória de Reconhecimento em Ratos

Dissertação apresentada como requisito para a obtenção do título de Mestre em Biologia Celular e Molecular, a ser apreciada pela Banca Examinadora do Programa de Pós Graduação em Biologia Celular e Molecular, da Faculdade de Biociências, da Pontifícia Universidade Católica do Rio Grande do Sul.

Orientadora: Prof.a Dr.a Nadja Schröder

Porto Alegre
2007

AGRADECIMENTOS

À Pontifícia Universidade Católica do Rio Grande do Sul, especialmente à Faculdade de Biociências por me acolher, tanto ao longo da graduação, quanto ao da pós-graduação.

Às meninas da secretaria do Programa de Pós Graduação em Biologia Celular e Molecular; Maria Luiza Moreira, Cátia Bonacina e Josilene Martins Rocha que, devido a sua competência, me mantiveram sempre bem informada a respeito de tudo sobre o programa nunca perdendo a paciência e oferecendo toda a atenção possível.

À professora Dr.a Clarice Alho que demonstrou-se sempre presente e disponível para resolver todos os problemas que apareciam ao longo desses anos.

Aos meus colegas e amigos do laboratório Marcelo Guimarães, Caroline Dias e Roberta Petry pela ajuda prestada em todas as horas, aos amigos Felipe Scalco, Vanessa Athaíde Garcia e Juliana Presti Torres que foram incansáveis em sua ajuda, sempre presentes em qualquer hora e principalmente à Maria Noêmia Martins de Lima que foi excepcional, pela paciência dissipada comigo no decorrer de todo este trabalho não medindo esforços para me auxiliar; muito obrigada.

À Glauramar Barbosa Martins e à Betânia Souza de Freitas, pessoas que conheci graças ao desenvolvimento deste trabalho e que, desde o início, estiveram dispostas a ajudar, não só na rotina laboratorial, mas sim, em todos os momentos nos quais precisei de amigas para desabafar. Estou certa de que este vínculo não acabará com a conclusão deste trabalho.

À Prof.a Dr.a Elke Bromberg por emprestar o computador, por ter colaborado com a realização do meu estágio docente e por descontrair o ambiente nos momentos de tensão mostrando que, por mais difícil que possa parecer a situação na qual nos encontremos, devemos agir de maneira calma e despreocupada.

À Prof.a Dr.a Nadja Schröder, que mesmo sem me conhecer, me recebeu de braços abertos no momento em que mais precisava de apoio; no momento em que parecia que o mundo tinha desabado e que não sabia mais o rumo que as coisas tomariam. Agradeço, por desde o início, ter acreditado e investido em mim apesar de tudo que estava acontecendo. No decorrer de todo este trabalho, conseguistes mudar o conceito que eu tinha de orientador, pois me mostrastes que é possível orientar com paciência e carinho. Vi que, apesar da quantidade de trabalho que tenha, um orientador pode e deve ser, não só um orientador, mas um amigo. Obrigada por me ensinar muito além da tarefa de reconhecimento do objeto novo, dos efeitos das drogas ou de como escrever um artigo; me ensinastes como devo ser e agir na profissão que escolhi, não só uma orientadora, mas principalmente uma amiga. Bom, poderia escrever páginas e mais páginas e não seria suficiente para agradecer, por que tu sabes que se não fosse por ti, talvez tivesse desistido do meu sonho.

Às minhas maravilhosas irmãs, Arielle e Ariany, que sempre me apoiaram desde o momento que escolhi a carreira que gostaria de seguir. Mesmo sem saberem, me ajudaram muito no decorrer desta caminhada pelo simples fato de estarem do meu lado me fazendo rir e esquecer das coisas ruins que aconteciam e das dificuldades que ainda teria de enfrentar. Tenho certeza de que sempre poderei contar com o apoio de vocês, não importando o quanto falte para atingir meus objetivos. Amo vocês!

Aos meus pais, Carlos e Virginia. Difícil encontrar palavras para agradecer tudo que fizeram por mim até hoje. Desde o apoio financeiro, sem o qual realmente não teria chegado até aqui, até o apoio emocional e incentivo que me deram, mesmo sem saber direito do que se tratava o meu trabalho. Estiveram do meu lado no decorrer da graduação, no ingresso e no decorrer do programa de pós-graduação, sempre me apoiando e incentivando nos momentos mais difíceis, não me deixando desistir nunca de atingir meus objetivos não importando o

quão difíceis fossem. Muito obrigada por serem esses pais maravilhosos que são; sei que poderei contar com a ajuda e com o apoio de vocês sempre. Amo vocês!

INDICE

1. RESUMO/ABSTRACT

Resumo

Evidências indicam que a adrenalina modula a consolidação da memória em testes de caráter aversivo/emocional tanto em animais quanto em humanos. Entretanto, pouco se sabe a respeito dos efeitos da adrenalina sobre a consolidação da memória de reconhecimento. Neste trabalho, constatamos que a administração sistêmica de adrenalina melhora a consolidação da memória na tarefa de reconhecimento do objeto novo sob diferentes condições de treino. Ratos machos controles que receberam injeção sistêmica de salina (NaCl 0,9%) imediatamente após treino mostraram retenção significativa de memória quando testados 1,5 ou 24 horas, mas não 96 horas após o treino. Em contraste, ratos tratados com injeção pós-treino de adrenalina mostraram retenção significativa de memória de reconhecimento do objeto novo em todos os intervalos de tempo testados. Em um segundo experimento utilizando uma condição de treino com maior grau de dificuldade, os ratos tratados com adrenalina, mas não os animais tratados com salina, mostraram retenção significativa na memória de reconhecimento do objeto novo quando testados 1,5 e 24 horas depois do treino. Depois, mostramos que a melhora de retenção na memória testada 96 horas depois do treino induzida por adrenalina foi prevenida pela administração sistêmica pré-treino do antagonista de β-adrenoreceptores, propranolol. Estes resultados sugerem que, como observado anteriormente em experimentos usando tarefas com caráter aversivo/emocional, a adrenalina modula a consolidação da memória de reconhecimento e que seus efeitos necessitam da ativação de receptores β-adrenérgicos.

Palavras-chave: Adrenalina; Adrenoreceptores; Memória de Reconhecimento; Consolidação da Memória.

Abstract

Extensive evidence indicates that epinephrine (EPI) modulates memory consolidation for emotionally arousing tasks in animals and human subjects. However, previous studies have not examined the effects of EPI on consolidation of recognition memory. Here we report that systemic administration of EPI enhances consolidation of memory for a novel object recognition (NOR) task under different training conditions. Control male rats given a systemic injection of saline (0.9% NaCl) immediately after NOR training showed significant memory retention when tested at 1.5 or 24, but not 96 h after training. In contrast, rats given a posttraining injection of EPI showed significant retention of NOR at all delays. In a second experiment using a different training condition, with a higher degree of difficulty, rats treated with EPI, but not SAL-treated animals, showed significant NOR retention at both 1.5 and 24-h delays. We next showed that the EPI-induced enhancement of retention tested at 96 h after training was prevented by pretraining systemic administration of the β-adrenoceptor antagonist propranolol. The findings suggest that, as previously observed in experiments using aversively-motivated tasks, epinephrine modulates consolidation of recognition memory and that the effects require activation of β-adrenoceptors.

Keywords: Epinephrine; Adrenoceptors; Recognition memory; Memory consolidation

2. APRESENTAÇÃO DO TEMA

Nas últimas décadas o interesse pelo estudo dos efeitos do estresse sobre órgãos e sistemas tem crescido de maneira significativa dentro da comunidade científica. Investigações a respeito de que maneira os eventos de conteúdo emocional podem influenciar a memória têm se destacado dentre as pesquisas sobre esse tema.

A idéia de que a ativação emocional pode melhorar a consolidação da memória tem sido muito discutida nos últimos anos. Eventos com conteúdo emocional causam liberação de adrenalina e aumento nos níveis de glicocorticóides circulantes, os quais são conhecidos por modular a memória.

Testes de memória com caráter aversivo/emocional já foram demonstrados sensíveis a neuromoduladores e hormônios relacionados ao aspecto emocional que governa a fase de treino desses testes. Contudo, pouco se sabe a respeito da influência da adrenalina sobre tipos de memória que não são diretamente moduladas pelos hormônios liberados em situações de estresse, nem que regiões cerebrais estão classicamente envolvidas na modulação da memória de conteúdo emocional.

A memória, uma das mais importantes funções cognitivas do ser humano, pode ser entendida como a incrível habilidade que possuímos de armazenar informações e conhecimentos sobre nós mesmos e o mundo que nos cerca. Ela é a base para o desenvolvimento da linguagem, do reconhecimento das pessoas e dos objetos que encontramos todos os dias, para sabermos quem somos e para termos a consciência da continuidade de nossas vidas. Sem a memória, a cada dia, ou a cada momento, estaríamos começando uma nova vida, sem podermos nos valer do que aprendemos anteriormente (Yassuda, 2002).

Em relação ao conteúdo, as memórias podem ser declarativas ou procedurais. As memórias procedurais são aquelas relacionadas às capacidades/habilidades motoras, ou sensoriais (Izquierdo, 2002).

As memórias que registram fatos, eventos, ou conhecimento são chamadas declarativas, porque nós, seres humanos, podemos declarar que existimos e podemos relatar como as adquirimos. Entre elas, as referentes a eventos aos quais presenciamos ou dos quais participamos são denominadas episódicas e, ainda, as de conhecimentos gerais são conhecidas como semânticas (Izquierdo, 2002), sendo que esta última abrange a memória do significado das palavras e é utilizada quando envolve conceitos, ou seja, quando aprendemos que a capital do Brasil é Brasília, por exemplo.

A formação da memória declarativa depende de um sistema de estruturas anatomicamente relacionadas no lobo medial temporal. Em humanos a memória declarativa dá suporte à capacidade de relembrar fatos e eventos e pode ser contrastada com o acúmulo das habilidades não declarativas de memória: hábitos e habilidades, formas simples de condicionamento, entre outros (Manns et al., 2003).

Um dos exemplos mais profundamente estudados da memória declarativa é a memória de reconhecimento (neutra, ou seja, não causadora de estresse nos animais), que é a capacidade de julgar um item recentemente encontrado como familiar. A capacidade da memória de reconhecimento tem sido também muito bem documentada em camundongos, ratos e macacos, assim como em humanos (Manns et al., 2003).

A memória de reconhecimento em seres humanos consiste de dois componentes: um episódico, que diz respeito à habilidade de lembrar do episódio (situação) no qual um objeto foi introduzido (objeto novo), e um componente familiar, que se relaciona com a habilidade de reconhecer um objeto como já conhecido (ou familiar), mas sem a necessidade da lembrança do próprio episódio (Manns et al., 2003).

Experimentalmente, podemos estudar a memória de reconhecimento em roedores através da tarefa de reconhecimento do objeto novo, a qual se baseia na tendência espontânea dos roedores de explorar um objeto novo. Tem sido proposto que essa tarefa apresenta analogia com testes de memória de reconhecimento que são amplamente utilizados em seres humanos para caracterizar síndromes amnésicas, pois fornecem um índice acurado do grau de severidade geral de prejuízos da memória declarativa (Dix & Aggleton, 1999; Reed & Squire, 1997).

Durante e imediatamente após situações emocionalmente intensas ou estressantes, vários sistemas neuro-humorais são ativados, incluindo a liberação de inúmeros hormônios (Quevedo et al., 2003), como é o caso da adrenalina, citada anteriormente. Foi observado que pacientes que sofrem da Síndrome do Estresse Pós-Traumático apresentam níveis circulantes aumentados de adrenalina (Yehuda, 2002). Interessantemente, estes pacientes apresentam uma inabilidade de apagar memórias de certos eventos traumáticos (van Praag, 2004).

A premissa de que eventos com caráter emocional são lembrados de forma mais marcante tem sido especialmente discutida. Portanto, é de extrema importância o aprofundamento dos estudos sobre quais são os tipos de memória que podem ser influenciados pela ação dos hormônios do estresse; em que fase da formação da memória esses hormônios atuam e de que forma (positiva ou negativa) se dá essa modulação.

Nesse contexto, analisamos os efeitos da adrenalina sobre a consolidação da memória de reconhecimento através da tarefa de reconhecimento do objeto novo em ratos.

3. OBJETIVOS

Avaliar os efeitos de manipulações farmacológicas do sistema adrenérgico sobre a consolidação da memória de reconhecimento em ratos.

3.1 OBJETIVOS ESPECÍFICOS

Avaliar o efeito do tratamento agudo com salina (grupo controle) ou adrenalina (i.p.) sobre a memória de curta e de longa duração na tarefa de reconhecimento do objeto novo.

Avaliar o efeito do tratamento agudo com salina (grupo controle) ou propranolol (i.p.) a memória de curta e de longa duração na tarefa de reconhecimento do objeto novo;

Verificar se a administração prévia de propranolol bloquearia os efeitos da adrenalina.

4. ARTIGO

Neurobiology of Learning and Memory

Research article

Adrenergic enhancement of consolidation of object recognition memory

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Abstract

Extensive evidence indicates that epinephrine (EPI) modulates memory consolidation for emotionally arousing tasks in animals and human subjects. However, previous studies have not examined the effects of EPI on consolidation of recognition memory. Here we report that systemic administration of EPI enhances consolidation of memory for a novel object recognition (NOR) task under different training conditions. Control male rats given a systemic injection of saline (0.9% NaCl) immediately after NOR training showed significant memory retention when tested at 1.5 or 24, but not 96 h after training. In contrast, rats given a posttraining injection of EPI showed significant retention of NOR at all delays. In a second experiment using a different training condition, rats treated with EPI, but not SAL-treated animals, showed significant NOR retention at both 1.5 and 24-h delays. We next showed that the EPI-induced enhancement of retention tested at 96 h after training was prevented by pretraining systemic administration of the β-adrenoceptor antagonist propranolol. The findings suggest that, as previously observed in experiments using aversively-motivated tasks, epinephrine modulates consolidation of recognition memory and that the effects require activation of β-adrenoceptors.

Keywords: Epinephrine; Adrenoceptors; Recognition memory; Memory consolidation

1. Introduction

Extensive evidence indicates that adrenal stress hormones, namely epinephrine (EPI) and glucocorticoids, modulate consolidation of emotionally-motivated memory in animals and human subjects (for reviews, see Cahill & McGaugh, 1998; McGaugh, 1983; 2004; McGaugh, Cahill, & Roozendaal, 1996; McGaugh & Roozendaal, 2002). In rodent models, the kind of stimulation typically used in learning experiments induces release of endogenous EPI (McCarty & Gold, 1981; McGaugh et al., 1996), and systemic administration of EPI shortly after training enhances consolidation of memory for arousing tasks (McGaugh, 1983; McGaugh et al., 1996; Nordby, Torras-Garcia, Portell-Cortes, & Costa-Miserachs, 2006). The memory-enhancing effects of peripheral administration of EPI require release of norepinephrine and activation of β-adrenoceptors in brain areas including the basolateral amygdala (BLA) (Liang, Juler, & McGaugh, 1986; McGaugh et al., 1996; McGaugh & Roozendaal, 2002). Together, these findings strongly indicate that endogenous EPI released during learning modulate the formation of long-lasting memories for arousing events (McGaugh, 1983; 2004; McGaugh et al., 1996; McGaugh & Roozendaal, 2002).

In contrast to the extensive evidence available from studies in emotionally motivated tasks, the role of the adrenergic system in modulating memory for tasks in which learning occurs under conditions of low arousal remains poorly understood. In the present study we investigated the effects of EPI on consolidation of memory for a novel object recognition (NOR) task. NOR training relies on a rodent's spontaneous tendency to explore a novel object more than a familiar one. During training for this task, rats or mice are presented with two identical or different novel objects, which they explore for some time. When animals are presented at a retention test trial carried out after training with two different objects, one of which was presented previously during training ("familiar"), and the other of which is novel,

animals that remember the familiar object will spend more time exploring the novel one (Ennaceur & Delacour, 1988; Steckler, Drinkenburg, Sahgal, & Aggleton, 1998). Since no explicit rewarding or aversive stimulation is used during training, NOR is considered to be a poorly motivated task involving low levels of arousal when compared to aversively-motivated tasks. Two recent studies evaluating the role of adrenal stress hormones on consolidation of NOR have indicated that corticosterone influences memory for NOR only when an experimental condition in which the level of experimental arousal associated with training was higher (Okuda, Roozendaal, & McGaugh, 2004; Rozendaal, Okuda, Van der Zee, & McGaugh, 2006). However, previous studies have not evaluated whether consolidation of memory for NOR can be affected by peripheral EPI. To address this issue, EPI was administered systemically immediately after NOR training to rats that were exposed to two identical objects during training and given retention test trials at different posttraining delays. A second experiment examined the effects of posttraining administration of EPI on memory for NOR training in rats exposed to two different objects during training (an experimental condition in which control rats showed no significant retention 1 day after training). To examine whether the effects of EPI depend upon β-adrenoceptors, a third experiment evaluated the effect of pretraining administration of propranolol on EPI-induced enhancement of consolidation.

2. Materials and methods

2.1 Subjects

Adult male Wistar rats (age: 60 days at the time of arrival) were used as experimental subjects. Animals were housed five to a cage with food and water available *ad libitum*, and were maintained on a 12-h light/dark cycle (lights on at 7:00 h). All behavioral procedures

were conducted between 9:00 and 16:00 h. All experimental procedures were performed in accordance with the NIH Guide for Care and Use of Laboratory Animals (NIH publication No. 80-23 revised 1996), and were approved by the Ethics Committee of the Pontifical Catholic University.

2.2 Drug administration

In Experiments I and II, animals received an intraperitoneal (i.p.) injection of either saline solution (SAL, NaCl 0,9%) or epinephrine (EPI, 2.5; 25 or 250 μg/kg) immediately after NOR training. In Experiment III, an i.p. injection of propranolol (2 mg/kg, PROP) was given 15 minutes prior NOR training, followed by an i.p. injection of SAL or EPI (25 μg/kg) immediately after training. Drugs were purchased from Sigma-Aldrich, São Paulo, Brazil, and were dissolved in SAL in a 1.0 ml/kg injection volume. Drug doses were chosen on the basis of previous studies (Nordby et al., 2006; Roozendaal, de Quervain, Schelling, & McGaugh, 2004; Sternberg, Isaacs, Gold, & McGaugh, 1985).

2.3 Behavioral apparatus and procedures

For NOR training, rats were left to explore two objects in a training box to which they had been previously familiarized. The apparatus and procedures for the object recognition task have been described elsewhere (de Lima, Luft, Roesler, & Schröder, 2006; de Lima, et al., 2005a; de Lima, Polydoro, Laranja, Bonatto, Bromberg, Moreira, Dal-Pizzol, & Schröder, 2005b; Schröder, O'Dell, & Marshall, 2003). Briefly, the task took place in a 40 cm x 50 cm open field surrounded by 50 cm high walls, made of plywood with a frontal glass wall, with a floor covered with sawdust. All animals were given a habituation session where they were left

to freely explore the open field for 2 minutes. No objects were placed in the box during the habituation trial. All objects used in training and testing trials presented similar textures, colors, and sizes, but distinctive shapes. Between trials the objects were washed with 10% ethanol solution. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. NOR procedures were conducted in a dimly lit room in order to minimize the influence of contextual information.

2.3.1. Experiment I

Twenty-four hours after habituation, training was conducted by placing individual rats for 2 minutes into the field, in which two identical objects (objects A1 and A2; Duplo Lego toys) were positioned in two adjacent corners, 10 cm from the walls. In a short-term retention test given 1.5 hours after training, the rats explored the open field for 2 minutes in the presence of one familiar (A) and one novel (B) object. In a long-term retention test given 24 hours after training, rats explored the field for 2 minutes in the presence of familiar (A) and a novel (C) object. An additional retention test was performed 96 hours after training, where rats again explored the field for 2 min in the presence of the familiar (A) and a novel (D) object. The same groups of animals were submitted to NOR testing trials at 1.5, 24, and 96 hours after training.

2.3.2. Experiment II

In Experiment II, we assessed the effects of posttraining administration of EPI in rats exposed to two different objects during training. In this and the following experiment, training duration was increased. Twenty-four hours after habituation, training was conducted by placing individual rats for 5 minutes into the field, in which two distinct objects (A and B) were positioned in two adjacent corners, 10 cm from the walls. In a short-term retention test

given 1.5 hours after training, the rats explored the open field for 5 minutes in the presence of one familiar (either A or B) and one novel object. In a long-term retention test given 24 hours after training, the rats explored the open field for 5 minutes in the presence of one familiar (either A or B) and one novel object. Short- (1.5 h) and long- (24 h) retention tests were conducted in two separate groups of rats.

2.3.2. Experiment III

In Experiment III, training and testing trials were conducted as described for Experiment I except that trial duration was 5 min.

Data for all three experiments were analysed by calculating a recognition index for each animal, expressed as the ratio $T_B/(T_A + T_B)$ [T_A = time spent exploring the familiar object A; T_B = time spent exploring the novel object B] (de Lima et al., 2005a; b; 2006; Schröder et al., 2003).

2.4 Statistical analysis

Data for recognition indexes were expressed as median (interquartile ranges). Comparisons among groups were performed using a Kruskal-Wallis analysis of variance followed by Mann-Whitney *U*-tests when necessary. Total exploration time during object recognition training is expressed as mean $\pm$ SEM, and comparison among groups was done using an analysis of variance (ANOVA) (de Lima et al., 2005a; b; 2006). In all comparisons, $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Experiment I

We first examined the effects of posttraining administration of EPI on NOR consolidation using a training condition in which rats were exposed to two identical objects for a brief period and tested for retention at several posttraining delays. SAL or EPI was given immediately after training. There was no significant difference among groups in the total time spent exploring both objects during training ($F(3,31) = 0.54$, $P = 0.66$; overall mean $\pm$ SEM time spent exploring both objects (s) was 8.16 ± 0.84). Results for exploratory preferences are shown in Fig. 1. Kruskall-Wallis analysis of variance revealed a significant difference in exploratory preference among groups in the 96-h retention test trial (df = 3, H = 16.73, $P < 0.001$), but not in the training (df = 3, H = 4.77, $P = 0.19$), 1.5-h retention (df = 3, H = 5.83, $P = 0.12$), or 24-h retention (df = 3, H = 1.07, $P = 0.78$) trials. Further analysis using Mann-Whitney tests showed that rats given EPI at either 25 or 250 μg/kg showed enhanced NOR retention when tested 96 h posttraining when compared to SAL-treated rats (both Ps < 0.01), whereas retention of animals given EPI at 2.5 μg/kg was similar to that in the SAL group ($P = 0.14$). The results indicate that posttraining administration of EPI induced a facilitation of long-term NOR retention tested at 96 h after training.

3.2. Experiment II

Using two different objects during NOR training might increase the difficulty of the task, thus preventing control animals from showing significant retention at a 24-h posttraining delay (Tang, Shimizu, Dube, Rampon, Kerchner, Zhuo, Liu, & Tsien, 1999). We thus

assessed the effects of posttraining administration of EPI on 1.5 and 24-h retention of NOR in rats exposed to two different objects during training. There were no significant differences among groups in the total time spent exploring both objects during training in animals tested at 1.5 ($F(3,34) = 1.82$, $P = 0.16$; overall mean ± SEM time spent exploring both objects (s) was 15.71 ± 0.75) or 24 h ($F(3,36) = 1.12$, $P = 0.35$; overall mean ± SEM time spent exploring both objects (s) was 14.36 ± 0.83) after training. In rats tested for retention at 1.5 h posttraining, there was a significant difference among groups in the retention test trial ($df = 3$, $H = 8.40$, $P < 0.0001$), but not in the training trial ($df = 3$, $H = 5.34$, $P = 0.15$). Mann-Whitney tests revealed that rats treated with any dose of EPI showed enhanced retention when compared to the SAL-treated group (all Ps < 0.0001) (Fig. 2A). In rats tested at 24 h after training, there was a significant difference among groups in the retention test trial ($df = 3$, $H = 7.72$, $P < 0.0001$), but not in the training trial ($df = 3$, $H = 7.07$, $P = 0.07$). Rats treated with any dose of EPI showed enhanced retention when compared to the SAL-treated group (all Ps < 0.0001) (Fig. 2B). The results show that, using a training condition in which rats were exposed to two different objects, animals treated with EPI, but not SAL-treated rats, showed significant NOR retention tested at 1.5 or 24 h after training, thus indicating that posttraining administration of EPI facilitated both formation of short-term memory and consolidation of long-term memory for NOR.

3.3. Experiment III

We next verified whether pretraining administration of the β-adrenoceptor antagonist propranolol would prevent the enhancing effect of posttraining EPI on NOR consolidation. Results are shown in Fig. 3. There was no significant difference among groups in the total time spent exploring both objects during training ($F(3,42) = 0.87$, $P = 0.47$; overall mean ±

SEM time spent exploring both objects (s) was 27.5 ± 2.1). Kruskal-Wallis analysis of variance revealed significant differences in exploratory preferences among groups in retention test trials carried out at 1.5 (df = 3, H = 8.41, $P < 0.05$), 24 (df = 3, H = 27.23, $P < 0.0001$), and 96 h (df = 3, H = 21.31, $P < 0.0001$) after training, but not in the training trial (df = 3, H = 2.57, $P = 0.46$). Further analysis revealed that the group given pretraining SAL and posttraining EPI showed a significant enhancement of 96-h retention when compared to the control group given pretraining SAL and posttraining SAL ($P < 0.0001$). Pretraining administration of PROP induced a significant impairment of retention at 24 h after training ($P < 0.0001$) but did not affect retention at the 1.5 and 96 h posttraining delays (Ps = 0.31 and 0.81 respectively). Importantly, pretraining administration of PROP prevented the EPI-induced enhancement of 96-h retention. There was no significant difference in the 96-h retention test performance between the control group given SAL both before and after training and the group treated with pretraining PROP and posttraining EPI ($P = 0.39$). The group given pretraining PROP and posttraining EPI showed a significantly impaired retention at both 1.5 ($P < 0.05$) and 24 h ($P < 0.01$) after training. The main finding of this experiment was that pretraining administration of PROP prevented the enhancing effect of EPI on NOR memory tested 96 h posttraining.

4. Discussion

The main findings of the present study can be summarized as follows: (1) rats treated with a posttraining systemic injection of EPI showed enhanced retention of memory of a NOR task 96 h after training when compared to control animals; (2) when rats were trained under a condition in which the difficulty of the task was increased (i.e., using two different objects during training), rats treated with a posttraining injection of EPI showed enhanced retention at

1.5 and 24 h after training in comparison to the control group; and (3) pretraining systemic administration of the β-adrenoceptor antagonist PROP prevented the EPI-induced enhancement of NOR retention. Together, these findings suggest that the adrenergic system modulates consolidation of recognition memory through a mechanism dependent on β-adrenoceptors. In addition, pretraining administration of PROP produced an impairment of retention tested 24 h posttraining, and rats given pretraining PROP followed by posttraining EPI showed a mild, yet significant, impairment of 1.5- 24-h retention. The impairing effect observed 1.5 h after training in rats treated with both PROP and EPI might be related to an effect of PROP and/or EPI on performance or retrieval since animals were tested around 1.5 h after drug injections.

Since Gold & van Buskirk (1975) provided the first finding that posttraining administration of EPI enhanced memory retention, extensive evidence has supported the view that peripheral release of EPI regulates consolidation of memory for emotionally-motivated tasks. Thus, it has long been known that systemic administration of EPI to rats modulates consolidation of memory for arousing tasks, and the modulatory effects of EPI are blocked by propranolol (Gold & van Buskirk, 1975; Izquierdo & Dias, 1983; Sternberg et al., 1985). Proposed mechanisms mediating the enhancing effects of EPI on consolidation include release of hepatic glucose and activation of β-adrenoceptors on vagal afferents, which ultimately would lead to noradrenergic activation in brain areas including the basolateral amygdala (for reviews, see McGaugh & Roozendaal, 2002; McGaugh et al., 1996).

The role of the adrenergic system in modulating tasks involving lower levels of emotional arousal is less clear. In the NOR task, two recent studies indicated that memory consolidation is regulated by corticosterone (which, like EPI, is an adrenal stress hormone regulating memory consolidation in rats) through a mechanism dependent on noradrenergic activation only under a condition (i.e., when rats were not habituated to the experimental

context) in which training stimulated novelty-induced emotional arousal (Okuda et al., 2004; Roozendaal et al., 2006). The present findings indicate that, in animals given a single session of habituation to the training context, after training EPI facilitated memory consolidation for the NOR task so that memory retention measured either at 24 (Experiment II) or 96 h (Experiment I) after training was increased in comparison to control animals. The use of a longer posttraining delay (i.e., 96 h) to measure retention in Experiment I, as well as the use of two different objects during training in Experiment II, enabled us to reveal the memory-enhancing effect of EPI under conditions in which control animals did not show high retention scores. These findings indicate that, as with tasks involving strong aversive stimulation (such as a footshock), peripheral release of EPI might modulate consolidation of memory for the NOR task through a mechanism dependent on β-adrenoceptors.

Further studies are required to investigate the brain systems and neurochemical mechanisms mediating the adrenergic modulation of NOR consolidation. NOR is a nonspatial memory task based on the ability to recognize a familiar object and the natural tendency to explore a novel one (Ennaceur & Delacour, 1988). Evidence indicates that brain areas involved in mediating or modulating NOR might include the dorsal hippocampus (Broadbent, Squire, & Clark, 2004; Clark, Zola, & Squire, 2000; de Lima et al., 2006;) and, at least under some experimental conditions, the basolateral amygdala (Roozendaal et al., 2006). We and others have proposed that molecular mechanisms involved in formation of NOR memory might include *N*-methyl-D-aspartate (NMDA) glutamate receptors (Baker & Kim, 2002; de Lima et al., 2005a; Puma & Bizot, 1998; Rampon, Tang, Goodhouse, Shimizu, Kyin, & Tsien, 2000; Sargolini, Roullet, Oliverio, & Mele, 2003; Tang et al., 1999). A number of studies suggest that the noradrenergic system modulation of memory consolidation depends on NMDA receptors (for a review, see McGaugh & Roozendaal, 2002). Thus, noradrenergic activation leads to facilitation of NMDA receptor-mediated synaptic plasticity in the

amygdala (Huang, Tsai, & Gean, 1994; Wang, Huang, Hsu, Tsai, Huang, & Gean, 1996), the memory-impairing effects of propranolol can be reversed by glutamate (Lennartz, Helems, Mook, & Gold, 1996), and NMDA receptor blockade prevents the enhancing effect of posttraining administration of EPI on memory consolidation (Roesler, Vianna, de Paris, & Quevedo, 1999). These findings suggest that an interesting possibility worth examining is whether the adrenergic modulation of NOR consolidation requires NMDA receptors.

In summary, the present study shows that posttraining systemic administration of EPI in rats can facilitate consolidation of memory for the NOR task through a mechanism dependent on activation of β-adrenoceptors. To our knowledge, this is the first evidence suggesting that the adrenergic system, and possibly peripheral release of EPI, modulate consolidation of object recognition memory.

Acknowledgements

This research was supported by FAPERGS grant 05/2179.8 to N.S.

References

Baker, K.B., & Kim, J.J. (2002). Effects of stress and hippocampal NMDA receptor antagonism on recognition memory in rats. *Learning and Memory, 9*(2), 58-65.

Broadbent, N.J., Squire, L.R., & Clark., R.E. (2004). Spatial memory, recognition memory, and the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America, 101*(40), 14515-14520.

Cahill, L., & McGaugh, J. L. (1998). Mechanisms of emotional arousal and lasting declarative memory. *Trends in Neurosciences, 21*(7), 294-9.

Clark, R.E., Zola, S.M., & Squire, L.R. (2000). Impaired recognition memory in rats after damage to the hippocampus. *Journal of Neuroscience, 20*(23), 8853-8860.

de Lima, M. N. M., Laranja, D. C., Bromberg, E., Roesler, R., & Schröder, N. (2005a). Pre- or post-training administration of the NMDA receptor blocker MK-801 impairs object recognition memory in rats. *Behavioral Brain Research, 156*(1), 139-43.

de Lima, M. N., Luft, T., Roesler, R., & Schröder N. (2006). Temporary inactivation reveals an essential role of the dorsal hippocampus in consolidation of object recognition memory. *Neuroscience Letters, 405*(1-2), 142-6.

de Lima, M. N., Polydoro, M., Laranja, D. C., Bonatto, F., Bromberg, E., Moreira, J. C., Dal-Pizzol, F., & Schröder, N. (2005b). Recognition memory impairment and brain oxidative stress induced by postnatal iron administration. *European Journal of Neuroscience, 21*(9), 2521-8.

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioral Brain Research, 31*(1), 47-59.

Gold, P.E., & Van Buskirk, R.B. (1975). Facilitation of time-dependent memory processes with posttrial epinephrine injections. *Behavioral Biology, 13*(2):145-153.

Huang, C.C., Tsai, J.J., & Gean, P.W. (1994). Actions of isoproterenol on amygdalar neurons in vitro. *Chinese Journal of Physiology, 37*(2), 73-78.

Izquierdo, I., & Dias, R.D. (1983). The influence of adrenergic receptor antagonists on the amnestic and antiamnestic actions of adrenaline and tyramine. *Psychopharmacology, 80*(2), 181-183.

Lennartz, R.C., Hellems, K.L., Mook, E.R., & Gold, P.E. (1996). Inhibitory avoidance impairments induced by intra-amygdala propranolol are reversed by glutamate but not glucose. *Behavioral Neuroscience, 110*(5), 1033-1039.

Liang, K. C., Juler, R. G., & McGaugh, J. L. (1986). Modulating effects of posttraining epinephrine on memory: involvement of the amygdala noradrenergic system. *Brain Research, 368*(1), 125-33.

McCarty, R., & Gold, P. E. (1981). Plasma catecholamines: effects of footshock level and hormonal modulators of memory storage. *Hormones and Behavior, 15*, 168-82.

McGaugh, J.L. (1983). Hormonal influences on memory. *Annual Review of Psychology, 34*, 297-323.

McGaugh, J.L (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annual Review of Neuroscience, 27*, 1-28.

McGaugh, J. L., Cahill, L., & Roozendaal, B. (1996). Involvement of the amygdala in memory storage: interaction with other brain systems. *Proceedings of the National Academy of Sciences of the United States of America, 93*(24), 13508-14.

McGaugh, J. L., & Roozendaal, B. (2002). Role of adrenal stress hormones in forming lasting memories in the brain. *Current Opinion in Neurobiology, 12*, 205-10.

Nordby, T., Torras-Garcia, M., Portell-Cortes, I., & Costa-Miserachs, D. (2006). Posttraining epinephrine treatment reduces the need for extensive training. *Physiology and Behavior, 89*(5), 718-23.

Okuda, S., Roozendaal, B., & McGaugh, J.L. (2004). Glucocorticoid effects on object recognition memory require training-associated emotional arousal. *Proceedings of the National Academy of Sciences of the United States of America, 101*(3):853-858.

Puma, C., & Bizot, J.C. (1998). Intraseptal infusions of a low dose of AP5, a NMDA receptor antagonist, improves memory in an object recognition task in rats. *Neuroscience Letters, 248*(3), 183-186.

Rampon, C., Tang, Y.P., Goodhouse, J., Shimizu, E., Kyin, M., & Tsien, J.Z. (2000). Enrichment induces structural changes and recovery from nonspatial memory deficits in CA1 NMDAR1-knockout mice. *Nature Neuroscience, 3*(3), 238-244.

Roesler, R., Vianna, M.R., de-Paris, F., & Quevedo, J. (1999). Memory-enhancing treatments do not reverse the impairment of inhibitory avoidance retention induced by NMDA receptor blockade. *Neurobiology of Learning and Memory, 72*(3), 252-258.

Roozendaal, B., de Quervain, D.J., Schelling, G., & McGaugh, J.L. (2004). A systemically administered beta-adrenoceptor antagonist blocks corticosterone-induced impairment of contextual memory retrieval in rats. *Neurobiology of Learning and Memory, 81*(2), 150-154.

Roozendaal, B., Okuda, S., Van der Zee, E.A., & McGaugh, J.L. (2006). Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America, 103*(17):6741-6746.

Sargolini, F., Roullet, P., Oliverio, A., & Mele A. (2003). Effects of intra-accumbens focal administrations of glutamate antagonists on object recognition memory in mice. *Behavioural Brain Research, 138*(2), 153-163.

Schröder, N., O'Dell, S. J., & Marshall, J. F. (2003). Neurotoxic methamphetamine regimen severely impairs recognition memory in rats. *Synapse, 49*(2), 89-96.

Steckler, T., Drinkenburg, W. H., Sahgal, A., & Aggleton, J. P. (1998). Recognition memory in rats--I. Concepts and classification. *Progress in Neurobiology, 54*(3), 289-311.

Sternberg, D.B., Isaacs, K.R., Gold, P.E., & McGaugh, J.L. (1985). Epinephrine facilitation of appetitive learning: attenuation with adrenergic receptor antagonists. *Behavioral and Neural Biology, 44*(3), 447-453.

Tang, Y.P., Shimizu, E., Dube, G.R., Rampon, C., Kerchner, G.A., Zhuo, M., Liu, G., & Tsien, J.Z. (1999). Genetic enhancement of learning and memory in mice. *Nature, 401*(6748), 63-69.

Wang, S.J., Huang, C.C., Hsu, K.S., Tsai, J.J., Huang, C.C., & Gean, P.W. (1996). Blockade of isoproterenol-induced synaptic potentiation by tetra-9-aminoacridine in the rat amygdala. *Neuroscience Letters, 214*(2-3), 87-90.

Legends for figures

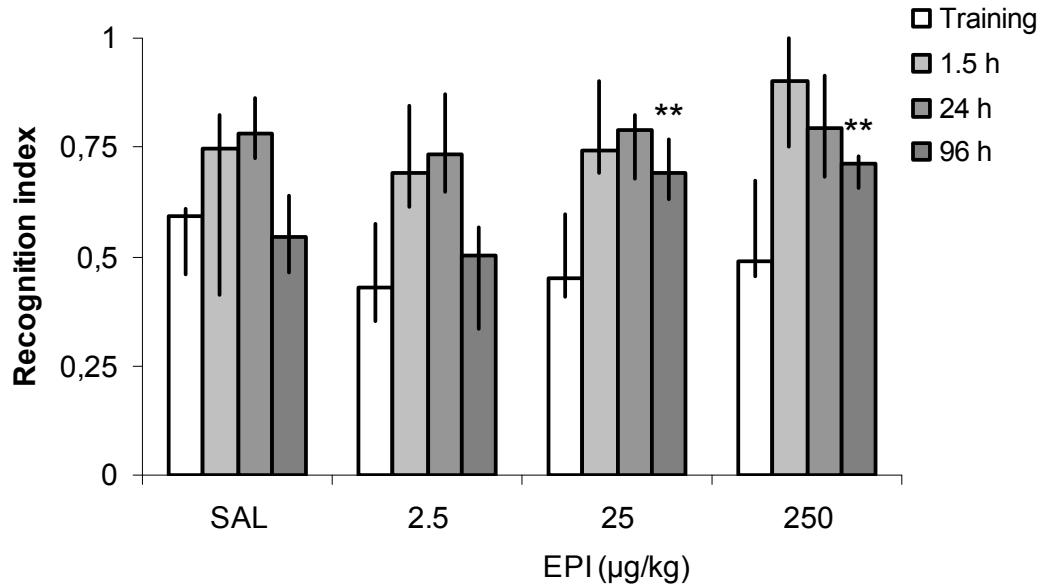
Fig. 1. Posttraining systemic administration of epinephrine (EPI) enhanced 96-h retention of memory for novel object recognition (NOR) in rats exposed to two identical objects during training. Rats received a single intraperitoneal (i.p.) injection of saline (SAL) or EPI (2.5; 25 or 250 μg/kg) immediately after training and were tested for retention at 1.5, 24, and 96 h after training. EPI administered in doses of 25 or 250 μg/kg significantly enhanced 96-h NOR memory. $^{**}P < 0.01$ compared with the SAL control group (N = 8-9 animals per group).

Fig. 2. Posttraining systemic administration of epinephrine (EPI) enhanced short- (1.5 h) and long-term (24 h) retention of memory for novel object recognition (NOR) in rats exposed to two different objects during training. Rats received a single intraperitoneal (i.p.) injection of saline (SAL) or EPI (2.5; 25 or 250 μg/kg) immediately after training and were tested for retention at 1.5 (**A**) or 24 h (**B**) after training. EPI administered at any of the three doses significantly enhanced 1.5- and 24-h retention of NOR memory. $\#P < 0.0001$ compared with the SAL control group (N = 9-10 animals per group).

Fig. 3. Pretraining administration of propranolol (PROP) prevents the enhancement of 96-h retention of novel object recognition (NOR) memory induced by posttraining administration of epinephrine (EPI). Rats received an intraperitoneal (i.p.) injection of saline (SAL) or PROP (2 mg/kg) 15 minutes before training and an i.p. injection of SAL or EPI (25 μg/kg) immediately after training. Two identical objects were used during training. Animals were tested for retention at 1.5, 24, and 96 h after training. EPI administration significantly enhanced 96-h retention of NOR memory. Pretraining administration of PROP prevented the EPI-induced memory enhancement. In addition, pretraining administration of PROP produced

a significant impairment of 24-h retention, and rats treated with pretraining PROP and posttraining EPI showed a significant impairment of both 1,5- and 24-h retention. *$P < 0.05$, **$P < 0.01$, and #$P < 0.0001$ compared with the control group treated with pretraining and posttraining injections of SAL (N = 10-11 animals per group).

Figure 1


Training
1.5 h
24 h
96 h
Recognition index
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0,75
0,5
0,25
0
**
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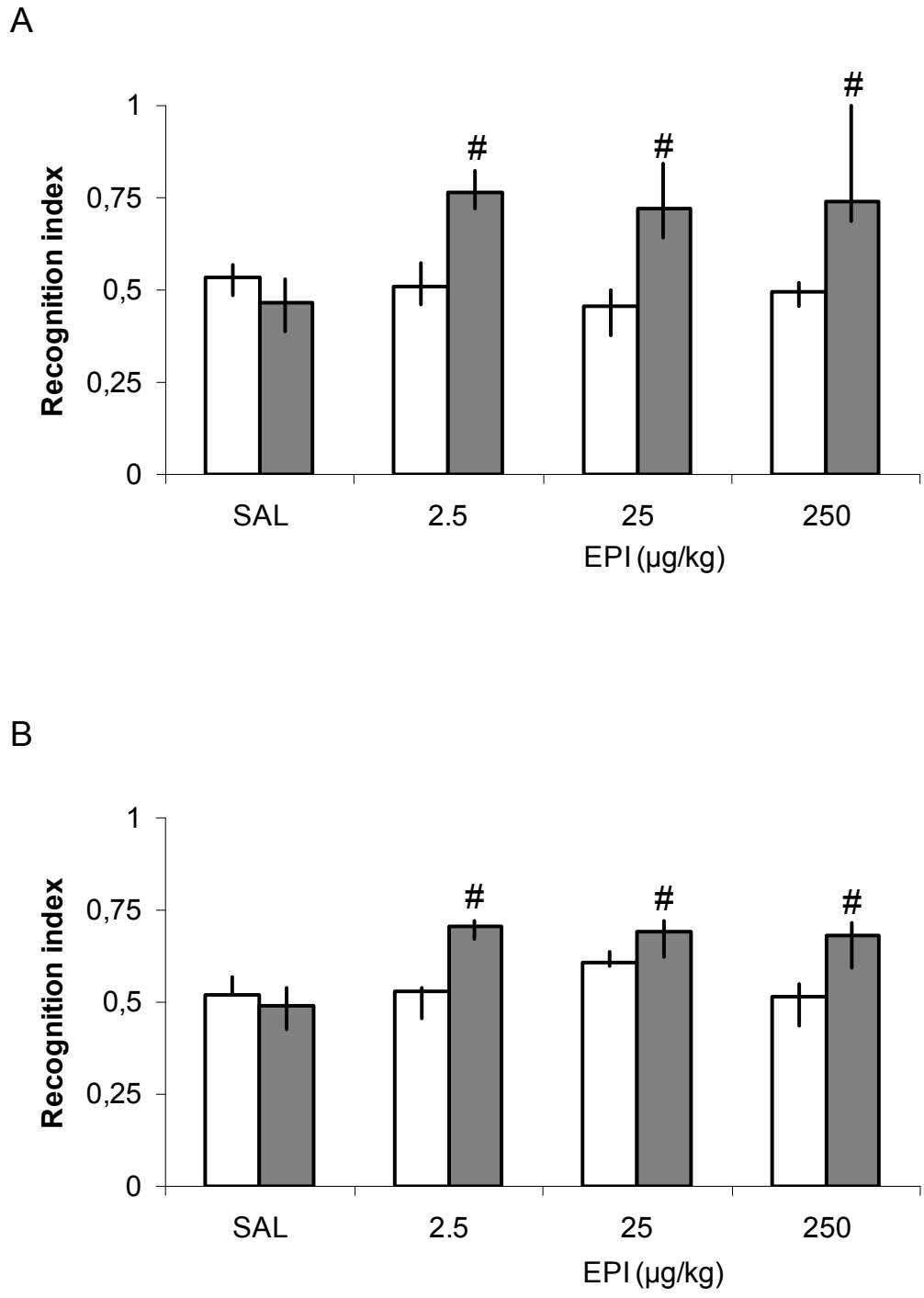
SAL
2.5
25
250
EPI (µg/kg)

Figure 2


A
Recognition index
1
0,75
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SAL
2.5
25
250
EPI (µg/kg)
B
Recognition index
1
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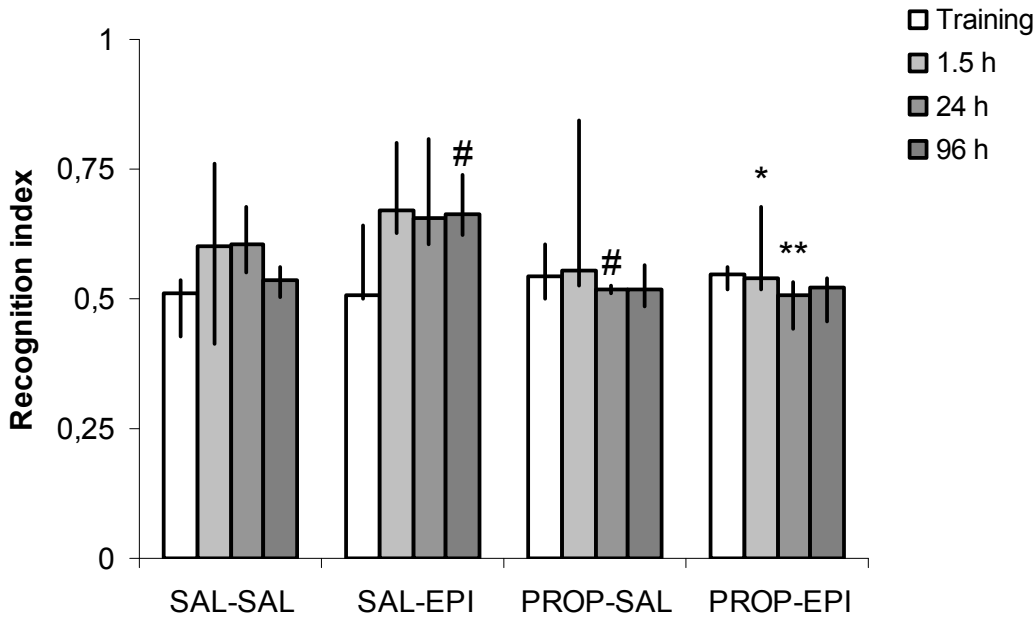
EPI (µg/kg)

Figure 3


Training
1.5 h
24 h
96 h
Recognition index
1
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SAL-SAL
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PROP-SAL
PROP-EPI

5. CONSIDERAÇÕES FINAIS

Com a realização deste trabalho foi possível constatar que os ratos tratados com injeção sistêmica pós-treino de adrenalina apresentaram melhora na retenção de memória na tarefa de reconhecimento do objeto novo 96 horas depois do treino quando comparados com os animais controles. Além disso, vimos que quando os ratos foram treinados em uma condição com um maior grau de dificuldade, na qual foram utilizados dois objetos diferentes durante o treino, os animais tratados com injeção pós-treino de adrenalina apresentaram maior retenção nos testes realizados 1,5 e 24 horas depois do treino em comparação ao grupo controle. Constatamos também que a administração sistêmica pré-treino de propranolol, um antagonista dos receptores β-adrenérgicos, inibiu a melhora na retenção da memória de reconhecimento do objeto novo induzida por adrenalina. Todas essas informações sugerem que o sistema adrenérgico modula a consolidação da memória de reconhecimento através de um mecanismo dependente dos receptores β-adrenérgicos. Ainda, a administração de propranolol pré-treino provocou um prejuízo no teste de retenção realizado 24 horas depois do treino e os ratos que receberam injeção pré-treino de propranolol seguida de uma injeção pós-treino de adrenalina também mostraram um leve, mas significativo, prejuízo na taxa de retenção quando testados 1,5 e 24 horas depois do treino. Esse efeito observado 1,5 horas depois do treino nos ratos tratados com propranolol e com adrenalina pode estar relacionado com o efeito do propranolol e/ou da adrenalina na performance ou evocação, já que os animais foram testados aproximadamente 1,5 horas depois das injeções.

Desde que Gold & van Buskirk (1975) forneceram o primeiro indício de que a administração pós-treino de adrenalina melhora a retenção da memória, muitas evidências têm dado suporte à idéia de que a liberação periférica de adrenalina regula a consolidação da memória para tarefas motivadas emocionalmente. Assim, é bem estabelecido que a

administração sistêmica de adrenalina em ratos modula a consolidação da memória para tarefas com caráter emocional e que os efeitos modulatórios da adrenalina são bloqueados pelo propranolol (Gold & van Buskirk, 1975; Izquierdo & Dias, 1983; Sternberg et al., 1985). Dentre os mecanismos propostos envolvidos na melhora da consolidação induzida pela adrenalina, se encontram a liberação de glicose hepática e a ativação dos receptores β-adrenérgicos nas aferências vagais, as quais podem levar a uma ativação noradrenérgica em áreas do cérebro incluindo a amígdala basolateral (McGaugh & Roozendaal, 2002; McGaugh et al., 1996). A amígdala, por sua vez, modula a consolidação da memória em outras regiões cerebrais, como o hipocampo, por exemplo. Portanto, se sugere que a amígdala seja sensível às mudanças nos níveis periféricos de adrenalina, ou seja, a amígdala pode estar relacionada com o efeito modulador da adrenalina sobre a memória, de acordo com modelo proposto por McGaugh, J. L. (2004), o qual pode ser observado na figura 4.

O papel do sistema adrenérgico na modulação de tarefas com baixo nível de envolvimento emocional ainda não é clara. Na tarefa de memória de reconhecimento do objeto novo, dois estudos recentes indicaram que a consolidação da memória é regulada por corticosterona (o qual, assim como a adrenalina é um hormônio adrenal do estresse que regula a consolidação da memória em ratos) através de um mecanismo dependente da ativação noradrenérgica somente sob uma condição na qual os ratos não estão habituados ao contexto experimental, sugerindo que o treino estimulado pela novidade induz uma situação com caráter emocional (Okuda et al., 2004; Roozendaal et al., 2006). Nossos dados indicam que, em animais que foram submetidos a uma única sessão de habituação ao contexto do treino, adrenalina administrada após o treino, facilitou a consolidação da memória para tarefa de reconhecimento do objeto novo de modo que a retenção da memória medida tanto 24 horas (Experimento II) quanto 96 horas (Experimento I) após o treino estava aumentada em comparação aos animais controles. O uso de um intervalo pós-treino mais longo (por

exemplo, 96 horas) para medir a retenção no Experimento I, assim como o uso de dois objetos diferentes durante a sessão de treino no Experimento II, nos permitiu revelar o aumento da memória causado pela adrenalina sob condições nas quais os animais controles não mostraram altas taxas de retenção. Esses achados indicam que, assim como nas tarefas envolvendo estimulação aversiva, como a esquiva inibitória, por exemplo, a liberação periférica de adrenalina pode modular a consolidação da memória para tarefa de reconhecimento do objeto novo através de um mecanismo dependente de receptores β-adrenérgicos.

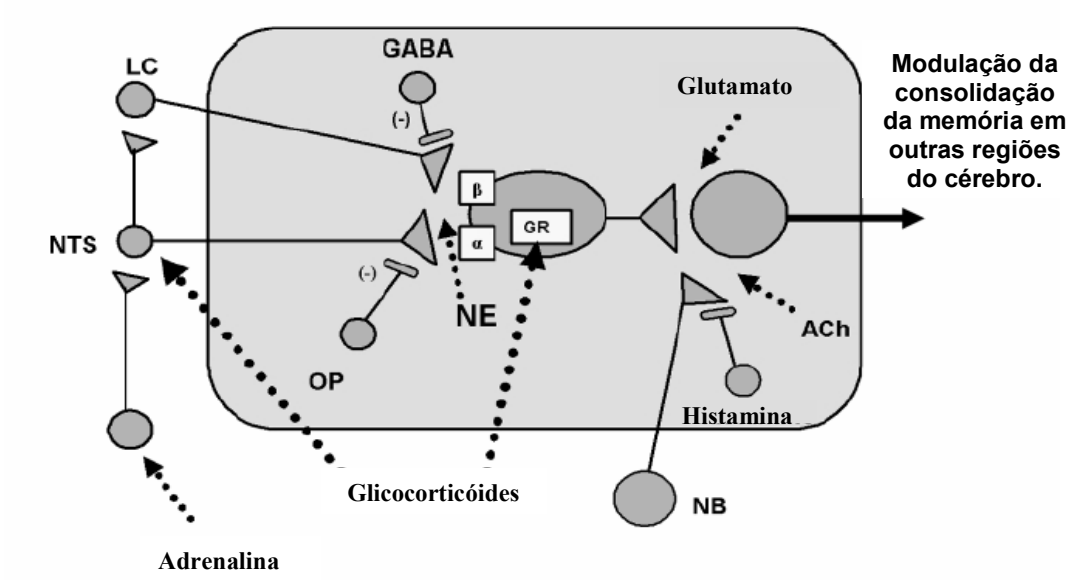

Figura 4. Esquema representativo da modulação da memória através do complexo basolateral da amígdala. Através da ilustração podemos ver a liberação de noradrenalina (NA) dentro da amídala é crítica para as influências da modulação da memória. A adrenalina liberada pela medula adrenal ativa receptores no nervo vago ascendente que projeta ao núcleo do trato solitário (NTS), o qual envia projeções noradrenérgicas à amídala assim como o locus coeruleus (LC). Peptídeos opióides (OP) e o ácido gama-amino-butírico (GABA) inibem a liberação de NA. Corticosterona liberada no córtex adrenal ativa receptores de glicocorticóides no NTS, no complexo basolateral da amídala (BLA) e em outros lugares no cérebro. No BLA, a corticosterona interage com a ativação β-adrenérgica. A ativação glutamatérgica e colinérgica no núcleo basal (NB) ocorre na etapa posterior da ativação noradrenérgica. Ativação de receptores de histamina regula liberação de acetilcolina (ACh). Estas influências modulatórias convergem em projeções da amídala e na ativação de outras regiões do cérebro envolvidas na consolidação de memória. *McGaugh, J. L. (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. Annual Review of Neuroscience, 27, 1-28.*

Estudos posteriores são necessários para investigar os sistemas cerebrais e os mecanismos neuroquímicos envolvidos na modulação adrenérgica da consolidação da tarefa de reconhecimento do objeto novo. Esta é uma tarefa de memória não espacial baseada na habilidade de reconhecer um objeto familiar e na tendência natural de explorar um objeto novo (Ennaceur & Delacour, 1988). Evidências indicam que áreas do cérebro envolvidas na modulação da memória de reconhecimento do objeto novo podem incluir o hipocampo dorsal (Broadbent, Squire, & Clark, 2004; Clark, Zola, & Squire, 2000; de Lima et al., 2006;) e, pelo menos sob algumas condições experimentais, a amígdala basolateral (Roozendaal et al., 2006). Tem sido proposto que mecanismos moleculares envolvidos na formação da memória de reconhecimento do objeto novo podem incluir receptores de glutamato N-metil-D-aspartato (NMDA) (Baker & Kim, 2002; de Lima et al., 2005a; Puma & Bizot, 1998; Rampon, Tang, Goodhouse, Shimizu, Kyin, & Tsien, 2000; Sargolini, Roullet, Oliverio, & Mele, 2003; Tang et al., 1999). Alguns estudos sugerem que a modulação da consolidação da memória pelo sistema noradrenérgico depende dos receptores NMDA (McGaugh & Roozendaal, 2002). Assim, a ativação noradrenérgica causa uma facilitação da plasticidade sináptica mediada pelos receptors NMDA na amígdala (Huang, Tsai, & Gean, 1994; Wang, Huang, Hsu, Tsai, Huang, & Gean, 1996), os efeitos prejudiciais do propranolol na memória podem ser revertidos por glutamato (Lennartz, Helems, Mook, & Gold, 1996), e o bloqueio dos receptores NMDA impede a melhora na consolidação da memória causada pela administração de adrenalina pós-treino (Roesler, Vianna, de Paris & Quevedo, 1999). Todas essas informações sugerem a importância de se examinar se a modulação adrenérgica da consolidação da memória de reconhecimento necessita de receptores NMDA.

Em suma, este estudo mostra que a administração sistêmica de adrenalina pós-treino em ratos pode facilitar a consolidação da memória para a tarefa de reconhecimento do objeto novo através de um mecanismo dependente da ativação de receptores β-adrenérgicos. Essa é a

primeira evidência sugerindo que o sistema adrenérgico, e possivelmente a liberação periférica de adrenalina, modula a consolidação da memória de reconhecimento do objeto.

A conclusão deste trabalho nos permite sugerir outros estudos como, por exemplo, a análise dos efeitos da adrenalina sobre a memória de reconhecimento do objeto novo, quando administrada após outros intervalos de tempo, e não imediatamente após a sessão de treino, como realizado no presente trabalho. Podem ainda ser analisados os efeitos de injeções intra-cerebrais tanto de adrenalina quanto de propranolol em regiões comprovadamente envolvidas com a consolidação deste tipo de memória, como o hipocampo a amígdala, utilizando nosso protocolo da tarefa de reconhecimento do objeto novo no qual os ratos são habituados ao contexto.

6. REFERENCIAS

Baker, K.B., & Kim, J.J. (2002). Effects of stress and hippocampal NMDA receptor antagonism on recognition memory in rats. *Learning and Memory, 9*(2), 58-65.

Broadbent, N.J., Squire, L.R., & Clark., R.E. (2004). Spatial memory, recognition memory, and the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America, 101*(40), 14515-14520.

Clark, R.E., Zola, S.M., & Squire, L.R. (2000). Impaired recognition memory in rats after damage to the hippocampus. *Journal of Neuroscience, 20*(23), 8853-8860.

de Lima, M. N. M., Laranja, D. C., Bromberg, E., Roesler, R., & Schröder, N. (2005a). Pre- or post-training administration of the NMDA receptor blocker MK-801 impairs object recognition memory in rats. *Behavioral Brain Research, 156*(1), 139-43.

de Lima, M. N., Luft, T., Roesler, R., & Schröder N. (2006). Temporary inactivation reveals an essential role of the dorsal hippocampus in consolidation of object recognition memory. *Neuroscience Letters, 405*(1-2), 142-6.

Dix, S. L., & Aggleton, J. P. (1999). Extending the spontaneous preference test of recognition: evidence of object-location and object location and object-context recognition. *Behavioural Brain Research*, *99*, 191-200.

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioral Brain Research, 31*(1), 47-59.

Gold, P.E., & Van Buskirk, R.B. (1975). Facilitation of time-dependent memory processes with posttrial epinephrine injections. *Behavioral Biology, 13*(2):145-153.

Huang, C.C., Tsai, J.J., & Gean, P.W. (1994). Actions of isoproterenol on amygdalar neurons in vitro. *Chinese Journal of Physiology, 37*(2), 73-78.

Izquierdo, I., 2002. Memória. Porto Alegre: Artes Médicas; p. 19-30.

Izquierdo, I., & Dias, R.D. (1983). The influence of adrenergic receptor antagonists on the amnestic and antiamnestic actions of adrenaline and tyramine. *Psychopharmacology, 80*(2), 181-183.

Lennartz, R.C., Hellems, K.L., Mook, E.R., & Gold, P.E. (1996). Inhibitory avoidance impairments induced by intra-amygdala propranolol are reversed by glutamate but not glucose. *Behavioral Neuroscience, 110*(5), 1033-1039.

Manns, J. R., Hopkins, R. O., Reed, J. M., Kitchener, E. G., & Squire, L. R. (2003). Recognition Memory and the Human Hippocampus. *Neuron, 37,* 171-180.

McGaugh, J.L (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annual Review of Neuroscience, 27*, 1-28.

McGaugh, J. L., Cahill, L., & Roozendaal, B. (1996). Involvement of the amygdala in memory storage: interaction with other brain systems. *Proceedings of the National Academy of Sciences of the United States of America, 93*(24), 13508-14.

McGaugh, J. L., & Roozendaal, B. (2002). Role of adrenal stress hormones in forming lasting memories in the brain. *Current Opinion in Neurobiology, 12*, 205-10.

Okuda, S., Roozendaal, B., & McGaugh, J.L. (2004). Glucocorticoid effects on object recognition memory require training-associated emotional arousal. *Proceedings of the National Academy of Sciences of the United States of America, 101*(3):853-858.

Puma, C., & Bizot, J.C. (1998). Intraseptal infusions of a low dose of AP5, a NMDA receptor antagonist, improves memory in an object recognition task in rats. *Neuroscience Letters, 248*(3), 183-186.

Quevedo, J., Feier, G., Agostinho, F. R., Martins, M. R., & Roesler R. (2003). Consolidação da memória e estresse pós-traumático. *Revista Brasileira de Psiquiatria, 25*, 25-30.

Rampon, C., Tang, Y.P., Goodhouse, J., Shimizu, E., Kyin, M., & Tsien, J.Z. (2000). Enrichment induces structural changes and recovery from nonspatial memory deficits in CA1 NMDAR1-knockout mice. *Nature Neuroscience, 3*(3), 238-244.

Reed, J. M., & Squire, L. R. (1997). Impaired recognition memory in patients with lesions limited to the hipocampal formation. *Behavioural Neurosciences, 111*, 667-675.

Roesler, R., Vianna, M.R., de-Paris, F., & Quevedo, J. (1999). Memory-enhancing treatments do not reverse the impairment of inhibitory avoidance retention induced by NMDA receptor blockade. *Neurobiology of Learning and Memory, 72*(3), 252-258.

Roozendaal, B., Okuda, S., Van der Zee, E.A., & McGaugh, J.L. (2006). Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America, 103*(17):6741-6746.

Sargolini, F., Roullet, P., Oliverio, A., & Mele A. (2003). Effects of intra-accumbens focal administrations of glutamate antagonists on object recognition memory in mice. *Behavioural Brain Research, 138*(2), 153-163.

Sternberg, D.B., Isaacs, K.R., Gold, P.E., & McGaugh, J.L. (1985). Epinephrine facilitation of appetitive learning: attenuation with adrenergic receptor antagonists. *Behavioral and Neural Biology, 44*(3), 447-453.

Tang, Y.P., Shimizu, E., Dube, G.R., Rampon, C., Kerchner, G.A., Zhuo, M., Liu, G., & Tsien, J.Z. (1999). Genetic enhancement of learning and memory in mice. *Nature, 401*(6748), 63-69.

van Praag, H.M..(2004) The cognitive paradox in posttraumatic stress disorder: a hypothesis. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*; 28, 923–935.

Wang, S.J., Huang, C.C., Hsu, K.S., Tsai, J.J., Huang, C.C., & Gean, P.W. (1996). Blockade of isoproterenol-induced synaptic potentiation by tetra-9-aminoacridine in the rat amygdala. *Neuroscience Letters, 214*(2-3), 87-90.

Yassuda, M. S. (2002). Memória e Envelhecimento Saudável. *In: Tratado de Geriatria e Gerontologia*. Rio de janeiro: Guanabara Koogan; 914-920.

Yehuda, R. (2002). Post-Traumatic Stress Disorder. *The New England Journal of Medicine*, 346 (2), 108-114.

7. ANEXOS

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Subject: Neurobiology of Learning and Memory Submission: Manuscript Number Assigned

> Ms. No.: NLM-06-176
> Title: Adrenergic enhancement of consolidation of object recognition
> memory
> Corresponding Author: Dr. Nadja Schroder
> Authors: Arethuza Dornelles; Maria Noemia M de Lima; Manoela Grazziotin;
> Juliana Presti-Torres; Vanessa A Garcia; Felipe S Scalco; Rafael Roesler;
>
> Dear Dr. Schroder,
>
> Your submission, referenced above, has been assigned the following
> manuscript number: NLM-06-176
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> Thank you for submitting your work to Neurobiology of Learning and Memory.
>
> Kind regards,
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> Susan Ikeda
> Journal Manager, Neurobiology of Learning and Memory
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XXII CONGRESO LATINOAMERICANO Y 1RO IBEROAMERICANO DE CIENCIAS FISIOLÓGICAS. BUENOS AIRES, 4 AL 7 DE NOVIEMBRE DE 2006
ENVIO DE RESUMENES

Utilizar el recuadro que se adjunta (11 x 17 cms), sin exceder los límites del mismo. Emplear letra Time New Roman 12 para el título, autores y lugar de trabajo. Para el cuerpo del resumen usar Time New Roman 11 siguiendo el modelo que se muestra (borrar el texto e incluír el propio). Enviar por mail *antes del 30 de agosto de 2006* a safisiol@safisiol.org.ar . Solo se acepta un resumen por participante inscripto (subrayar su nombre). Utilizar castellano, portugués o inglés.

Adrenaline modulates object recognition memory

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Extensive findings from animal experiments indicate that stress hormones modulate memory storage of emotionally arousing information. Over the last decades a number of studies using aversively motivated learning tasks indicated that posttraining administration of adrenaline modulates memory consolidation. However, little is known about adrenaline influences on memory tasks that do not involve aversive/emotional content. Thus, the aim of the present study was to evaluate the effects of the acute adrenaline on a nonaversive memory test: the novel object recognition task (NOR). In order to do that, adult male Wistar rats received intraperitoneal injections of saline (control group) or epinephrine (2,5; 25,0 and 250,0 µg/kg) immediately after the NOR training session. The behavioral task consisted of a training session, where rats explored two identical copies of the same object for 2 minutes (Experiment I) or 2 distinct objects for 5 minutes (Experiments II) in an open field box. In retention tests, one of the familiar objects was replaced by a novel object. A recognition index calculated for each animal was expressed by the ratio T_N/T_F+T_N (T_F= time spent exploring the familiar object; T_N= time spent exploring the novel object). Results have indicated that adrenaline facilitated recognition memory, as assessed by comparison of recognition indexes among groups. These findings provide evidence that, as found with emotionally arousing tasks, recognition memory consolidation requires posttraining adrenergic activity.