Universidade Federal do Rio Grande do Sul

Instituto de Ciências Básicas da Saúde

Departamento de Bioquímica

Curso de Pós-Graduação em Bioquímica

Estudo do Processamento das Memórias de Curta e Longa Duração

JOÃO QUEVEDO

Orientador:

Prof. Dr. Ivan Izquierdo

Tese apresentada ao Curso de Pós-Graduação em Ciências Biológicas – Bioquímica como requisito parcial para a obtenção do título de Doutor em Bioquímica.

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Mestre, Prof. Dr. Ivan Izquierdo – procurei nele um orientador; encontrei um amigo, modelo de correção ética e moral. Muitos dos meus sonhos só se concretizaram porque pude contar com seu apoio.

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Departamento de Bioquímica e Curso de Pós-Graduação em Ciências Biológicas – Bioquímica – é um centro de ciência, capacidade de superação e competência. Uma prova de que a universidade pública, e conseqüentemente o Brasil, podem dar certo.

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onde o amor é a pedra fundamental e, por conseqüência,

a capacidade de sonhar e produzir

conhecimento dele resulta.

A principal impressão, a mais forte e a mais constante, que se tem ao estudar a atividade nervosa superior pelo nosso método, é a extrema plasticidade dessa atividade, as suas imensas possibilidades: nela, nada permanece na imobilidade, nada é inflexível, tudo pode ser conseguido e aperfeiçoado, desde que sejam satisfeitas certas condições necessárias.

Ivan Pavlov, 1932, Resposta de um fisiologista aos psicólogos.

Resumo

(João Quevedo - Estudo do Processamento das Memórias de Curta e Longa Duração) - Este trabalho apresenta a compilação dos 4 principais experimentos carreados ao longo de 1999-2002: 3 deles envolvem o modelo animal e um quarto utiliza-se de voluntários humanos. Entretanto, o uso desses diferentes paradigmas não prejudica a unidade do conjunto. O **Capítulo 1** apresenta sucintamente o marco teórico dos 4 trabalhos. Inicialmente são discutidos aspectos modulatórios da consolidação da memória. Após, alguns elementos da bioquímica da consolidação da memória são apresentados no intuito de permitir establecer um entendimento das vias da PKA e da MAPK e suas correlações com a via final comum – a síntese protéica. Adicionalmente, a dissociação STM e LTM é discutida a partir do referencial farmacológico. Uma última unidade apresenta conceitos primitivos do papel da amígdala na modulação da memória e das evidências da implicação das emoções, via amígdala, na modulação da memória em humanos. Os experimentos utilizando a esquiva inibitória como paradigma e o rato como sujeito ocupam os **Capítulos 2, 3 e 4.** No **Capítulo 2** é apresentado um corpo de resultados que permite observar uma dissecção farmacológica da STM e LTM. Os dados demonstram um envolvimento de fenômenos dependentes de PKA em ambas STM e LTM, dependentes de MAPK apenas na STM, e dependentes de síntese protéica apenas na LTM. O **Capítulo 3** apresenta um trabalho realizado em colaboração com o Prof. Steven P. R. Rose (Open University, UK), que envolve a determinação dos momentos sensíveis à inibição da síntese protéica na consolidação da LTM. Foram observados dois momentos: um inicial, junto ao treino, e um tardio apos 3h. Além disso, foi possível

demonstrar que um treino prévio de baixa intensidade, mas não a pré-exposição ao aparato, pode impedir o estabelecimento de amnésia induzida pelo bloqueio da síntese protéica. O **Capítulo 4** estende os achados com anisomicina observados no **Capítulo 3**, estudando também o inibidor da PKA, Rp-cAMPs, e o inibidor da MAPKK, PD 098059. Os dados obtidos confirmam também para essas cascatas a indução em um treino prévio de baixa intensidade de algum fenômeno celular de longa duração que torna o aprendizado de um segundo treino independente de PKA, MAPK ou síntese protéica. O estudo da dissociação da STM e LTM foi ampliado, agora no modelo humano, no experimento descrito no **Capítulo 6**. Nesse experimento, observamos uma clara influência do conteúdo emocional na LTM, mas a ausência desse efeito na STM. A discussão geral (**Capítulo** 7) busca integrar esses achados descritos nos capítulos anteriores dentro da nova perspectiva molecular da neurobiologia da memória. Além disso, abre discussão acerca de possíveis novas possibilidades de pesquisa.

Abstract

(João Quevedo – Pharmacological Study of Short- and Long-Term Memory) – The main purpose of the research presented in this thesis was evaluate the involvement of protein synthesis, protein kinase A (PKA) and mitogen-activated protein kinase (MAPK) in fear-motivated memory in rats given different training conditions. Additionally, an experiment was carried out in order to elucidate the dissociation between short- and long-term memory in human subjects. In the experiments described in **Chapter 2**, we studied the involvement of hippocampal protein synthesis-, PKA- and MAP kinase-dependent processes in short- (STM) and long-term memory (LTM) for inhibitory avoidance task. Fifteen minutes before or immediately after training rats received intrahippocampal infusions of vehicle, the protein synthesis inhibitor anisomycin, the PKA inhibitor Rp-cAMPs or the MAPKK inhibitor PD098059. Results demonstrated STM recruits PKA and MAPK while LTM depends on PKA activity and protein synthesis during the initial postraining period. In the **Chapters 3 and 4**, we have studied the effect of training conditions on hippocampal protein synthesis-, protein kinase A- and Mitogen-Activated Protein Kinase-dependent processes in consolidation of the inhibitory avoidance task. Adult male Wistar rats were trained and tested in a step-down inhibitory avoidance task (0.4 mA footshock, 24 h training-test interval). Fifteen minutes before or 0, 1.5 or 3 hours after training, animals received a 0.8 μl intrahippocampal infusion of the protein synthesis inhibitor anisomycin (80 μg), protein kinase A (PKA) inhibitor Rp-AMPs (0.05 μg), mitogen-activated protein kinase kinase (MAPKK) inhibitor PD 098059 (50 μM solution) or vehicle (phosphate buffer in saline, pH 7.4).

The infusion of anisomycin impaired retention test performance in animals injected 15 min before and 3 h after the training session, but not at 0 or 1.5 h post-training. The infusion of Rp-AMPs impaired retention test performance in animals injected 15 min before and 0 or 3 h after the training session, but not at 1.5 h post-training. PD 098050 impaired retention test performance only when injected at 3 h after training session. Pretraining with a low footshock intensity (0.2 mA) 24 h before training prevented the amnestic effect of all drugs studied at 15 min before or 0, 1.5 and 3 h after training. However, simple preexposure to the inhibitory avoidance apparatus did not alter the amestic effects of all drugs. The results suggest that memory processing requires hippocampal mechanisms dependent on protein synthesis, PKA and MAPKK at different times after training. A prior weak training session, however, is sufficient to prevent the amnestic effect of anisomycin, Rp-AMPs or PD 098050 suggesting that weak training must be sufficient to produce some lasting cellular expression of the experience. In the **Chapter 5**, thirty-one healthy volunteers were divided into two major groups. In the first group long-term memory (LTM) was evaluated, with the testing session one week after training. The second group was tested one hour after training, where short-term memory (STM) was evaluated. Each group was divided in to two subgroups. One half of the volunteers was exposed to an emotionally neutral story, and the other half of each group was exposed to a closely matched but more emotionally arousing story. The testing session consisted of a questionary containing 80 questions of multiple choices. The results were evaluated through percentage of correct answers. Results showed that correct answers were increased, in LTM measures, in the subjects that were given the emotional version of the test. In STM measures, no differences were found between the

emotional and neutral version. However, the presentation of emotional story caused an emotional reaction in both groups. The lack of effect of emotional arousal in STM suggests that amygdala is not related to STM mechanisms. Further studies using different approaches are needed to elucidate if STM processes are influenced by emotional arousal.

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Capítulo 1. Introdução Geral

Capítulo 1. Introdução Geral

O reconhecimento de que as memórias de longa duração (LTMs, do inglês *long-term memory*) não se estabelecem rapidamente em sua forma final, mas levam algum tempo para serem consolidadas, são lábeis durante a consolidação, e que sistemas de curta duração (memória de cura duração) podem potencialmente estabelecer LTM deriva de observações de Müller e Pilzecker (1900) e William James (1890), respectivamente (McGaugh, 1966; 2000). A observação de que traumatismos cranianos induzem amnésia retrograda proveram as primeiras vagas evidências das bases neurobiológicas da consolidação. A popularização do uso de estimulações elétricas e convulsões induzidas por fármacos para o tratamento de transtornos psiquiátricos nos anos 30 a 40 demonstrou que esses tratamentos também induzem amnésia retrógrada (McGaugh, 1966). No final dos anos 50 e início dos anos 60, estudos dos efeitos de drogas, então chamados "estimuladores centrais" (picrotoxina, por exemplo) administradas em ratos imediatamente após o treino mostraram que essas drogas facilitavam a memória; ou seja, induziam facilitação "retrógrada" da memória (Breen and McGaugh, 1961; MacGaugh, 1966; Flood et al., 1977).

Os dados iniciais com "estimuladores centrais" sugeriam que a descoberta de receptores ativados por essas drogas poderia prover evidências mais precisas dos processos envolvidos na consolidação da memória. O progresso foi inicialmente lento devido ao desconhecimento dos mecanismos de ação daquelas drogas.

1.1. Modulação da memória

Entre as décadas de 60 e 80, o interesse foi dado aos efeitos amnésicos induzidos pela administração sistêmica ou intracerebral de uma variedade de antibióticos, os quais demonstraram a necessidade de síntese protéica para a consolidação nas primeiras horas que se seguiam após a aquisição (Cherkin, 1969; Agranoff et al., 1965; Flexner et al., 1967; Matthies, 1982; 1989). Posteriormente, foi demonstrado que os níveis de RNAm hipocampais, proteínas e síntese de glicoproteínas apresentavam dois picos após o treino: o primeiro em cerca de 1 h e o segundo entre 3-6h após o treino, em ratos (Matthies, 1982; 1989) e em pintos (Rose, 1995b). Os dois picos são essenciais para a formação da LTM: inibidores da síntese proteica administrados ao tempo de cada pico provocam amnésia. Então, tornou-se claro que a memória de curta duração (STM, do inglês *short-term memory*) não requer síntese proteica (Agranoff et al., 1965; Frey et al., 1995; Rose, 1995a,b; Wustenberg et al., 1998).

Não houve nenhum modelo para memória dominante nos anos 50 e 60. As hipóteses variavam de limitadas (cada memória induz a síntese de uma proteína específica) a especulativas (uma variedade de circuitos reverberatórios). Uma séria análise dos mecanismos celulares/moleculares não parecia promissora. Nos anos 60 e 70, muitos laboratórios envolvidos no estudo da memória tomaram duas diferentes direções. Alguns tentaram identificar, usualmente através de estudos com lesões, aquelas estruturas cerebrais envolvidas em diferentes tipos de memórias. Os resultados foram importantes e

levaram a definição de memória declarativa e procedural, as quais são processadas por diferentes estruturas cerebrais (Squire, 1992).

A descrição de Scoville e Milner (1957) da perda de memória do paciente H.M., que teve a porção anterior de seu lobo temporal excisada cirurgicamente por Scoville para o tratamento de epilepsia, demonstrou o papel crucial desta região na formação da memória. A perda foi inicialmente, e por muitos anos, atribuída a lesão hipocampal; entretanto, estudos recentes de ressonância nuclear magnética demonstraram que a perda anatômica de H.M. compreendeu muito da amígdala e do córtex entorrinal, além da metade do complexo hipocampal (giro denteado, hipocampo e subículo) (Corkin et al., 1997). Então, a princípio, a amnésia de H.M. pode ser atribuída a perda de qualquer uma dessas estruturas, ou da perda combinada de algumas delas. A amnésia de H.M. foi extremamente restrita aquela que nós conhecemos como memória declarativa ou explícita (Cohen et al., 1985).

Estudos com lesões em primatas demonstraram que o hipocampo, córtex ento e perirrinal são todos cruciais para as memórias declarativas (Zola-Morgan et al., 1993). A necessidade de o hipocampo e suas conexões estarem intactos para a formação de memórias declarativas em animais foi particularmente bem estudado em tarefas que se utilizam dicas espaciais (Morris et al., 1986), em tarefas utilizando dicas espaciais e/ou dicas seqüenciais, como o *delayed matching-to-sample* (Squire, 1992; Zola-Morgan et al., 1993), e para memórias contextuais (Eichenbaum, 1996, 1999; Eichenbaum et al., 1996), incluindo a esquiva inibitória e suas variantes (Lorenzini et al., 1996; Izquierdo e Medina, 1997a; Izquierdo et al., 1999a), além do *contextual fear* (Izquierdo et al., 1979, 2000; Vazdarjanova e McGaugh, 1999), o qual foi chamado inicialmente de *"any-way*

avoidance" (Izquierdo et al., 1979). Os estudos com lesões forneceram importantes informações anatômicas, mas foram pouco claros quanto aos mecanismos (Izquierdo e Medina, 1998).

Os laboratórios dedicados à farmacologia entre os anos 60 e 80 empenharam-se na análise da modulação da memória, ou seja, o efeito de drogas, hormônios, neurotransmissores e neuromoduladores na consolidação da memória. Foi rapidamente compreendido que substâncias moduladoras podiam influenciar os mecanismos básicos do processamento da memória (Bär et al., 1982). Dessa forma, o estudo da modulação foi considerado uma útil abordagem indireta para a investigação dos mecanismos da memória. Foi uma infelicidade o fato de que esses estudos não foram combinados com estudos de lesões; mas ambos foram realizados por grupos distintos de cientistas.

Após o sucesso dos estudos iniciais usando "estimulantes" para aumentar a consolidação da memória, as drogas passaram a ser administradas imediatamente após o treino para evitar confusão de efeitos na aquisição, performance ou evocação (McGaugh, 1966). Por esta razão, a esquiva inibitória (ou passiva) tornou-se o paradigma de aprendizado mais largamente utilizado neste tipo de estudo (McGaugh, 1966; Gold, 1986; Rose, 1995a,b, 2000; Izquierdo e Medina, 1997a,b). Como é rapidamente aprendida, esta tarefa permite a determinação precisa do momento após o aprendizado no qual os tratamentos afetam a consolidação, algo difícil de discernir em tarefas que requerem muitos treinos (Gold, 1986). Entretanto, a esquiva inibitória representa uma forma de memória constantemente utilizada em humanos e necessária para a sobrevivência: uma forma que evita que coloquemos os dedos em uma tomada elétrica, que atravessemos a rua sem olhar para os lados, que andemos por lugares perigosos, etc.

Os estudos farmacológicos proveram muitos achados de valor clínico no que diz respeito à modulação dos estágios iniciais da consolidação da memória, além da evocação da memória. A consolidação da memória é regulada pela adrenalina circulante, noradrenalina, ACTH e fragmentos de ACTH, vasopressina, ocitocina, glicocorticóides (De Wied, 1964, 1993; Gold e Buskirk, 1975, 1976; McGaugh, 1983; Bohus, 1994; Rose, 1995b, 2000; Roozendaal e McGaugh, 1996; McGaugh et al., 1996; Ferry et al., 1999; Setlow et al., 2000) e opióides endógenos, sendo a β-endorfina o mais importante (Izquierdo, 1989, 1991). Esses agentes agem através de influências no complexo basolateral do núcleo amigdalóide (McGaugh et al., 1996; Roozendaal and McGaugh, 1996; Cahill and McGaugh, 1998) e, no caso dos opióides, talvez também no septo medial (Izquierdo, 1989, 1991). O efeito amnésico dos opióides, incluindo a morfina, justifica o uso de opióides como medicação pré-anestésica. Parte do efeito amnésico dos opióides é devido à indução de dependência de estado, como se observa com altas doses de ACTH, vasopressina ou adrenalina (Izquierdo, 1984, 1989, 1991).

A compreensão do modo de ação da picrotoxina levou a uma importante reinterpretação dos resultados iniciais nos quais ela era usada com um mero "estimulador central" para aumentar a consolidação da memória (McGaugh, 1966). Após o entendimento de que a picrotoxina, age pelo bloqueio do canal de Cl^- do receptor $GABA_A$, tornou-se claro que esses receptores são os responsáveis pelo maior sistema de *down-regulation* da consolidação da memória (Izquierdo et al., 1992, Izquierdo e Medina, 1997a). Esse é talvez o mecanismo modulatório mais importante da fase inicial da consolidação que determina rapidamente se o processo de consolidação será completado (Brioni, 1993). Estudos com microinfusão do agonista GABAérgico

muscimol ou de picrotoxina no septo medial, amígdala ou hipocampo (Brioni et al., 1990; Izquierdo et al., 1992), demonstrou que mecanismos GABAérgicos atuam como reguladores precoces da evolução da formação da memória nestas três regiões (Brioni, 1993). Por exemplo, parece claro que memória da esquiva inibitória (Bianchin et al., 1999; Barros et al., 1999; Izquierdo e Medina, 1997a,b; Izquierdo et al., 1997a, 1998c) e o aprendizado espacial em um labirinto aquático (Morris et al., 1986) é feito no hipocampo e suas conexões com o neocórtex; mas infusões de muscimol intraseptal (Brioni et al., 1990) ou intramígdala (Izquierdo et al., 1992) bloqueia ambos os tipos de memória.

A descoberta farmacológica do papel dos receptores GABA na memória ajudou a elucidar os efeitos dos barbitúricos, álcool e benzodiazepínicos, os quais agem pela potencialização dos receptores $GABA_A$ (Izquierdo e Medina, 1991).

Alerta (Cahill e McGaugh, 1990), ansiedade e estresse (Gold, 1986) tem sido implicados como importantes moduladores das fases iniciais da formação da memória (McGaugh, 1966; 1983, 2000) e evocação (Izquierdo, 1989). Há evidências (Izquierdo, 1984, 1989; Bruins Slot e Colpaert, 1999; Izquierdo et al., 2000 a) que alguns aspectos neurohumorais e hormonais presentes no momento da consolidação (β-endorfina cerebral, hormônios do estresse) devem ser reptidos no momento da evocação para que essa seja melhor. Experiências traumáticas, por exemplo, são usualmente melhor lembradas em situações estressantes (para esclarecimentos adicionais, ver Izquierdo 1984, 1989; Bruins Slot e Colpaert, 1999).

A simples exposição a uma nova situação ou ambiente, sem estresse, pode também ter profundos efeitos modulatórios. Quando ocorre em 1 h após o treino ou

menos, essa é amnésica (Izquierdo et al., 1999b) e bloqueia a LTP hipocampal (Xu et al., 1998). Quando ocorre entre 0-2h antes do teste, a exposição à novidade facilita a evocação (Izquierdo e McGaugh, 1985; Izquierdo et al., 2000a). Os efeitos da novidade são bloqueados por naloxone ou naltrexone sistêmicos, o que indica que opióides endógenos estão envolvidos (Izquierdo e McGaugh, 1985), e por AP5 e pelo inibidor da CaMKII, KN62, o que indica que os receptores NMDA e a CaMKII são necessários para a habituação à novidade esteja envolvida (Izquierdo et al., 1999, 2000a).

Três a 6 h após a aquisição, mecanismos β-adrenérgicos, 5HT-1_A e D_1/D_5-dopaminérgicos na região CA1, córtex entorinal e córtex parietal posterior, mas não na amígdala, fortemente regulam o armazenamento da memória de longa duração através da regulação da adenilato ciclase e, portanto, indiretamente da atividade da proteína kinase dependente de AMPc (PKA) (Ardenghi et al., 1997; Bevilaqua et al., 1997). Esses sistemas monoaminérgicos também regulam a formação da STM separadamente da LTM, e frequentemente em direções opostas; o sistema dopaminérgico, por exemplo, bloqueia a STM da esquiva inibitória e ao mesmo tempo facilita a LTM (Izquierdo et al., 1998b).

1.2. A separação da memória de curta duração (STM) da memória de longa duração (LTM)

Um dos maiores questionamentos levantados por William James em 1890 é se a STM é um passo necessário para o estabelecimento da LTM, ou uma forma separada de memória. A única forma de responder possível para essa questão seria demonstrar se a STM poderia ser bloqueada sem alterar a LTM para uma mesma tarefa no mesmo animal,

ou que isso seria impossível (Izquierdo et al., 1998a,b). Partindo do pressuposto de que os mecanimos moleculares da STM são, por definição, simultâneos daqueles da formação da LTM, tanto experimentos bioquímicos como o uso de procedimentos com animais transgênicos não podem ser aplicados a esse tipo de estudo (Izquierdo e Medina, 1998). Apenas experimentos farmacológicos poderiam prover essa resposta.

Muitos experimentos foram conduzidos nesse sentido (Izquierdo et al., 1998a, b, c). Dezesseis diferentes tratamentos farmacológicos administrados no hipocampo, córtex entorrinal e córtex parietal posterior seletivamente suprimiram STM sem afetar LTM, e dois outros tratamentos seletivamente facilitaram a STM enquanto prejudicaram a LTM. Tratamentos administrados no córtex pré-frontal afetaram especificamente a *working memory* e a LTM, mas não a STM (Izquierdo et al., 1998c). As drogas utilizadas foram agonistas específicos e antagonistas de vários receptores e proteínas quinases. Em conclusão, STM e LTM operam através de mecanimos separados, mas simultâneos (Izquierdo et al., 1998a, b, c; 1999a).

A relevância clínica desses achados é óbvia. Warrington (1972) e seu grupo (Warrington e Shallice, 1969; Shallice e Warrington, 1970), e muitos outros têm descrito uma dissociação clínica entre memória de curta duração e memòria de longa duração que tem valor diagnóstico Um específico distúrbio da memória de curta duração com pouca ou nenhuma alteração da memória de longa-duração é típica do delirium e de certas formas de depressão e demência.

É importante observar que várias áreas corticais (CA1, córtex entorrinal, córtex parietal, cingulado anterior) tem se mostrado por métodos farmacológicos serem

crucialmente envolvidos na formação da memória de curta e de longa duração (Izquierdo et al., 1998c; Mello e Souza et al., 1999), mas não a amígdala (Bianchin et al., 1999).

Em relação ao hipocampo, a STM tem algumas similaridades com o fenômeno conhecido como potenciação de curta duração (STP, do inglês *short-term potentiation*) a qual também requer CaMKII e PKC, mas não uma ativação tardia da PKA, e é claramente independente da LTP (Bliss e Collingridge, 1993).

1.3. Via cAMP/PKA e síntese protéica

É sabido que a proteína quinase dependente de cAMP (PKA) é necessária para a manutenção da LTP na região CA1 (Matthies e Reymann, 1993; Huang et al., 1994) e da LTM (Carew, 1996; Bernabeu et al., 1997a), em muitas espécies (Bourtchouladze et al., 1994; Yin e Tully, 1996). No caso da LTM, a via cAMP/PKA é crucial em dois tiferentes momentos: primeiro, imediatamente após a induçào do treino, e novamente 3-6 h mais tarde (Vianna et al., 2000b). O segundo pico de atividade da PKA correlaciona-se com um aumento do cAMP endógeno e ambos os picos de atividade da PKA correlacionam-se com um aumento do $CREB_1$ nuclear fosforilado na ^{135}Ser (Bernabeu et al., 1997a). O aumento tardio pós-treino da unidade catalítica da PKA não é observado em animais que recebem AP5 na região CA1 imediatamente após o treino (Cammarota et al., 2000).

Recentemente, foi observado que, 2 h após o treino, há um pico de $CREB_1$ fosforilado em mitocôndrias extraídas de sinapses. CREB fosforilado na ^{135}Ser foi recentemente descrito na mitôndria de vários tecidos (Cammarota et al., 1999). Recentemente, Freeman e Young (1999) observaram que a administração intracerebral de

cloranfenicol causa amnésia para esquiva inibitória em pintos. Cloranfenicol é um inibidor específico da síntese proteica mitocondrial.

O segundo pico de aumento da atividade da PKA e de P-CREB é altamente sensível à infusão local de drogas que afetam a adenilato ciclase (dopaminérgicas D!, β-adrenérgicas e agonistas e antagonistas 5HT1A), ou os níveis endógenos de cAMP (forskolin, 8-Br-cAMP) (Ardenghi et al., 1997; Bernabeu et al., 1997a, Bevilaqua et al., 1997).

Várias linhas de evidência indicam que o segundo e tardio pico de atividade de PKA e níveis de P-CREB é necessário para a manutenção de LTP e LTM por volta das 3-6h; ou seja, permite que se torne de fato de longa duração (Matthies e Reymann, 1993; Huang et al., 1994; Bernabeu et al., 1997a; Vianna et al., 2000b). Síntese protéica é também necessária neste momento com esse mesmo propósito (Frey et al., 1988; Bourchouladze et al., 1998; Quevedo et al., 1999). Adicionalmente, o aumento de várias glicoproteínas relacionadas à adesão celular têm sido relacionado ao segundo pico de PKA/P-CREB 5-7 h após o treino em esquiva inibitória em pintos (Rose, 1995a) e 6-8 h e de novo 10-12 h em ratos (Ni Dhuill et al., 1999; O'Malley et al., 1997).

Um papel crucial na consolidação da memória é atribuído a todas essas modificações seqüenciais, desde o cAMP, passando pela ativação da PKA, a fosforilação do CREB, a síntese inespecífica de proteínas, até a síntese de glico- e sialoproteínas especializadas (Rose, 1995b; 2000). As evidências em todos os casos são farmacológicas. Inibidores da adenilato ciclase admnistrados no CA1 ou no córtex entorrinal imediatamente após o treino bloqueiam a memória (Ardenghi et al., 1997). Inibidores da PKA administrados no momento do primeiro pico o do segundo pico de atividade da

PKA bloqueiam a formação da LTM (Bernabeu et al., 1997a; Ardenghi et al., 1997; Bevilaqua et al., 1997; Bourchouladze et al., 1998). Antisense para CREB administrado no CA1 bloqueia a formação de memória espacial por volta das 4 h (Guzowski e McGaugh, 1997). Inibidores da síntese protéica dados no momento do treino ou 2-3 h após o treino (Bourchouladze et al., 1998; Quevedo et al., 1999) também bloqueiam a LTM. Anticorpos contra glicoproteínas de adesão celular administrados tardiamente após o treino em ratos ou em pintos bloqueiam a memória para esquiva inibitória (Rose, 1995a).

É importante observar que o primeiro a demonstrar dois picos de síntese protéica e sua sensibilidade a anisomicina em ratos foram Grecksch e Matthies em 1980. Posteriormente, eles demonstraram que estas duas ondas também incluíam síntese de mRNA e glicoproteínas (Matthies, 1989).

1.4. A via da MAPK

A cascata da proteína quinase ativadora de mitogênese (*mitogen-activated protein kinase*) (MAPK) foi proposta como um via chave na formação da memória baseado em observações em LTP e em camundongos transgênicos mutantes que expressam membros alterados dessa via (English e Sweatt, 1997; Atkins et al., 1998). A demonstração efetiva do papel crucial dessa enzima na formação da STM e LTM requereu o uso de microinfusões de diferentes doses de um inibidor seletivo de um ponto crucial da cascata, MAPKK (PD 098059) em vários tempos após o treino. Utilizando a esquiva inibitória, a administração de PD 098059 na área dorsal do CA1 de ratos imediatamente após o treino bloqueou seletivamente a STM de um modo dose-dependente, mas não a LTM. PD

098059 não teve efeito sobre a STM e LTM quando administrado no CA1 30,90 ou 120 min após o treino. Entretanto, bloqueou seletivamente a LTM (mas não a STM) quando administrado 180 min após o treino. Em outras tarefas, incluindo tarefas espaciais de múltiplos treinos, PD 098059 também mostrou-se amnésico (Atkins et al., 1999; Blum et al., 1999; Shafe et al., 1999; Walz et al., 1999a, b, 2000).

Um aumento da forma ativada (fosforilada) de MAPK no CA1/CA2 foi recentemente relatado após múltiplas sessões de treino no labirinto aquático de Morris (Blum et al., 1999).

A via MAPK também fosforila CREB na ^{135}Ser (Micheau e Riedel, 1999; Roberson et al., 1999), e seu momento de intervenção (Bourchouladze et al., 1998; Walz et al., 1999a, b) e seu papel diferenciado na STM e LTM são correlacionáveis com a PKA (Walz et al., 2000). Há um considerável "cross-talk" entre as vias da PKA e MAPK em vários níveis (Micheau e Riedel, 1999).

1.5. A esquiva inibitória

A metodologia enpregada para a realização dos experimentos, incluindo animais e drogas utilizadas, procedimentos cirúrgicos, micro-injeções intracerebrais, verificação histológica, ensaios bioquímicos, análise e discussão dos resultados são apresentados de forma pertinente em cada um dos capítulos que se seguem. Da mesma forma o embasamento teórico para cada experimento é apresentado no início de cada capítulo.

Na tarefa de EI (ver descrição nos capítulos subsequentes) o animal aprende a relacionar a descida de uma plataforma com um leve choque aplicado nas patas. Com isso, numa segunda exposição à caixa de esquiva ele evita um comportamento inato de descer da

plataforma para explorar a caixa. O aprendizado de EI envolve vários estímulos, incluindo percepção espacial e visual, sensibilidade à dor, acompanhados de um componente emocional amplamente modulado por hormônios relacionados ao estresse (Gold, 1986; Izquierdo, 1989; Izquierdo e Medina, 1997). A EI é uma tarefa ideal para estudar processos de memória iniciados durante e após o treino porque:

1) Em geral pode ser aprendida com uma única sessão de treino;
2) Não é contaminada por sessões prévias ou subsquentes, como ocorre no "water-maze", radial-maze ou esquiva ativa de duas vias; (Gold, 1986; Izquierdo, 1989);
3) Não é um aprendizado inato;
4) A farmacologia envolvida na sua modulação é bastante conhecida.

Ao longo dos últimos 10 (dez) anos, nosso grupo vem utilizando abordagens farmacológicas específicas para o estudo de aspectos neuroquímicos envolvidos no aprendizado da EI, incluindo:

1) Agonistas e antagonistas específicos para diferentes receptores;
2) Ativadores e bloqueadores específicos de segundos mensageiros intracelulares;
3) Ativadores e bloqueadores específicos de proteínas quinase;
4) Ativadores e bloqueadores específicos de mesageiros retrógrados;
5) Bloqueadores de síntese protéica.

A abordagem farmacológica, unida a ensaios bioquímicos realizados em diferentes tempos após o treino de EI vem permitindo estabelecer um perfil bioquímico relacionado com o aprendizado desta tarefa, incluindo variações neuroquímicas tempo-dependentes na amígdala, septo, hipocampo, córtex, entorrinal e córtex parietal. Assim, demonstramos modificações na quantidade e/ou afinidade de receptores, atividade de diversas enzimas e

expressão gênica durante o período pós-treino (Ardenghi et al., 1997; Bevilaqua et al., 1997; Bernabeu et al., 1997; Izquierdo e Medina 1997; Jerusalinsky et al; 1992,1993, 1994 ; Walz et al., 1999 a ,b, 2000).

1.6. O estudo da memória emocional em humanos

Não há dúvida de que a memória para eventos com componente emocional é melhor do que a para eventos neutros. Isso é claramente adaptativo, porque estímulos emocionais, sejam prazerosos ou aversivos, são geralmente mais importantes para a sobrevivência das espécies. Evidências indicam que as memórias emocionais estabelecem-se através da amígdala e são mais resitentes a extinsão e esquecimento (Cahill, 1995; Ledoux, 1992). Estes achados são consistentes com a hipótese de que as respostas emocionais influenciam a memória, pelo menos em parte, através da amígdala, modulando o armazenamento da memória de longa duração (Cahill e McGaugh, 1998; Bianchin et al., 1999). Durante e imediatamente após situações emocionalmente intensas ou estressantes, vários sistemas fisiológicos são ativados, incluindo a liberação de vários hormônios (Stratakis e Chrousos, 1995; Roozendaaal et al., 1999).

Há fortes evidências de que a amígdala está envolvida na modulação da memória de longa duração em estudos utilizando animais e humanos (McGaugh, 2000). Estudos utilizando β-bloqueadores em voluntários saudáveis ou pacientes com lesões de amígdala demonstraram redução da influência das emoções na memória (Cahill et al., 1994,1998). Adicionalmente, uma evocação de 3 semanas de material emocional foi altamente correlacionável com ativação da amígdala direita observada na tomografia por emissão de pósitrons (McGaugh et al., 1996).

Apesar da forte correlação do papel da amígdala na consolidação da LTM, os resultados de Bianchin e colaboradores (1999) utilizando a esquiva inibitória em ratos mostrou ausência de envolvimento da amígdala na memória de curta duração.

1.7. Objetivos e organização dos trabalhos apresentados na tese

Este trabalho apresenta a compilação dos 4 principais experimentos carreados ao longo de 1999-2002. Claramente, uma primeira observação é o fato de que 3 deles envolvem o modelo animal e um quarto utiliza-se de voluntários humanos. Entretanto, o uso desses diferentes paradigmas não prejudica a unidade do conjunto.

O **Capítulo 1** apresenta sucintamente o marco teórico dos 4 trabalhos. Inicialmente são discutidos aspectos modulatórios da consolidação da memória. Após, alguns elementos da bioquímica da consolidação da memória são apresentados no intuito de permitir establecer um entendimento das vias da PKA e da MAPK e suas correlações com a via final comum – a síntese protéica. Adicionalmente a dissociação STM e LTM é discutida a partir do referencial farmacológico. Uma última unidade apresenta conceitos primitivos do papel da amígdala na modulação da memória e das evidências da implicação das emoções, via amígdala, na modulação da memória em humanos.

Os experimentos utilizando a esquiva inibitória como paradigma e o rato como sujeito ocupam os **Capítulos 2, 3 e 4.** No **Capítulo 2** é apresentado um corpo de resultados que permite observar uma dissecção farmacológica da STM e LTM. Os dados demonstram um envolvimento de fenômenos dependentes de PKA em ambas STM e LTM, dependentes de MAPK apenas na STM, e dependentes de síntese protéica apenas na LTM.

O **Capítulo 3** apresenta um trabalho realizado em colaboração com o Prof. Steven P. R. Rose (Open University, UK), que envolve a determinação dos momentos sensíveis à inibição da síntese protéica na consolidação da LTM. Foram observados dois momentos: um inicial, junto ao treino, e um tardio após 3h. Além disso, foi possível demonstrar que um treino prévio de baixa intensidade, mas não a pré-exposição ao aparato, pode impedir o estabelecimento de amnésia induzida pelo bloqueio da síntese protéica.

O **Capítulo 4** estende os achados com anisomicina observados no **Capítulo 3**, estudando também o inibidor da PKA, Rp-cAMPs, e o inibidor da MAPKK, PD 098059. Os dados obtidos confirmam também para essas cascatas a indução em um treino prévio de baixa intensidade de algum fenômeno celular de longa duração que torna o aprendizado de um segundo treino independente de PKA, MAPK ou síntese protéica.

O estudo da dissociação da STM e LTM foi ampliado, agora no modelo humano, no experimento descrito no **Capítulo 6**. Nesse experimento, observamos uma clara influência do conteúdo emocional na LTM, mas a ausência desse efeito na STM.

A discussão geral (**Capítulo** 7) busca integrar esses achados descritos nos capítulos anteriores dentro da nova perspectiva molecular da neurobiologia da memória. Além disso, abre discussão acerca de possíveis novas possibilidades de pesquisa.

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Capítulo 2.

Protein synthesis, PKA and MAP Kinase are differentially involved in short- and long-term memory in rats.

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Short Communication

Protein synthesis, PKA and MAP Kinase are differentially involved in short- and long-term memory in rats

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Abstract

We studied the involvement of hippocampal protein synthesis-, PKA- and MAP kinase-dependent processes in short- (STM) and long-term memory (LTM) for inhibitory avoidance task. Fifteen minutes before or immediately after training rats received intrahippocampal infusions of vehicle, the protein synthesis inhibitor anisomycin, the PKA inhibitor Rp-cAMPs or the MAPKK inhibitor PD098059. Results demonstrated STM recruits PKA and MAPK while LTM depends on PKA activity and protein synthesis during the initial postraining period.

Theme: Neural Basis of Behaviour

Topic: Learning and Memory: Pharmacology

Keywords: Short-term memory, Long-term memory, hippocampus, protein synthesis, cAMP-dependent kinase (PKA), mitogen-activated protein kinase (MAPK)

Memory is not a unitary process and a single learning experience can originate processes with different duration and therefore specific biological purposes. According to duration there are two major forms of memory: Short-term memories (STMs) lasting from minutes to hours and long-term memories (LTMs) lasting days, weeks and even a life time. Since William James (1890) made the distinction between these two memories a major question on neurobiology was whether STM was merely an initial stage of LTM formation an independent phenomena. For almost a century attempts to disengage STM from LTM failed mostly due to methodological limitations that unable the separation of simultaneous processes sharing common neuroanatomical and neural substrates.

For these reasons most of the efforts to understand the neuroanatomical, cellular and molecular basis of memory processing concentrated on LTM. A unique characteristic of LTM is the requirement of a consolidation period during which synaptic structural and functional modifications take place to enable its long-lasting maintenance. During this period LTM is being consolidated the STM system operates and is responsible for the behavioral responses related to the learned information.

Recent studies from our laboratory have shown that, for the one-trial inhibitory avoidance task, a STM memory system operates separately and parallel to LTM formation in the hippocampus and structures related to this area (Izquierdo et al., 1998a, b,c, 1999, Vianna et al., 1999). A series of studies have shown that several pharmacological treatments block STM leaving LTM intact, when given into the hippocampus, entorhinal, parietal cortex and prefrontal cortex (Izquierdo et al., 1999). Some mechanisms underlying STM overlap with those of LTM consolidation, but several

others are independent or operate at specific time periods (Izquierdo et al., 1998a,b,c, 1999, Vianna et al., 1999).

A distinguishing characteristic of long-term memory (LTM) is its sensitivity to inhibitors of protein synthesis (Davis and Squire, 1984). Most findings argue that the critical time for protein synthesis is during or immediately after training (Freemar et al., 1995; Schafe et al., 1999). More recent reports have shown a second time window, hours after training, where protein synthesis inhibition can also cause amnesia (Freeman et al., 1995; Grecksch and Matthies, 1980; Quevedo et al., 1999). LTM consolidation for inhibitory avoidance indeed has been shown to have two periods sensitive to protein synthesis inhibition: first around the training and later 3 h after training (Quevedo et al., 1999).

LTM consolidation also requires the intervention of the cAMP –dependent protein kinase (PKA) and Mitogen-activated protein kinase (MAPK) pathways. Pharmacological inhibition of both catalytic and regulatory subunits of PKA block STM when administered immediately or 3 hour after training session in the hippocampus (Vianna et al., 1999; 2000). Both periods of memory sensitiveness to PKA inhibition are accompanied by PKA enhanced activity in hippocampal CA1 region of animals submitted to inhibitory avoidance training (Bernabeu et al.1997, Vianna et al.,1999; 2000). Moreover, the transcription factor CREB has been shown to be activated during the second period, probably due to PKA phosphorylation on Ser133 (Bernabeu et al.,

1997). Several other authors reported PKA and CREB activation following learning (Bourtchouladze et a., 1998).

The mitogen-actiated protein kinase (MAPK) pathway has been proposed to also contribute to LTM formation and inhibition of MAP kinase kinase (MAPKK), the upstream activator of MAPK, blocks LTM when injected 3 h after inhibitory avoidance training (Walz et al., 1999). Other authors confirmed this findings in other tasks (English and Sweatt, 1997).

The goal of the present experiments was therefore to utilize the inhibitory avoidance task to evaluate the role of PKA- and MAP kinase-signaling pathways, and protein synthesis-dependent mechanisms in the consolidation process of STM and LTM. We used intrahippocampal infusions Rp-cAMPs, a PKA inhibitor, PD098056, a MAP kinase kinase inhibitor, or anisomycin protein synthesis inhibitor, during the acquisition and/or consolidation period of the inhibitory avoidance and evaluated STM and LTM.

Materials and methods

Subjects

A total of 240 male Wistar rats (age, 3-4 months; weight, 220-310 g) were obtained from our breeding colony (Departamento Bioquímica, ICBS, UFRGS, Porto Alegre). They 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 AM). Behavioral procedures were conducted between 13:00 and 16:00 h.

Surgery

Animals were bilaterally implanted under thionembutal anesthesia (30 mg/kg, i.p.) with 9.0-mm guide cannulae aimed 1.0 mm above the dorsal CA1 region of hippocampus. Stereotaxic coordinates were according to the atlas of Paxinos and Watson (1986): A -4.3, L ± 4.0, V 3.4.

Behavioral Procedures

Animals were trained 3 to 7 days after surgery. The inhibitory avoidance apparatus was a 50 X 25 X 25-cm acrylic box whose floor consisted of parallel stainless steel bars (1 mm diameter) spaced 1 cm apart. A 7-cm-wide, 2.5-cm-high platform was placed on the floor of the box against the left wall. Animals were placed on the platform and their latency to step down on the grid with all four paws was measured with an automatic device. In training sessions, immediately after stepping down on the grid, the animals received a 0.4 mA 2.0-s scrambled footshock (Izquierdo et al., 1992; 1997; Quevedo et al., 1999). In test sessions no footshock was administered and the step-down latency (maximum 180 s) was used as a measure of retention (Izquierdo et al., 1992; 1997; Jerusalinsky et al., 1992; Quevedo et al., 1999). In Experiment 1, training-test interval was 1.5 h in order to investigate short-term memory (Izquierdo et al., 1998). Long-term memory was evaluated with a 24 h training-test interval (Izquierdo et al., 1992; 1997; Quevedo et al., 1999) (Experiment 2).

Drugs and Infusion Procedures

Fifteen minutes before or immediately after the inhibitory avoidance training session, an infusion cannula was fitted into the guide cannula in both experiments. The tip of the infusion cannula protruded 1mm beyond the guide cannula and was aimed at the CA1 area of the dorsal hippocampus. The animals received a bilateral 0.8 μl infusion of vehicle (phosphate buffer in saline, pH 7.4), Rp-cAMPs (0.05 μg/side) (Vianna et al., 1999), PD 098059 (50 μM solution) (Walz et al., 1999a,b), or anisomycin (80 μg/side) (Sigma) (Quevedo et al., 1999) via the infusion cannula. Anisomycin was dissolved in a minimal volume of 3N HCl and the solution adjusted to pH 7.2 and brought to a concentration of 100 μg/l by addittion of 3N NaOH (Tiunova et al., 1996). The specific doses of the different drugs used in the present study were chosen precisely because they had all been previously shown to have clear effects on memory formation of a one trial inhibitory avoidance task (Vianna et al., 1999; Walz et al., 1999a,b; Quevedo et al., 1999).

Histology

Postmortem verification of cannulae placements was performed as described in previous papers (Izquierdo et al., 1992; 1997). Briefly, animals were killed by decapitation and 0.8 μl of a solution of 5% methylene blue in saline was infused through the cannulae. Brains were stored in formalin for at least 72 h and cannulae placements were verified by histological examination as explained elsewhere (Bernabeu et al., 1997; Izquierdo et al., 1992, 1997; Quevedo et al., 1999). Infusions spread with a radius of less than 1.0 mm^3, as described before (Martin 1991; Bernabeu et al., 1997; Izquierdo et al., 1992; 1997; Quevedo et al., 1999), and were found to be correct (i.e., within 1.5 mm^3 of a intended

site) in 92 % of the animals. Only data from these animals were included in the final analysis.

Statistical analysis

Data for inhibitory avoidance are shown as median (interquartile range) of step-down latencies. Comparisons of both training and test session step-down latencies between groups were performed using a Mann-Whitney U test. Comparisons between training and test sessions were done using a Wilcoxon test (Quevedo et al., 1999).

Results

Experiment 1: Role of protein synthesis, PKA, and MAP kinase on short-term memory for inhibitory avoidance task.

The first experiment was designed to evaluate the effects of pretraining (- 15 min) or immediate post-training (0 min) infusions of the protein synthesis inhibitor anisomycin, PKA inhibitor Rp-cAMPs, and MAPKK inhibitor PD098059 into the hippocampus on short-term retention of an inhibitory avoidance response (1.5 h training-test interval). The results are shown in Figure 1. There were no significant differences between groups in training performance in any group studied. However, Rp-cAMPs and PD098059 impaired STM retention when injected at either -15 min or 0 min post-training (Mann-Whitney U test, $p < 0.01$). Anisomycin was ineffective in both times of infusion. In saline- and anisomycin-injected groups, but not in animals injected with Rp-cAMPs e PD098059, there were significant training-test differences (Wilcoxon test, $p < 0.01$).

Experiment 2: Role of protein synthesis, PKA, and MAP kinase on long-term memory for inhibitory avoidance task.

The second experiment was designed to evaluate the effects of pretraining (- 15 min) or immediate post-training (0 min) infusions of the protein synthesis inhibitor anisomycin, PKA inhibitor Rp-cAMPs, and MAPKK inhibitor PD098059 into the hippocampus on long-term retention of an inhibitory avoidance response (24 h training-test interval). The results are shown in Figure 2. There were no significant differences between groups in training performance in any group studied. Rp-cAMPs caused impairment of retention

when injected at either -15 min or 0 min (Mann-Whitney U test, $p < 0.01$). Anisomycin was amnestic only when injected at –15 min (Mann-Whitney U test, $p < 0.01$). PD098059 was ineffective in both times of infusion. In saline- and PD098059-injected groups, but not in animals injected with Rp-cAMPs, there were significant training-test differences (Wilcoxon test, $p < 0.01$). Animals injected with anisomycin at 0 min, but not at –15 min, also showed significant training-test differences (Wilcoxon test, $p < 0.01$).

Discussion

The findings of the first experiment reinforce previous demonstrations of STM independence from *de novo* protein synthesis and suggest that STM might be maintained by cellular process involving covalent modifications of synaptic substrates. Our results also suggest that, among the signaling pathways involved in STM processing, the cAMP/PKA and the MAPK cascade might contribute.

The fact that some treatments affected exclusively STM reinforce the recent notion of STM and LTM (Izquierdo et al., 1998a,b,c; 1999; Vianna et al., 1999; 2000), providing additional evidences that both memories can be independently manipulated and respond specifically to pharmacological interference on the cellular events involved on its parallel processing.

The results from the second experiment confirm previous findings from PKA and protein synthesis involvement in the early consolidation period (Bernabeu et al., 1997; Quevedo et al; 1999; Vianna et al., 1999). Moreover, it demonstrates that in the

hippocampus, MAP kinase cascade is involved only in STM events during this period of STM and LTM simultaneous processing.

Taken together these findings reinforce the individual identity of STM and LTM as independent process, and demonstrate that some of the mechanisms involved on their maintainace overlap whereas others are independent or contributes specifically during the post-training period (Izquierdo et al., 1998a,b,c, 1999, Vianna et al., 1999).

Legends to Figures

Figure 1. Role of protein synthesis, PKA, and MAP kinase on short-term memory for inhibitory avoidance task. (footshock intesity, 0.4 mA; training-test interval, 1.5 h). Data are expressed as median (interquartile range) training (white columns) and test (gray columns) session latencies, in seconds. Animals received bilateral 0.8 μl infusions of vehicle, anisomycin (80 μg/side), Rp-cAMPs (0.5 μg/side), or PD098059 (50 μM solution) in the CA1 region of the dorsal hippocampus 15 min before (upper graph) or immediately after (down graph) training. n = 10 to 13 animals per group. * significant difference when compared to control group (Mann Whitney U test, $p < 0.01$). All groups, except Rp-cAMPs and PD098059, showed significant training-test differences (Wilcoxon test, $p < 0.01$).

Figure 2. Role of protein synthesis, PKA, and MAP kinase on long-term memory for inhibitory avoidance task. (footshock intesity, 0.4 mA; training-test interval, 24 h). Data are expressed as median (interquartile range) training (white columns) and test (gray columns) session latencies, in seconds. Animals received bilateral 0.8 μl infusions of vehicle, anisomycin (80 μg/side), Rp-cAMPs (0.5 μg/side), or PD098059 (50 μM solution) in the CA1 region of the dorsal hippocampus 15 min before (upper graph) or immediately after (down graph) training. n = 10 to 13 animals per group. * significant difference when compared to control group (Mann Whitney U test, $p < 0.01$). All groups,

except Rp-cAMPs –15, Rp-cAMPs 0 min and anisomycin –15 min, showed significant training-test differences (Wilcoxon test, $p < 0.01$).

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Figura 1

Figura 2

Capítulo 3.

Two time-windows of anisomycin-induced amnesia for inhibitory avoidance training in rats: protection from amnesia by pre-exposure to the task apparatus.

João Quevedo, Mônica R.M. Vianna, Rafael Roesler, Fernanda de-Paris, Ivan Izquierdo, and Steven P.R. Rose.

Learning & Memory (1999) 6: 600-607.

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Capítulo 4.

Pretraining but not prexposure to the task apparatus prevents the memory impairment induced by blockade of protein synthesis, PKA or MAP kinase in rats.

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Manuscrito em preparação.

Pretraining but not preexposure to the task apparatus prevents the memory impairment induced by blockade of protein synthesis, PKA or MAP kinase in rats.

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Abstract

We have studied the effect of training conditions on hippocampal protein synthesis-, protein kinase A (PKA), and mitogen-activated protein kinase (MAPK)-dependent processes in consolidation of the inhibitory avoidance task. Adult male Wistar rats were trained and tested in a step-down inhibitory avoidance task (0.4 mA footshock, 24 h training-test interval). Fifteen minutes before or 0, 1.5 or 3 hours after training, animals received a 0.8 μl intrahippocampal infusion of the protein synthesis inhibitor anisomycin (80 μg), the PKA inhibitor Rp-AMPs (0.05 μg), the MAPK kinase inhibitor PD 098059 (50 μM solution) or vehicle (phosphate buffer in saline, pH 7.4). The infusion of anisomycin impaired retention test performance in animals injected 15 min before and 3 h after the training session, but not at 0 or 1.5 h post-training. The infusion of Rp-AMPs impaired retention test performance in animals injected 15 min before and 0 or 3 h after the training session, but not at 1.5 h post-training. PD 098050 impaired retention test performance only when injected at 3 h after training session. Pretraining with a low footshock intensity (0.2 mA) 24 h before training prevented the amnestic effect of all drugs studied at 15 min before or 0, 1.5 and 3 h after training. However, simple preexposure to the inhibitory avoidance apparatus did not alter the amestic effects of all drugs. The results suggest that memory processing requires hippocampal mechanisms dependent on protein synthesis, PKA and MAPK kinase at different times after training. A prior weak training session, however, is sufficient to prevent the amnestic effect of anisomycin, Rp-AMPs or PD 098050. These findings suggest that weak training must be sufficient to produce some lasting cellular expression of the experience so that the

enhancement of consolidation of a previously acquired memory is not dependent on protein synthesis, PKA or MAPK.

Introduction

The molecular events required for long-term memory (LTM) consolidation are dependent on protein synthesis (Davis and Squire, 1984). Most findings argue that the critical time for protein synthesis is during or immediately after training (Freemar et al., 1995; Schafe et al., 1999). More recent reports have shown a second time window, hours after training, where protein synthesis inhibition can also cause amnesia (Freeman et al., 1995; Grecksch and Matthies, 1980; Quevedo et al., 1999). LTM consolidation for inhibitory avoidance indeed has been shown to have two periods sensitive to protein synthesis inhibition: first around the training and later 3 h after training (Quevedo et al., 1999).

LTM consolidation also requires the intervention of the cAMP –dependent protein kinase (PKA) and Mitogen-activated protein kinase (MAPK) pathways. Pharmacological inhibition of both catalytic and regulatory subunits of PKA block STM when administered immediately or 3 hour after training session in the hippocampus (Vianna et al., 1999; 2000). Both periods of memory sensitiveness to PKA inhibition are accompanied by PKA enhanced activity in hippocampal CA1 region of animals submitted to inhibitory avoidance training (Bernabeu et al.1997, Vianna et al.,1999; 2000). Moreover, the transcription factor CREB has been shown to be activated during the second period, probably due to PKA phosphorylation on Ser133 (Bernabeu et al.,

1997). Several other authors reported PKA and CREB activation following learning (Bourtchouladze et a., 1998).

The mitogen-actiated protein kinase (MAPK) pathway has been proposed to also contribute to LTM formation and inhibition of MAP kinase kinase (MAPKK), the upstream activator of MAPK, blocks LTM when injected 3 h after inhibitory avoidance training (Walz et al., 1999). Other authors confirmed this findings in other tasks (English and Sweatt, 1997).

The involvement of biochemical events in the hippocampus related to long-term memory formation has been extensively studied in rats using a one trial step-down inhibitory avoidance task (for review see Izquierdo and Medina, 1997). The protein synthesis inhbitor anisomycin was amnestic for the avoidance if injected into the hippocampus 15 min before and 3 h after, but not immediately or 6 h after the training session (Quevedo et al., 1999). However it was recently found that pretraining, but not preexposure to the task apparatus, could prevent the amnesia induced by intahippocampal infusion of anisomycin (Quevedo et al., 1999). These led us to think that different neurochemical mechanisms are involved in the formation of memory of a new training and of a second training in a task in which the animal has been previously trained. While the cascade of neurochemical events induced by a new, single training trial in the step-down inhibitory avoidance task are now well known (Izquierdo and Medina, 1997), the mechanisms involved in processing of a second training and their biological significance deserve further investigation.

The goal of the present experiments was therefore to utilize the inhibitory avoidance task to extend the evaluation of the effects of pretraining on consolidation. For

this prurpose, we studied the effects of pretraining on mechanisms dependent of protein synthesis, protein kinase A and mitogen-activated protein kinase. To do this, we explored the time-dependent interactions between experience of the task apparatus, training, and infusions of anisomycin, RP-cAMPs or PD 098050 on recall of the inhibitory avoidance.

Materials and methods

Subjects

A total of 690 male Wistar rats (age, 3-4 months; weight, 220-310 g) were obtained from our breeding colony (Departamento Bioquímica, ICBS, UFRGS, Porto Alegre). They 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 AM). Behavioral procedures were conducted between 13:00 and 16:00 h.

Surgery

Animals were bilaterally implanted under thionembutal anesthesia (30 mg/kg, i.p.) with a 9.0-mm guide cannulae aimed 1.0 mm above the dorsal CA1 region of hippocampus. Stereotaxic coordinates were according to the atlas of Paxinos and Watson (1986): A -4.3, L ± 4.0, V 3.4.

Behavioral Procedures

Animals were trained 3 to 7 days after surgery. The inhibitory avoidance apparatus was a 50 X 25 X 25-cm acrylic box whose floor consisted of parallel stainless steel bars (1 mm

diameter) spaced 1 cm apart. A 7-cm-wide, 2.5-cm-high platform was placed on the floor of the box against the left wall. Animals were placed on the platform and their latency to step down on the grid with all four paws was measured with an automatic device. In training sessions, immediately after stepping down on the grid, the animals received a 2.0-s scrambled footshock (Izquierdo et al., 1992; 1997; Jerusalinsky et al., 1992; Roesler et al., 1998). The shock intensity was 0.4 mA for animals given only one training session (Experiment 1 and Experiment 3) and 0.2 mA for animals given two training sessions (Experiment 2) (Roesler et al., 1998; Quevedo et al., 1999). In test sessions no footshock was administered and the step-down latency (maximum 180 s) was used as a measure of retention (Izquierdo et al., 1992; 1997; Jerusalinsky et al., 1992; Roesler et al., 1998). In Experiment 1, animals were given a single training session followed by a retention test session 24 h later. In Experiment 2, the animals were given two training sessions separated by a 24-h interval followed by a retention test session carried out 24 h after the second training (Roesler et al., 1998; Quevedo et al., 1999). In Experiment 3, the animals were preexposed to the task apparatus 24 h before training. In this preexposure session, animals were placed on the platform and allowed to explore the box freely for 5 min without footshock (Roesler et al., 1998; Quevedo et al.,1999). A training session was carried out 24 h after preexposure, followed by a test session 24 h after training.

Drugs and Infusion Procedures

Fifteen minutes before or 0, 1.5 or 3 hours after the inhibitory avoidance training session, an infusion cannula was fitted into the guide cannula. The tip of the infusion cannula protruded 1mm beyond the guide cannula and was aimed at the CA1 area of the dorsal

hippocampus. In Experiment 1, infusions were made at 15 min pre, or 0, 1.5 and 3 hr posttraining. In the other experiments, infusions were made at 15 min before or 0, 1.5 and 3 hr after either the single (Experiment 3) or the second (Experiment 2) training session. The animals received a bilateral 0.8 µl infusion of vehicle (phosphate buffer in saline, pH 7.4), anisomycin (80 µg/side) (Sigma) Quevedo et al., 1999), Rp-cAMPs (0.05 µg/side) (Vianna et al., 1999), or PD 098059 (50 µM solution) (Walz et al., 1999) via the infusion cannula. Anisomycin was dissolved in a minimal volume of 3N HCl and the solution adjusted to pH 7.2 and brought to a concentration of 100 µg/l by addittion of 3N NaOH (Tiunova et al., 1996). The dose we used is likely to inhibit most protein synthesis in the hippocampus since it is much higher than doses showed to inhibit protein synthesis in the chick brain (Freeman et al., 1995), and in rat hippocampal slices (Frey and Morris, 1998). The specific doses of different drugs used in the present study were chosen precisely because they had all been previously shown to have clear effects on memory formation of a one trial inhibitory avoidance task (Vianna et al., 1999; Walz et al., 1999; Quevedo et al., 1999).

Histology

Postmortem verification of cannulae placements was performed as described in previous papers (Izquierdo et al., 1992; 1997; Jerusalinsky et al., 1992). Briefly, animals were killed by decapitation and 0.8 µl of a solution of 5% methylene blue in saline was infused through the cannulae. Brains were stored in formalin for at least 72 h and cannulae placements were verified by histological examination. Cannulae were found to be correctly placed in the CA1 region of the dorsal hippocampus in 621 rats (Figure 1). Only

data from these animals were included in the final analysis.

Statistical analysis

Data for inhibitory avoidance are shown as median (interquartile range) of step-down latencies. Comparisons of both training and test session step-down latencies between groups were performed using a Mann-Whitney U test. Comparisons between training and test sessions were done using a Wilcoxon test (Roesler et al., 1998; Quevedo et al., 1999).

Results

Experiment 1: Involvement of protein synthesis, protein kinase A and mitogen-activated protein kinase in memory consolidation for one-trial step-down inhibitory avoidance task

The first experiment was designed to evaluate the effects of pretraining (- 15 min) or post-training (0, +1.5 or +3 h) infusions of the protein synthesis inhibitor anisomycin, the PKA inhibitor Rp-cAMPs, and the MAPK kinase inhibitor PD 098050 into the hippocampus on retention of an inhibitory avoidance response. The results are shown in Figure 2. There were no significant diferences between groups in training performance in any group studied. However, anisomycin caused impairment of retention when injected at either -15 min or +3 h (Mann-Whitney U test, $p < 0.01$) although not at 0 or +1.5 h. Rp-cAMPs induced amnesia when injected at all times, except at +1.5 h (Mann-Whitney U test, $p < 0.01$). PD 098050 was amnestic only when injected at +3 h (Mann-Whitney U test, $p < 0.01$). In all groups, except that were amestic, there were significant training-test differences (Wilcoxon test, $p < 0.01$). This result is consistent with previous studies showing two time windows of anisomycin-induced amnesia in passive avoidance for chicks (Freeman et al., 1995) and rats (Quevedo et al., 1999), and in contextual fear conditioning for mouse (Bourtchouladze et al., 1998). Additionally, we reproduced and extended previous results from our lab showing that Rp-cAMPs induced two time windows of amnesia (Vianna et al., 1999) and PD 098050 was amestic only late after training (Walz et al., 1999).

Experiment 2: Pretraining protects against amnesia induced by anisomycin, Rp-cAMPs and PD 098050

Experiment 2 was designed to determine whether pretraining of the rats on a weaker form of the task altered the amnestic effects of anisomycin, Rp-cAMPs and PD 098050. The results are shown in Figures 3A and B. Infusion of all drugs 15 min before or 0, 1.5 and 3 h after the second training did not affect retention test performance (Mann-Whitney U test, $p > 0.10$). The difference between latencies in the second training compared with the test session was significant in all groups (Wilcoxon test, $p < 0.01$). This result indicates that pretraining prevents the amnesia induced by anisomycin, Rp-cAMPs and PD 098050.

Experiment 3: Preexposure to the task apparatus does not prevent amnesia induced by anisomycin, Rp-cAMPs and PD 098050

In order to determine whether the prevention of amnesia by pretraining observed in experiment 2 is due to the contextual or the aversive component of the inhibitory avoidance task in the next experiment we merely preexposed the animals to the task apparatus without applying footshock, followed 24hr later by a standard training session. Drugs were injected 15 min before or 0, 1.5 or 3 h after the training session. The results are shown in Figure 4 A and B. There were no significant differences between groups in training latencies (Mann-Whitney U test, $p > 0.10$). However, anisomycin caused

impairment of retention when injected at either -15 min or +3 h (Mann-Whitney U test, $p < 0.01$) although not at 0 or +1.5 h. Rp-cAMPs induced amnesia when injected at all times, except at +1.5 h (Mann-Whitney U test, $p < 0.01$). PD 098050 was amnestic only when injected at +3 h (Mann-Whitney U test, $p < 0.01$). In all groups, except that were amestic, there were significant training-test differences (Wilcoxon test, $p < 0.01$). Thus the abolition of the amnesia by pretraining is not a consequence simply of familiarity with the contextual component of the inhibitory avoidance task, but must relate to its aversive element.

Discussion

Our findings lead to three major observations: a) in a single-training procedure, the infusion of anisomycin impaired retention test performance in animals injected 15 min before and 3 h after the training session, but not at 0 or 1.5 h post-training (two time-windows). The infusion of Rp-cAMPs impaired retention test performance in animals injected 15 min before and 0 or 3 h after the training session, but not at 1.5 h post-training (two time-windows). PD 098059 impaired retention test performance only when injected at 3 h after training session (a single time-window); b) pretraining, 24 hr previous to the training experience, blocks the amnestics effects of anisomycin, Rp-cAMPs and PD 098059; c) simple preexposure to the task apparatus does not prevent amnesia induced by anisomycin, Rp-cAMPs and PD 098059.

Pharmacological data have demonstrated the importance of protein synthesis for long-term memory (Davis and Squire, 1984; Freeman et al., 1995; Bourtchouladze et al., 1998; Quevedo et al., 1999). The two time windows for the amnestic effect of protein synthesis blockade that we have found, the first around the time of training and the second at least 3hr but less than 6hr subsequently are in accord with previous studies in chicks (Freeman et al., 1995), rats (Grecksch and Matthies, 1980; Quevedo et al., 1999), and mice (Bourtchouladze et al., 1998). In addition, we have demonstrated that cAMP/PKA pathway is also involved in memory consolidation in a two time-window manner (Bernabeu et al., 1997; Bourtchouladze et al., 1998; Vianna et al., 1999, 2000). MAPK-dependent mechanisms are required in a single late period (Walz et al., 1999). Bourtchouladze et al. (1998) using contextual fear conditioning have reported that the first period of cAMP/PKA-protein synthesis, around the time of training, is common to both weak and strong training protocols, whereas only in the weak protocol are there two sensitive periods, the first around and the latter after training. A similar conclusion has been arrived at using the one-trial passive avoidance task in chicks (Freeman et al 1995), and it has been suggested that while the first wave of protein synthesis is concerned with enhanced expression of immediately-early genes and transcription factors, the second involves the structural proteins, including cell adhesion molecules, required for more lasting synaptic modulation (Anokhin and Rose, 1991; Rose 1995; Bernabeu et al., 1997; Izquierdo and Medina, 1997).

We have reported recently that pretraining, but not preexposure to the task apparatus, prevents the amnesia otherwise induced by intrahippocampal infusion of anisomycin (Quevedo et al., 1999). In the present report, pretraining on a weaker form of

the task, prevented also the amnesia induced by intrahippocampal infusion of Rp-cAMPs and PD 098059.

Together with our previous finding that intrahippocampal infusion of AP5 blocks retention of a new training trial but not of a second training, and with the early finding by Netto and Maltchik (1990) that opioid receptors modulate the first, but not the second, training of inhibitory avoidance, the present results indicate that different neurochemical mechanisms are involved in the formation of memory of a new training and of a second training in a task in which the animal has been previously trained. While the cascade of neurochemical events induced by a new, single training trial in the step-down inhibitory avoidance task are now well known (Izquierdo and Medina, 1997), the mechanisms involved in processing of a second training and their biological significance are, now, also under investigation.

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Legends to Figures

Figure 1. Schematic drawing of plane A - 4.3 of the atlas of Paxinos and Watson (1986), showing, sttipled, the extend of the area reached buy the infusion in the dorsal hippocampus.

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Table 1 - Effects of anisomycin, Rp-cAMPs, and PD 098050 on retention of one trial step-down inhibitory avoidance task in rats (footshock intesity, 0.4 mA; training-test interval, 24 h). Data are expressed as median (interquartile range) training and test session latencies, in seconds. Animals received bilateral 0.8 µl infusions of vehicle, anisomycin (80 µg), Rp-cAMP (0.05 µg), or PD 098050 (50 µM solution) in the CA1 region of the dorsal hippocampus at different times before or posttraining. n = 12 to 15 animals per group. * significant difference when compared to control group (Mann Whitney U test, $p < 0.01$). All groups, except that were amnestic, showed significant training-test differences (Wilcoxon test, $p < 0.01$).

Group	Time of infusion	*N*	Training	Test
V ehicle	- 15 min	15	2.50 (2.00/5.10)	45.05 (38.00/86.06)
Anisomycin	-15 min	13	2.52 (2.00/3.59)	2.69 (2.21/3.62)*
Rp-cAMPs	- 15 min	12	2.49 (2.25/5.33)	2.74 (2.00/6.03)*
PD 098050	- 15 min	14	2.30 (2.06/3.67)	45.35 (29.47/59.56)
V ehicle	0 h	13	2.82 (2.00/4.25)	42.02 (28.27/81.66)
Anisomycin	0 h	12	3.27 (2.57/5.33)	45.00 (32.00/73.71)
Rp-cAMPs	0 h	13	2.60 (1.58/4.27)	3.00 (2.00/5.95)*
PD 098050	0 h	13	3.03 (2.70/5.24)	57.64 (30.23/80.79)
V ehicle	1.5 h	13	5.51 (3.05/7.59)	47.00 (37.64/91.19)
Anisomycin	1.5 h	12	4.88 (3.38/7.32)	50.00 (35.02/81.62)
Rp-cAMPs	1.5 h	12	5.34 (3.37/7.21)	45.23 (43.61/84.28)
PD 098050	1.5 h	13	3.60 (2.79/5.94)	41.70 (32.98/85.36)
V ehicle	3 h	13	4.99 (2.88/8.40)	43.00 (32.63/80.63)
Anisomycin	3 h	12	5.57 (3.15/7.74)	6.28 (4.79/12.01)*
Rp-cAMPs	3 h	12	4.50 (2.63/6.16)	5.24 (4.39/11.96)*
PD 098050	3 h	13	3.91 (2.92/6.86)	4.39 (3.39/9.87)*

Table 2 - Effects of anisomycin, Rp-cAMPs, and PD 098050 on memory of step-down inhibitory avoidance task in rats given previous training (footshock intesity, 0.2 mA; interval between sessions, 24 h). Data of step-down inhibitory avoidance is expressed as median (interquartile range) session latencies, in seconds. Animals received bilateral 0.8 µl infusions of vehicle, anisomycin (80 µg), Rp-cAMP (0.05 µg), or PD 098050 (50 µM solution) in the CA1 region of the dorsal hippocampus at different times before or after the second training session. n = 12 to 14 animals per group. There are no significant difference between groups in any of the three sessions (Mann Whitney U test, $p < 0.10$). All groups showed significant difference between second training and test sessions (Wilcoxon test, $p < 0.01$).

Group	Time of infusion	N	Training 1	Training 2	Test
V ehicle	- 15 min	13	2.50 (1.75/5.35)	19.04 (12.16/31.12)	59.06 (36.52/180.00)
Anisomycin	-15 min	13	2.69 (2.00/3.59)	18.69 (13.25/28.28)	60.03 (34.07/150.02)
Rp-cAMPs	- 15 min	13	2.48 (2.25/5.41)	20.03 (16.22/29.29)	53.23 (35.00/180.00)
PD 098050	- 15 min	13	2.39 (2.11/4.17)	15.13 (10.01/29.32)	58.09 (33.47/180.00)
V ehicle	0 h	14	3.14 (2.09/4.41)	18.14 (10.09/28.41)	55.43 (33.10/155.23)
Anisomycin	0 h	12	3.56 (2.72/5.21)	17.77 (13.83/28.39)	48.88 (32.00/153.67)
Rp-cAMPs	0 h	13	2.73 (1.73/4.50)	15.38 (11.23/29.50)	53.00 (35.61/180.00)
PD 098050	0 h	13	3.22 (2.63/5.39)	15.72 (10.24/27.13)	57.52 (30.27/121.75)
V ehicle	1.5 h	13	5.00 (2.75/7.19)	12.73 (11.77/28.00)	49.00 (35.86/180.00)
Anisomycin	1.5 h	13	4.62 (3.13/6.74)	13.76 (9.03/25.90)	58.86 (35.05/180.00)
Rp-cAMPs	1.5 h	14	5.08 (3.37/6.89)	14.08 (11.37/22.89)	55.16 (43.61/180.00)
PD 098050	1.5 h	12	3.86 (2.82/6.09)	15.00 (9.75/26.22)	53.10 (39.64/180.00)
V ehicle	3 h	14	4.62 (2.69/7.77)	15.52 (9.69/27.07)	61.02 (35.63/150.03)
Anisomycin	3 h	13	5.57 (3.15/7.74)	18.76 (10.65/29.17)	66.28 (39.79/180.00)
Rp-cAMPs	3 h	12	4.50 (2.63/6.16)	15.90 (11.13/29.66)	59.24 (39.39/180.00)
PD 098050	3 h	13	3.90 (2.76/6.86)	17.93 (10.88/29.36)	54.53 (33.39/165.02)

Table 3 - Effects of anisomycin, Rp-cAMPs, and PD 098050 on retention of one trial step-down inhibitory avoidance task in rats preexposed to the task apparatus (footshock intesity, 0.4 mA; training-test interval, 24 h). Retention of step-down inhibitory avoidance is expressed as median (interquartile range) session latencies, in seconds. Animals received bilateral 0.8 µl infusions of vehicle, anisomycin (80 µg), Rp-cAMP (0.05 µg), or PD 098050 (50 µM solution) in the CA1 region of the dorsal hippocampus at different times before and after the training session. N = 12 to 15 animals per group. *significant difference when compared to control group (Mann Whitney U test, $p < 0.01$). All groups, except that were amnestic, showed significant training-test differences (Wilcoxon test, $p < 0.01$).

Group	Time of infusion	*N*	Training	Test
V ehicle	- 15 min	13	2.50 (1.75/5.35)	46.35 (37.07/87.57)
Anisomycin	-15 min	12	2.88 (2.56/4.44)	2.73 (2.21/6.86)*
Rp-cAMPs	- 15 min	13	2.40 (2.25/5.41)	2.78 (2.25/7.55)*
PD 098050	- 15 min	13	2.35 (2.13/4.67)	43.06 (32.97/69.56)
V ehicle	0 h	15	3.07 (2.00/5.20)	45.02 (30.02/85.23)
Anisomycin	0 h	13	3.08 (2.77/5.30)	42.54 (35.00/65.37)
Rp-cAMPs	0 h	12	2.71 (1.63/4.50)	3.71 (2.50/6.00)*
PD 098050	0 h	14	3.10 (2.95/5.74)	55.23 (30.22/85.56)
V ehicle	1.5 h	13	5.10 (3.05/7.79)	47.52/40.64/86.19)
Anisomycin	1.5 h	12	5.00 (3.48/7.82)	51.23 (35.02/83.62)
Rp-cAMPs	1.5 h	13	4.85 (3.88/7.26)	46.00 (41.61/79.28)
PD 098050	1.5 h	13	3.57 (2.83/5.99)	42.70 (33.68/85.61)
V ehicle	3 h	13	5.00 (2.85/8.31)	42.02 (32.64/79.63)
Anisomycin	3 h	13	5.48 (3.12/7.62)	6.30 (4.54/11.01)*
Rp-cAMPs	3 h	13	4.83 (2.99/6.44)	5.53 (4.46/10.46)*
PD 098050	3 h	13	3.98 (3.02/6.91)	4.50 (3.36/10.32)*

Figura

Capítulo 5.

Differential effects of emotional arousal in short- and long-term memory in health adults

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Brief Report

Differential Effects of Emotional Arousal in Short and Long-Term Memory in Healthy Adults*

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ABSTRACT

Recent studies demonstrated important differences between short- and long-term memory mechanisms. Besides, the emotional component has a crucial role in memory formation. This study was carried out to answer whether there is a differential influence of emotional arousal in short and long-term memory in healthy adults. Thirty-one healthy volunteers were divided into two major groups. In the first group long-term memory (LTM) was evaluated, with the testing session one week after training. The second group was tested one hour after training, where short-term memory (STM) was evaluated. Each group was divided in to two subgroups. One half of the volunteers was exposed to an emotionally neutral story, and the other half of each group was exposed to a closely matched but more emotionally arousing story. The testing session consisted of a questionary containing 80 questions of multiple choices. The results were evaluated through percentage of correct answers. Results showed that correct answers were increased, in LTM measures, in the subjects that were given the emotional version of the test. In STM measures, no differences were found between the emotional and neutral version. However, the presentation of emotional story caused an emotional reaction in both groups. The lack of effect of emotional arousal in STM suggests that amygdala is not related to STM mechanisms. Further studies using different approaches are needed to elucidate if STM processes are influenced by emotional arousal.

There is little doubt that memory for emotional arousing events is better than for neutral stimuli. This is clearly adaptative, because emotional stimuli, whether pleasant or aversive are generally more important to species survival (Hamann, Ely, Grafton, Kilts, 1999). Evidence indicates that emotional memories established through the amygdala are impervious to extinction and forgetting processes (Cahill, 1995; Ledoux 1996). These findings are consistent with the hypothesis that emotional responses influence memory, at least in part, through amygdala by modulating long-term memory storage (Cahill & McGaugh, 1996; Bianchin, Mello-e-Souza, Medina & Izquiedo, 1999). During and immediately after emotionaly arousing or stressful situations, several physiological systems are activated, including the release of many hormones (Stratakis, Chrousos, 1995, Roozendaal, Quirarte & McGaugh, 1998). Several of these substances like adrenal hormones, corticotropin, prolactin, vassopressin and opioid peptides are known to modulate memory storage (McEwen & Magarinos, 1997; Newcomer, Craft, Hershey, Askins & Bardgett, 1994; Kirschbaum, Wolf, May, Wippich, Hellhammer, 1996; Cahill, 1998; McGaugh, 2000). Evidence indicates that epinephrine and glucocorticoid effects on memory are mediated by influences involving amygdala (Roozendaal, Quirarte & McGaugh, 1998). The basolateral nucleus of the amygdala is critically involved in the glucocorticoid influence since lesions or of infusion of B-adrenergic receptor antagonist at this area block the effects of glucocorticoids (McGaugh, 2000). Considerable evidence indicates that amygdala has a crucial role in enhancing the strength of long-term memory for emotional events through the interaction of peripheral adrenenergic systems with cholinergic, opioid-peptidergic and GABAergic systems (Hamann, Ely, Grafton, Kilts, 1999).

There is strong evidence that amygdala is involved in modulating long-term storage, for many reasons, like the compatibility between studies using human subjects and those of animal experiments (McGaugh, 2000). Studies using B-blockers and amygdala lesions showed a reduced effect of emotional arousal on memory (Cahill, Prins, Weber & McGaugh, 1994). Most current evidence indicates that the amygdala is not a site of storage of memory processes, but has a modulatory function (McGaugh & Izquierdo, 2000). Barros et. Al. described that drugs that facilitate memory given 3h posttraining into rat CA1 region reverse the amnestic effect of KN62, an inhibitor of memory biochemical cascade, given into amygdala 0 h after training, but not that of KN62 given into CA1 0 h posttraining (Barros, Izquierdo, Sant´Anna, Quevedo, Medina, McGaugh & Izquierdo, 1999). Additionally, 3-week recall of emotional material is highly correlated with positron emission tomography activation of the right amygdala during encoding (McGaugh, Cahill & Roozendaal, 1996). Furthermore, studies examing the consequences of visual cortex lesions on the acquisition and extinction of fear responses conditioned to visual stimuli showed that the animals failed to extinguish over the period of one month (Ledoux, 1992). This suggested that emotional memories involving subcortical inputs to the amygdala are highly resistant to extinction. The amygdala is believed to influence memory through its direct connection with hippocampus and entorhinal cortex (Izquierdo & Medina, 1997). This connection appears to have a time-limited role in memory formation.

Recent findings described differences between short-term memory and long-term memory in step-down inhibitory task, suggesting that different mechanisms are involved in each one (Izquierdo, Barros, Mello-e-Souza, Izquierdo & Medina, 1998;

Izquierdo, Medina, Vianna, Izquierdo & Barros, 1999). Short-term memory was defined as that measured while LTM becomes effectively consolidated, i.e., in the first 3-6 h after acquisition (Izquierdo & Medina, 1997). During this time, biochemical events take place in the hippocampus and elsewhere, which culminate by gene transcription and protein synthesis that are necessary for LTM consolidation. This definition of STM stems from the classical studies of James and McGaugh (James, 1890; McGaugh, 1966). Long-term memory becomes fully consolidated only several hours after acquisition. Many studies suggest that this process involves hippocampus, entorhinal cortex, and other cortical regions and that it is modulated early after training by the amygdala (Cahill & McGaugh, 1996; Izquierdo & Medina, 1997). Emerging evidence from Bianchin et al. suggests that the influence of amygdala on long-term memory occurs independent of working memory and short-term memory and that amygdala is uninvolved in the last two kinds of memory in step-down inhibitory avoidance task (Bianchin, Mello-e-Souza, Medina & Izquierdo, 1999).

Considered together, these findings provide support to investigate the influence of emotional arousal on STM and LTM in healthy adults. Since amygdala, that appears to mediate most of the emotional influence on memory, have been shown different involvement in STM and LTM, this allows to evaluate the importance of the emotional content to memory formation in these two different moments in the time window. According to the memory consolidation hypothesis and experimental animal data, we expect that emotional arousing increases long-term memory, possibly through amygdala modulation of hippocampal activity, but do not affect short-term memory, since little

consolidation has taken place at this period and the two kinds of memory may have different mechanisms.

Thirty-one healthy volunteers (male, University students, age between 18 – 30) had an individual explanation about study objectives, which persuade the subjects to believe it was a cardiologic physiology evaluation in variable conditions. Then, the informed consent was obtained. University Hospital Ethics Committee approved the protocol. Subjects were exposed to an emotionally neutral story, or a closely matched but more emotionally arousing story (Cahill, Prins, Weber & McGaugh, 1994). The stories were presented as a brief (about 5 min) narrated slide show. The entire presentation consisted of 11 slides accompanied by narration, which could be emotional or neutral (Cahill, Prins, Weber & McGaugh, 1994). The story was divided into three phases; the first including the slides 1-4, the second including slides 5 –8 and the final with slides 9–11. In phase one the narration was identical in the neutral and in the emotional version of the story, in phase three it was nearly identical. The emotionally arousing narration occurred in the middle (phase 2) of the story. Immediately after training, each subject was asked to match in a zero to ten scale how emotional he thought the story was. Subjects watched the slide presentation individually and heart rate and blood pressure were measured while viewing the story. They were told that the study was conducted in order to evaluate physiological responses to different types of stimuli. One hour or one week later volunteers were submitted to a surprise memory-recall test. Subjects were divided into two major groups in which the difference between them was the time they did the testing session. In the first group (n=14) long-term memory was evaluated, with the testing session one week after slide show. The second group (n=17) was tested one hour

after training, considering STM. In the period of time between slide presentation and test session, the group that had to wait one hour was conducted to a neutral environment where they were allowed to read books or previous selected magazines and watch some documentary on VCR. The group that performed the memory test one week later went back home and came next week at right time of testing. Both groups were recommended not to comment about the presentation they had just seen. The testing session consisted of a questionary containing 80 questions of multiple choices. The questionary consisted of 5 to 8 questions for each slide, and was presented in the same order of the story. The questions were presented only once and the subject was asked to choose one answer and then go on to the next one.

Results are presented as length measure in centimeters for self-rating emotional scale and as % of correct answers for the questionary of multiple choices. Data for emotional scale and % for correct answers are shown as media ± S.E.M. Comparisons between emotional and neutral groups were performed using an independent t-test. Differences between each phase were performing using a paired t-test. In all comparisons, $p < 0.05$ was considered to indicate statistical significance.

The results for LTM are shown in figure 1. Percentage for correct answers was enhanced in phase 2 of emotional version ($p < 0.05$). Phase 1 and phase 3 have similar % for correct answers. Comparisons within groups showed similar % for correct answers across phases in neutral version, while % for correct answers in phase 2 of emotional version was enhanced when compared to phase 1 and 3. Self-rating for emotionally shown an enhancement of subjective emotion for subjects submitted to emotional version ($p < 0.05$).

Figure 2 presents the results for STM. There were no differences in % for correct answers between groups and across phases within groups. However, self-rating for emotionally shown an enhancement of subjective emotion for subjects submitted to emotional version ($p < 0.05$).

Our results demonstrated that emotional arousal enhanced LTM but not STM. Several lines of evidence indicates that amygdala play a key role in modulation of emotional memory (Cahill & McGaugh, 1996; Ledoux, 1992) for both human (Cahill & McGaugh, 1998; McGaugh, Cahill & Roozendaal, 1996) and non-human subjects (Bianchin, Mello-e-Souza, Medina & Izquierdo, 1999; Barros, Izquierdo Sant´Anna, Quevedo, Medina, McGaugh & Izquierdo, 1999). Recent data from our (Izquierdo, Barros, Mello-e-Souza, Souza, Izquierdo & Medina, 1998) and other laboratories (Mcgaugh, 2000) demonstrated that STM and LTM require different neuroanatomical (Bianchin, Mello-e-Souza, Medina & Izquierdo, 1999) and biochemical (Izquierdo, Medina, Izquierdo, Barros, Souza & Mello-e-Souza, 1998; Medina, Schröder & Izquierdo, 1999) components. Consistent with studies of patients with amygdala lesions, positron emission tomography (PET) studies provide additional support for the hypothesis that the amygdala is selectively involved with long-term memory for emotionally arousing events. Studies using PET scans to assess amygdala activity induced by emotionally arousing stimuli found that long term memory correlates with the degree of amygdala activation during original encoding (McGaugh, 2000; Cahill & McGaugh, 1998; Hamann, Ely, Grafton, Kilts, 1999). Other PET study demonstrated that viewing emotionally arousing films activated amygdala, but recall of previous learned emotional events does not, consistent with the time limited role of amygdala in memory

formation (Cahill & McGaugh, 1998). Results from Bianchin et al. (1999) using step-down inhibitory avoidance paradigm showed an absence of involvement of amygdala for both work- and short-term memory despite a strong role for LTM. The lack of effect of emotional arousal in STM points an additional evidence to theory of a non–involvement of amygdala in STM mechanisms. Further studies using different approaches are needed to elucidate if STM processes are influenced by emotional arousal.

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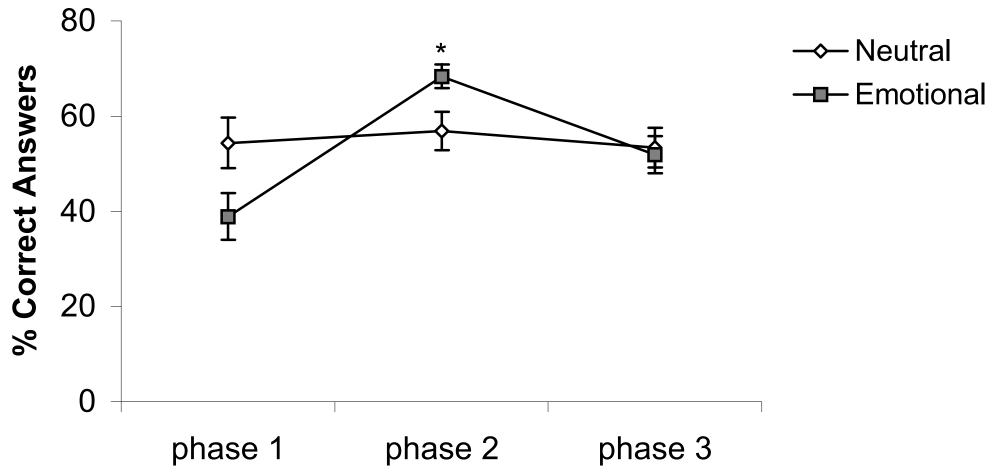
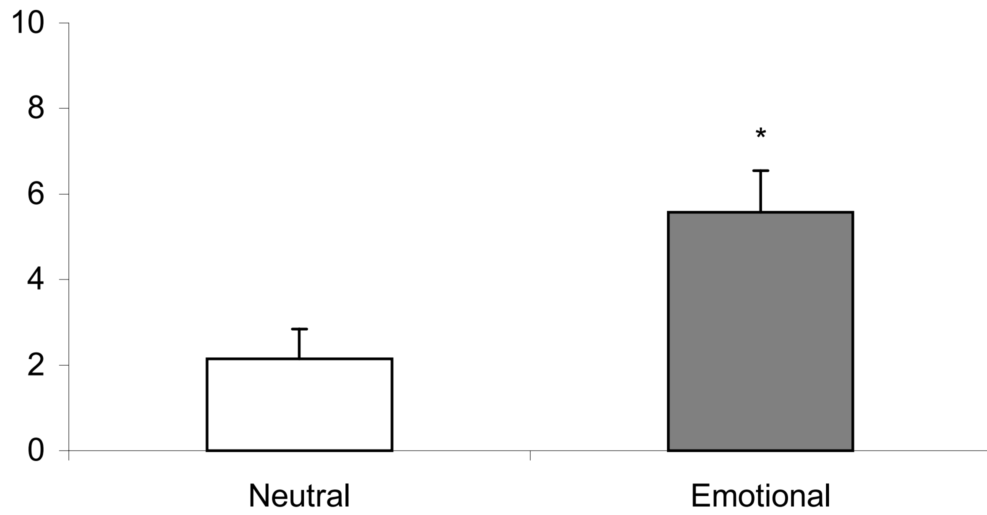
LEGENDS TO FIGURES

FIG 1. Long-Term Memory was measured (i.e., test session after one week). A. There was a significant ($p<0,05$) difference between the group, which attempt to neutral version and the group, which saw emotional one in relation to % of correct answers during phase 2. B. In the self-rating scale about emotional impact of the story, there was a significant ($p<0,05$) difference between neutral and emotional group.

FIG 2. Short-Term Memory was measured (i.e., test session after one hour). A. There was no ($p>0,05$) difference between the neutral group and the emotional one in relation to % of correct answers. B. In the self-rating scale about emotional impact of the story, there was a significant ($p<0,05$) difference between neutral and emotional group.


1 Week Memory
80
60
40
20
0
% Correct Answers
*
Neutral
Emotional
phase 1
phase 2
phase 3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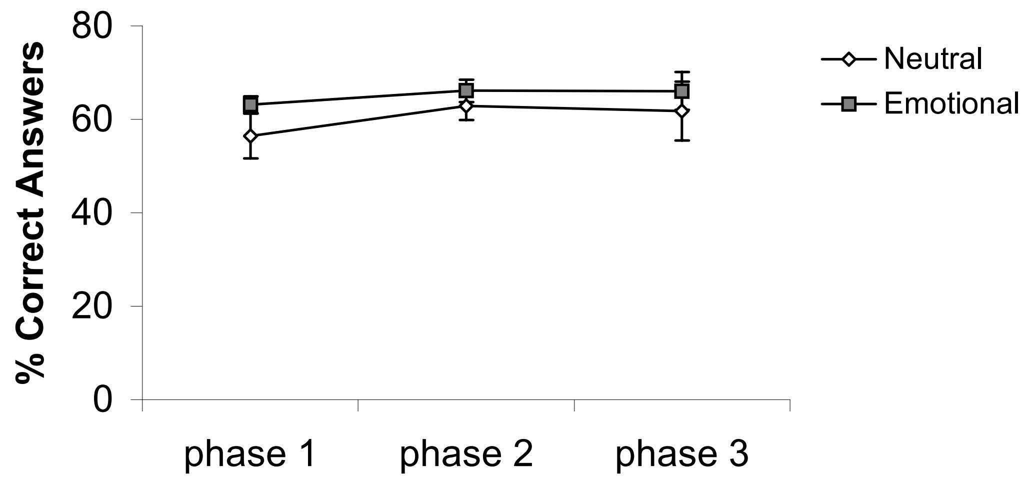
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% Correct Answers
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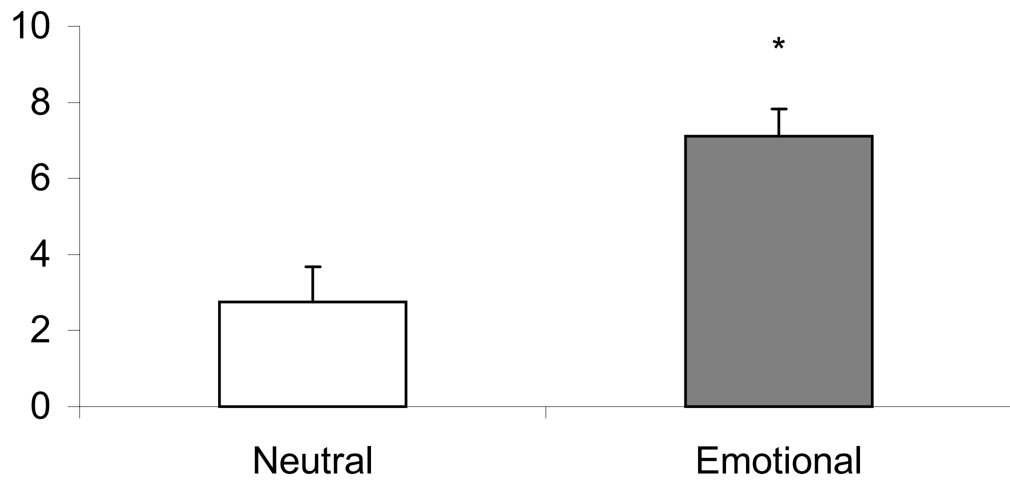
phase 1
phase 2
phase 3
Neutral
Emotional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Capítulo 6. Discussão Geral

Capítulo 6. Discussão Geral

6.1. STM x LTM: esquiva inibitória

Os resultados do primeiro experimento descrito no **Capítulo 2** reforçam demonstrações prévias de que a STM independe da síntese de novas proteínas e sugere que a LTM deve ser mantida por processos celulares envolvendo modificações covalentes de substratos sinápticos. Nossos resultados também sugerem que, entre as vias envolvidas no processo da STM, as vias das cascatas da cAMP/PKA e MAPK devem contribuir.

O fato de que alguns tratamentos afetam exclusivamente a STM reforça os recentes conceitos de STM e LTM (Izquierdo et al., 1999 a,b,c; 1999; Vianna et al., 1999; 2000), provendo evidências adicionais de que ambos os tipos de memórias podem ser independentemente manipuladas e respondem especificamente a interferências farmacológicas nos eventos celulares e seu processamento paralelo.

Os resultados do segundo experimento descrito no **Capítulo 2** confirmam achados prévios do envolvimento da PKA e da síntese protéica nos períodos iniciais da consolidação (Bernabeu et al., 1997; Quevedo et al., 1999; Vianna et al., 1999). Além disso, é demonstrado que, no hipocampo, a cascata da MAP kinase está envolvida apenas nos eventos relacionados à STM nesse período em que ambas STM e LTM se processam simultaneamente.

Tomados conjuntamente, esses dados reforçam a identidade individual da STM e LTM como processos independentes, e demonstram que alguns dos mecanismos envolvidos na sua manutenção sobrepõem-se enquanto outros são independentes e

contribuem especificamente durante o processo pós-treino (Izquierdo et al., 1998a, b, c, 1999; Vianna et al., 1999).

6.2. Síntese protéica, PKA e MAPK

Os resultados apresentados nos **Capítulos 3 e 4** levam a 3 conclusões principais. Primeiro, há duas janelas de ação da anisomicina (síntese protéica) e do Rp-cAMPs (PKA) no processo de consolidação da memória para esquiva inibitória, ao passo que o PD 098059 (MAPK) tem apenas uma ação tardia (3 h). Segundo, pré-treino, 24 h antes da sessão de treino, impede o efeito amnésico da anisomicina, Rp-cAMPs e PD 098059, inclusive quando injetados naqueles momentos em que sabidamente são amnésicos no paradigma convencional. Terceiro, a pré-exposição simples ao aparato não previne esses efeitos amnésicos.

Vários estudos recentes têm demonstrado a importância da síntese protéica para a memória de londa duração (Davis e Squire, 1984; Freeman et al., 1995; Bourtchouladze et al., 1998). Duas janelas de efeito amnésico do bloqueio da síntese proteica foram encontradas – a primeira no período do treino e o segundo 3 h, mas menos de 6 h – e estão consistentes com estudos prévios em ratos e pintos (Grecksch e Matthies, 1980; Freeman et al., 1995) . Bourtchouladze et al. (1998), usando *contextual fear conditioning*, relataram recentemente que o primeiro período, junto ao treino, é comum tanto a protocolos com treinos de baixa e de alta intensidade, enquanto apenas nos protocolos com treino de pouca intensidade ocorrem dois períodos sensíveis ao bloqueio da síntese protéica, o primeiro junto ao treino, e o segundo mais tardiamente. Uma conclusão similar foi encontrada para esquiva inibitória e pintos (Freeman et al., 1995; Scholey et

al., 1995), e tem sido sugerido que enquanto a primeira onda de síntese protéica está relacionada com o aumento da expressão de *immediately early genes* e fatores de transcrição, a segunda envolve proteínas estruturais, incluindo moléculas de adesão celular, necessárias para modulação sináptica de mais longa duração (Anokhin et al., 1991; Rose, 1995a, b; Bernabeu et al., 1997; Izquierdo e Medina, 1997).

Recentemente, nós havíamos descrito que tanto pré-treino quanto pré-exposiçào ao aparato de esquiva inibitória prevenia a amnésia induzida pela administração intra-hipocampal de AP5 (Roesler et al., 1998). Nesses experimentos aqui descritos, apenas o pré-treino em uma forma menos intensa da tarefa preveniu a amnésia induzida pela infusão intra-hipocampal de anisomicina, Rp-cAMPs e PD 098059. Está claro que os efeitos descritos em nossos experimentos são treino específicos, pois a simples pré-exposição ao aparato não foi capaz de prevenir a amnésia, levando-nos à conclusão de que a pré-exposição por si só não resulta em relevante síntese protéica.

Em resumo, foi demonstrado que a consolidação normal da esquiva inibitória requer duas ondas de síntese protéica, mas em uma versão menos intensa da tarefa distribuída ao longo de duas sessões de treino, tratamentos bloqueadores da síntese protéica, PKA ou MAPK administrados após o segundo treino não exercem efeito sobre a memória.

6.3. STM x LTM: memória emocional em humanos

Nossos resultados descritos no **Capítulo 5** demonstram que o componente emocional facilita a LTM, mas não a STM. Várias evidências indicam que a amígdala tem um papel chave na modulação da memória emocional (Cahill e McGaugh, 1996;

Ledoux, 1992) para humanos (Cahill e McGaugh, 1998; McGaugh et al., 1996) e não-humanos (Bianchin et al., 1999; Barros et al., 1999). Dados recentes de nosso laboratório (Izquierdo et al., 1998 a.,b,c; 1999) e outros laboratórios (McGaugh, 2000) demonstram que STM e LTM requerem diferentes substratos neuroanatômicos (Bianchin et al., 1999) e bioquímicos (Izquierdo et al., 1999). Consistente com estudos compacientes com lesões de amígdala, estudos com PET proveram um suporte adicional para a hipótese de que a mígdala está seletivamente envolvida com a memória de longa duração para eventos com componente emocional. Estudos utilizando PET para avaliar a atividade da amígdala induzida por estímulos de conteúdo emocional demonstraram que a LTM está correlacionada com o grau de ativação da amígdala durante a consolidação (McGaugh, 2000; Cahill e McGaugh, 1998). Outros estudos com PET demonstraram que a exposição a filmes com conteúdo emocional, mas não a evocação de eventos emocionais aprendidos, ativam a amígdala (Cahill e McGaugh, 1998). Os resultados de Bianchin et al. (1999) utilizando a esquiva inibitória demonstraram a ausência de envolvimento da amígdala para a memória de trabalho e para a STM, apesar de um envolvimento importante na LTM. Nosso resultado é consistente com esses achados no modelo animal. Novos estudos deverão ser empreendidos para determinar se as emoções exercem algum papel modulatório sobre a STM.

6.4. Considerações gerais e perspectivas futuras

A compreensão, mesmo que ainda não definitiva, dos fenômenos neurobiológicos envolvidos na consolidação da memória evoluiu sobremaneira nesta última década. Os conceitos, contudo, não foram alterados; ocorreu, de fato, um aprofundamento do

entendimento de seu substrato biológico. Os resultados compilados nesta dissertação compreendem uma pequena fração desse corpo de novos conhecimentos, mas depõem fortemente no sentido de dissociar as memórias de curta e longa duração quanto aos seus mecanismos de consolidação. O novo desafio é, no modelo humano, demonstrar essa mesma fenomenologia. Novos estudos devem ser realizados utilizando outras tarefas e/ou intervenções farmacológicas. Um dos ramos mais fascinantes da neurobiologia, o estudo da memória, é uma fonte inesgotável de mistérios biológicos a serem esclarecidos.

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Anexo.

Produção científica durante o doutoramento (1999-2002)

Anexo. Produção científica durante o doutoramento (1999-2002)

1. FORMAÇÃO ACADÊMICA

Especialização:

Curso de Especialização em Psiquiatria, Departamento de Psiquiatria e Medicina Legal, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul - UFRGS, no período de 1999 a 2000, Porto Alegre-RS.

2. TRABALHOS APRESENTADOS EM EVENTOS CIENTÍFICOS

2.1. Internacionais:

"What may go wrong when memory fails: the main biochemical events underlying memory consolidation and retrieval in rats", International Meeting of Neurotoxicology, Degeneration and Protection in Brain Disease States, Fundación Cerebro y Mente, October 15-19, 1999, Mojacar-Espanha.

2.2. Nacionais:

"Ação da gabapentina na ansiedade e memória em modelos animais", XVII Congresso Brasileiro de Psiquiatria, promovido pela Associação Brasileira de Psiquiatria, no período

de 13 a 16 de outubro de 1999, Fortaleza-CE.

“Efeito ansiolítico dos extratos hidroetanólicos das folhas de passiflora edulis em ratos”, XVII Congresso Brasileiro de Psiquiatria, promovido pela Associação Brasileira de Psiquiatria, no período de 13 a 16 de outubro de 1999, Fortaleza-CE.

“Mirtazapina versus fluoxetina no tratamento do transtorno do pânico: ensaio clínico randomizado”, XVII Congresso Brasileiro de Psiquiatria, promovido pela Associação Brasileira de Psiquiatria, no período de 13 a 16 de outubro de 1999, Fortaleza-CE.

“Use and abuse of benzodiazepines in Latin America”, XVII Congresso Brasileiro de Psiquiatria, promovido pela Associação Brasileira de Psiquiatria, no período de 13 a 16 de outubro de 1999, Fortaleza-CE.

3. ATUAÇÃO PROFISSIONAL

Professor Titular, Departamentos de Medicina, Farmácia e Psicologia, Universidade do Extremo Sul Catarinense - UNESC, Criciúma-SC, ingresso em agosto de 2000.

4. PUBLICAÇÕES

4.1. Artigos em periódicos indexados:

4.1.1. Internacionais:

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but not haloperidol, increases striatal ecto-5'-nucleotidase activity in rats. Neuropsychobiology 44(2): 99-102, 2001.

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DE-PARIS, F.; NEVES, G.; SALGUEIRO, J.B.; **QUEVEDO, J.L.**; IZQUIERDO, I.; RATES, S.M. Psychopharmacological screening of Pfaffia glomerata Spreng. (Amarathanceae) in rodents. Journal of Ethnopharmacology 73(1-2): 261-9, 2000.

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ROESLER, R.; VIANNA, M. R.; PARIS, F.; **QUEVEDO, J. L**. 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, 1999.

ROESLER, R.; WALZ, R.; **QUEVEDO, J. L.**; PARIS, F.; ZANATA, S. M.; GRANER, E.; IZQUIERDO, I.; MARTINS, V. R.; BRENTANI, R. R. Normal inhibitory avoidance learning and anxiety, but increased locomotor activity in mice devoid of PrPc. Molecular

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BARROS, D. M.; IZQUIERDO, L. A.; SANT'ANNA, M. K.; **QUEVEDO, J. L.**; MEDINA, J. H.; MCGAUGH, J. L.; IZQUIERDO, I. Stimulators of the cAMP cascade reverse amnesia induced by intra-amygdala but not intrahippocampal KN-62 administration. Neurobiology of Learning and Memory, 71(1): 94-103, 1999.

QUEVEDO, J. L.; VIANNA, M. R. M.; ROESLER, R.; PARIS, F.; IZQUIERDO, I.; ROSE, S. P R. Two time windows of anisomycin-induced amnesia for inhibitory avoidance training in rats: protection from amnesia by pretraining but not preexposure to the task apparatus. Learning Memory, 6:600-607, 1999.

4.1.2. Nacionais:

SHANSIS, F. M.; BUSNELLO, J. V.; **QUEVEDO, J. L.**; FORSTER, L.; YOUNG, S.; IZQUIERDO, I.; KAPCZINSKI, F. Effects of acute tryptophan depletion in healthy male volunteers. Revista de Medicina ATM, 20(1): 26-30, 2000.

QUEVEDO, J. L.; AMARAL, O. B.; WALZ, R.; KAPCZINSKI, F. Pathogenesis of hepatic encephalopathy - a role for the benzodiazepine receptor? Medicina, 32:82-96, 1999.

4.2. Capítulos de livros:

4.2.1. Internacional:

IZQUIERDO, I.; **QUEVEDO, J. L.**; IZQUIERDO, L.A.; VIANNA, M.R.M.; SZAPIRO, G.; BARROS, D.M.; ROESLER, R.; ALONSO, M.; DE SOUZA, M.M.; WALZ, R.; MEDINA, J.H. **What can go wrong when memory fails: the main biochemical events underlying consolidation and retrieval in rats.** In: PALOMO, T.; BENINGER, R.J.; ARCHER, T. Neurodegenerative Brain Disorders. Madri: Editorial Sintesis, 2000.

IZQUIERDO, I.; MEDINA, J.; ARDENGHI, P.; BARROS, D.; BEVILAQUA, L.; IZQUIERDO, L.; SOUZA, T. M.; **QUEVEDO, J. L.**; SCHRÖDER, N. **Memory processing and its shifting maps: interactions between monoamines and events dependent on glutamatergic transmission**. In: PALOMO, T.; BENINGER, R. J.; ARCHER, T. Interactive Monoaminergic Disorders. Madri:Editorial Sintesis, 1999.

4.2.2. Nacionais:

VIEIRA, R. M.; TREVISAN, J.; **QUEVEDO, J.L.;** KAPCZINSKI, F. Intoxicação e efeitos adversos graves dos psicofármacos. In: KAPCZINSKI, F.; QUEVEDO, J. L.; SCHMITT, R.; CHACHAMOVICH E. Emergências Psiquiátricas. Porto Alegre:Artes Médicas, 2001.

RIBEIRO, L.; BUSNELLO, J.V.; **QUEVEDO, JL.;** KAPCCZINSKI, F. Ansiedade

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SABBI, E.H.; **QUEVEDO, J.L.;** ALMEIDA, J.T.A. Emergências psiquiátricas no idoso. In: KAPCZINSKI, F.; QUEVEDO, J. L.; SCHMITT, R.; CHACHAMOVICH E. Emergências Psiquiátricas. Porto Alegre:Artes Médicas, 2001.

SABBI, E.H.; **QUEVEDO, J.L.;** MAJOLA, R.R. Atendimento domiciliar e remoção psiquiátrica emergencial. In: KAPCZINSKI, F.; QUEVEDO, J. L.; SCHMITT, R.; CHACHAMOVICH E. Emergências Psiquiátricas. Porto Alegre:Artes Médicas, 2001.

BUSNELLO, J.V.; MADRUGA, M.; SANT'ANNA, M.K.; TRAMONTINA, J.; **QUEVEDO, J.L.** Apêndice. In: KAPCZINSKI, F.; QUEVEDO, J. L.; SCHMITT, R.; CHACHAMOVICH E. Emergências Psiquiátricas. Porto Alegre:Artes Médicas, 2001.

PARIS, F.; VIANNA, M. R. M.; ROESLER, R.; **QUEVEDO, J. L. Neurotransmissores**. In: KAPCZINSKI, F.; QUEVEDO, J. L.; IZQUIERDO, I. Bases Biológicas dos Transtornos Psiquiátricos. Porto Alegre:Artes Médicas, 2000.

SANT'ANNA, M. K.; **QUEVEDO, J. L.**. **Psiconeuroendocrinologia**. In: KAPCZINSKI, F.; QUEVEDO, J. L.; IZQUIERDO, I. Bases Biológicas dos Transtornos Psiquiátricos. Porto Alegre: Artes Médicas, 2000.

ROESLER, R.; **QUEVEDO, J. L.**; IZQUIERDO, I. **Neurobiologia da memória**. In: KAPCZINSKI, F.; QUEVEDO, J. L.; IZQUIERDO, I. Bases Biológicas dos Transtornos Psiquiátricos. Porto Alegre: Artes Médicas, 2000.

PIZZOL, F. D.; KLAMT, F.; **QUEVEDO, J. L.**. **Demências**. In: KAPCZINSKI, F.; QUEVEDO, J. L.; IZQUIERDO, I. Bases Biológicas dos Transtornos Psiquiátricos. Porto Alegre: Artes Médicas, 2000.

4.3. Livros organizados:

KAPCZINSKI, F.; **QUEVEDO, J.L.;** SCHMITT, R.; CHACHAMOVICH E. (Org.). **Emergências Psiquiátricas**. Porto Alegre: Artes Médicas, 2001.

KAPCZINSKI, F. P.; **QUEVEDO, J. L.**; IZQUIERDO, I. A. (Org.). **Bases Biológicas dos Transtornos Psiquiátricos**. Porto Alegre: Artes Médicas, 2000.