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PARTICIPAÇÃO DO CÓRTEX CINGULADO E DO CÓRTEX PRÉ-FRONTAL NA MEMÓRIA DA TAREFA DE ESQUIVA INIBITÓRIA EM RATOS

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Tese apresentada ao Curso de Pós-Graduação em Ciências Biológicas - Bioquímica do Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul para obtenção do título de Doutor em Bioquímica.

Porto Alegre
2001

In Memoriam

Carl Sagan

AGRADECIMENTOS

NO DEPARTAMENTO DE BIOQUÍMICA

Ao Prof. Iván Izquierdo, que, além de MESTRE é, sobretudo, AMIGO.

Aos AMIGOS do Departamento (ou do CPG), pela honra de conhecê-los. Em especial, à Beti, ao Jorge e ao Rod, por orientações passadas.

A todos que colaboraram de alguma forma com o meu trabalho. Em especial, aos integrantes do Centro de Memória.

À Cléia, do Pós-Graduação, por sempre carregar o árduo piano da burocracia.

ÀS INSTITUIÇÕES...

pela universidade pública e gratuita.

pelo apoio financeiro do CNPq e do PRONEX.

AOS AMIGOS DE TODOS OS CANTOS DO MUNDO,

Meu muito obrigado a VOCÊS.

Em especial, à família Vencato, pela confiança e auxílio nos mais diversos momentos

AO AMOR...

de meus pais (Geraldo e Hedwiges), que sempre acreditaram, que sempre têm saudade, e que sempre comigo estarão presentes

de meu irmão Thiago

da minha querida e linda Inajara. Graças a ti, enfim, tornei-me um girassol.

ÍNDICE

1. INTRODUÇÃO

"Nada me lembra mais a igreja de São Raimundo do que o cheiro denso de suor mesclado com solas, cachaça e urina de animais deixados atrás do adro, nos sábados de feira. Os anjos, a pia dos altares, e o grave esvoaçar dos véus das noivas aderiam àquele cheiro híbrido que escalava cinqüenta e dois místicos degraus para se misturar com parábolas, salmos, incenso e à água encardida dos batismos."

Cândido Rolim, em *"Bacia de Rostos"*

1.1 Memória, um processo dinâmico

Aprendizagem e **memória** são propriedades básicas do Sistema Nervoso Central (SNC). Com elas, desenvolvemos estratégias mais adequadas de acordo com experiências passadas. Ambas estão intimamente interligadas e inexistem sem experiências: "Não há nada no intelecto que não tenha estado previamente nos sentidos" (Aristóteles, há dois mil anos, conforme Marshall, 1988) ou pelo menos que não se tenha desenvolvido com a ajuda dos mesmos (Hume, 1748).

Definimos memória, neste trabalho, basicamente como o armazenamento e a evocação de uma informação aprendida (Izquierdo, 1992). Esta se diferencia de outros tipos de memória, tais como a genética e a extra-somática (pinturas gravadas em cavernas, livros, mensagens gravadas em disquetes etc.), igualmente importantes na evolução humana, mas que, mais do que experiências individuais, refletem nossa experiência ao nível de espécie ou de diferentes culturas, respectivamente.

A formação da memória é um processo dinâmico que pode ser dividido basicamente em três fases: (1) **aquisição** da informação através da experiência, seja ela interna ou externa, (2) **consolidação**, onde o traço mnemônico, ainda instável, é processado para o seu (3) **armazenamento** (McGaugh, 1966 e 2000; Izquierdo, 1989; Quillfeldt,

1994). A abordagem fenomenológica nos faculta estudar este processo, bem como avaliá-lo, através da **evocação** da memória, quando observamos a mudança de comportamento do animal devido ao processo de memorização (Quillfeldt, 1994).

1.2 Plasticidade Sináptica, Ontogenia do SNC e Memória

O SNC é constituído de agrupamentos de neurônios, que por sua vez formam sistemas que se interligam entre si de uma forma organizada, formando vários sistemas de processamento de informação (Edelman e Mountcastle, 1978). A comunicação entre os neurônios é feita através das sinapses, conforme já evidenciado por Ramón y Cajal há aproximadamente um século (Bliss e Collingridge, 1993). Modificações das sinapses de uma forma dependente de sua atividade exercem um papel fundamental na ontogenia dos agrupamentos neuronais e, por sua vez, dos sistemas de processamento que estes formam. Esta é uma forma de relevar os fenômenos epigenéticos na ontogenia do SNC, não restringindo o seu desenvolvimento à imposição dos genes (Changeux, 1991). Da mesma forma, e através da experiência, estas modificações modelam a rede neuronal, mesmo após o seu amadurecimento, de forma a torná-la, a princípio, cada vez mais funcional e adaptativa. A memória seria uma das propriedades que emergiriam da integração destas modificações ao longo da rede (Bliss e Collingrigde, 1993; Changeux, 1991; Izquierdo e Medina, 1997; Tsumoto, 1992 e 1993). Uma memória, portanto, tem um traço, que é fisicamente marcado na rede neuronal pelas sinapses e seus neurônios portadores. Este traço foi denominado de *engrama* primeiramente pelo biólogo alemão Richard Semon (Quillfeldt, 1994; Schacter, 1982), termo que utilizaremos.

Um dos primeiros defensores da existência dos fenômenos de plasticidade sináptica dependentes de atividade foi Donald Hebb, em seu livro "*The Organization of Behavior*", em 1949: "Quando uma célula A excita através do seu axônio uma célula B e repetida e persistentemente participa na gênese de um impulso em B, ocorre um processo de crescimento ou uma alteração metabólica em uma ou em ambas as células, de tal modo que é aumentada a eficácia de A em desencadear um impulso em B em relação às outras células que possam fazê-lo". Nesta hipótese, memória e processamento, portanto, estariam intrinsecamente relacionados, desde a origem do traço até a elaboração de novos processamentos e, por conseguinte, de novas respostas comportamentais.

Como as memórias podem durar anos, acredita-se que as alterações sinápticas que lhe sejam o correlato fisiológico também sejam duradouras. Algumas alterações sinápticas dependentes de atividade neuronal não atendem a este requisito, tais como a Potenciação Pós-Tetânica, a Potenciação de Curta Duração (PCD ou, no inglês, STP — "Short-Term Potentiation") e a Facilitação por Pulso Pareado; porém, como a Potenciação de Longa Duração (PLD ou, no inglês, LTP — "Long-Term Potentiation") e a Depressão de Longa Duração (DLD ou, no inglês, LTD — "Long-Term Depression") duram semanas ou meses, tornaram-se fortes candidatas a mediadoras da formação de memórias de longa duração (Bliss e Collingridge, 1993; Izquierdo e Medina, 1997; Tsumoto, 1992 e 1993).

A PLD é classicamente definida como um aumento duradouro (maior que uma hora) nos potenciais excitatórios pós-sinápticos de células individuais ou populações neuronais, induzido por um breve período de estimulação tetânica de alta freqüência (Bliss e Collingridge, 1993). A PLD foi descrita primeiramente por Bliss e colaboradores (Bliss e Gardner-Medwin, 1973 e Bliss e Lømo, 1973). Estes trabalhos utilizaram a via perforante que inerva o giro denteado como alvo de tetanizações. Inúmeros trabalhos atualmente relatam a existência da PLD e da DLD em diversas estruturas além do hipocampo, tais como no isocórtex (Artola e Singer, 1987; Crair e Malenka, 1995; Tsumoto, 1992), na amígdala (Chapman *et al.*, 1990; Clugnet e LeDoux, 1990), no núcleo dorsolateral do septo (Zheng e Gallagher, 1992), no núcleo acúmbeo (Kombian e Malenka, 1994) e nos córtices cingulados anterior (Sah e Nicoll, 1991) e posterior (Hedberg e Stanton, 1995). Devemos ressaltar que a PLD não é um fenômeno homogêneo, mas varia em suas propriedades de estrutura a estrutura, ou mesmo de via a via dentro de uma mesma estrutura (exemplos em Bliss e Collingridge, 1993, e Nicoll e Malenka, 1995).

1.3 Neuroanatomia Funcional da Memória

1.3.1 Sistemas de Memórias ou Memórias de Sistemas?

Squire e Zola (1996) classificam as memórias em **sistemas de memórias**, dividindo-as basicamente entre memórias declarativas (ou explícitas) e não-declarativas (ou implícitas). Por esta classificação, a memória declarativa está acessível à consciência e dela necessita, ao contrário da memória não-declarativa. Portanto, esta abordagem releva o papel

exercido pela consciência na memória. Em acréscimo, esta classificação associa cada memória a uma determinada estrutura (por exemplo, hábitos e habilidades com o estriado, "priming" com o neocórtex).

Entretanto, Fuster (1997) defende a noção que a organização cortical da memória se baseia em **memórias de sistemas**. Segundo Fuster, a memória seria apenas uma propriedade emergente advinda do processamento da informação pelos sistemas cerebrais e, portanto, deve ser classificada conforme o processamento de seus conteúdos. Assim, as memórias estariam distribuídas por sistemas corticais e, não, em estruturas específicas, como defendem Squire e Zola (1996). Segundo Fuster (1997), as memórias seriam classificadas em dois tipos básicos: sensória e motora.

As memórias sensoriais têm como conteúdo fatos, eventos, objetos, pessoas, nomes, conceitos. Estas variam em complexidade: das mais simples, como as das sensações elementares, às mais complexas, como as dos conceitos abstratos. Estas últimas, com o tempo, podem se tornar independentes das primeiras através das operações cognitivas (Fuster, 1997). Este é um tema complexo e antigo. Por sinal, Hume (1748) defende a noção de que estas operações cognitivas jamais seriam realizadas sem a prévia existência da experiência sensorial.

À medida que crescem em complexidade, as memórias sensoriais vão requerendo maior número de sistemas de processamento corticais: as mais simples, apenas áreas sensoriais ou parasensoriais, enquanto as representações de nossa memórias pessoais envolveriam largas porções do córtex associativo (Fuster, 1997). Entretanto, estas memórias também requerem, para sua formação, estruturas diencefálicas e o lobo temporal mesial (Fuster, 1997; Izquierdo e Medina, 1997; Squire e Zola, 1996), que é composto pela formação hipocampal (hipocampo, giro denteado, subículo e córtex entorrinal) e pelos córtices perirrinal e parahipocampal (Zilles e Wree, 1995).

As memórias motoras, por sua vez, relacionam-se a atos motores, como o de aprender a dirigir um carro ou andar de bicicleta. As memórias motoras são mediadas pelas estruturas frontais, dentre as quais: em sua forma mais primitiva, o *córtex motor primário*, que representa e processa os atos motores elementares (Fuster, 1997); o **córtex pré-motor**, que codifica atos e programas motores definidos por uma meta, seqüência ou trajetória (Bear *et al.*, 1995; Fuster, 1997); e o **córtex pré-frontal**, considerado o córtex associativo

do lobo frontal, que codifica a representação de ações complexas (conceitos de ações, planos e programas), bem como atua nas operações da memória de trabalho, que viabilizam a realização destas representações (Fuster, 1997).

A maior complexidade de uma informação processada torna confuso o limite entre as memórias sensoriais e as motoras. Sistemas responsáveis pelas memórias sensoriais têm associações motoras, estendendo-se ao lobos frontais, bem como as ações motoras não-elementares podem ser executadas em conjunto com regiões corticais posteriores (Fuster, 1997; Hikosaka *et al.*; 1999). A conectividade entre ambos os sistemas faz-se essencial na memória de trabalho ou quando é necessária uma integração sensório-motora (Fuster, 1997), como veremos logo adiante (em 1.3.2.1).

1.3.2 Dinamismo e Polimorfismo do Engrama

O dinamismo e o polimorfismo do engrama evidenciam-se em diversas abordagens experimentais, indicando que, assim como o processamento da informação, a memória recebe contribuição diferencial, ao longo do tempo, de múltiplas áreas. Analisaremos alguns modelos experimentais que evidenciam estes conceitos.

1.3.2.1 No Aprendizado de Procedimento Seqüencial (APS)

No *aprendizado de procedimento seqüencial* (APS), macacos aperfeiçoam-se, de acordo com uma ordem pré-estabelecida, no ato de tocar duas teclas que são simultaneamente iluminadas dentro de uma matriz 4x4. Cada par simultaneamente apresentado, por sua vez, está contido em uma seqüência ainda maior, de cinco pares, cujo término só é possível se todos os pares são corretamente respondidos. Portanto, o animal tem que apresentar uma boa integração sensório-motora para ter um bom desempenho na referida tarefa (Hikosaka *et al.*, 1999).

Hikosaka e colaboradores (1999) postulam que a memória do APS utiliza dois sistemas, que interagem entre si: o sistema de coordenadas espaciais (SCE), que corresponde ao circuito fechado formado pelo córtex associativo (especialmente o **pré-frontal**) e a porção anterior dos gânglios da base (especialmente o caudato); e o sistema de coordenadas motoras (SCM), que corresponde ao circuito fechado formado pelo córtex pré-motor e motor (especialmente a **área suplementar motora**) e a porção intermediária dos

gânglios da base (especificamente, o putâmen).

No início, o procedimento motor é realizado passo a passo, em seqüências consecutivas de transformações de coordenadas espaciais em coordenadas motoras. Com a repetição em uma ordenação constante, novas conexões se formam dentro de cada sistema de coordenadas (Figura 1). O SCE é mais flexível, amplo e efêmero em sua plasticidade; o SCM, mais lento, restrito e robusto. Assim, as primeiras alterações ocorrem a nível do SCE, onde o engrama fica inicialmente mais representado. Ao longo do tempo, porém, estas alterações desaparecem, enquanto que as alterações no SCM vão surgindo e vingando, por sua maior robustez. O engrama transforma-se, em seu dinamismo. O resultado final seria que, aparentemente, a memória migraria de áreas motoras e pré-motoras corticais para áreas subcorticais, um caminho inverso ao das memórias declarativas (Fuster, 1997; Hikosaka, 1999). Portanto, os primeiros estágios memorizam passos, em separado; os finais, a seqüência como um todo. Em acréscimo, o SCE não especifica o efetor, ao contrário do SCM: uma memória motora em seus primeiros passos é mais facilmente transferível de um membro treinado a um membro não treinado do que nos estágios finais, cuja transferência, muitas vezes, é inviável (Hikosaka, 1999). Por outro lado, o SCE detém a propriedade da analogia e da generalidade (diante de certos limites, pois se trata de memória não-declarativa), ao contrário do SCM. Entretanto, o SCM permite um desempenho mais apurado, preciso e veloz em tarefas repetitivas. Desta forma, a partir de um largo repertório (adquirido, inclusive, pela experiência), ocorre uma gradual seleção da quantidade de informação processada, o que culmina com uma resposta precisa e rápida. Em termos evolutivos, labilidade e generalidade nas fases iniciais e rapidez e eficácia nas fases finais devem conferir maior adaptabilidade ao sistema.

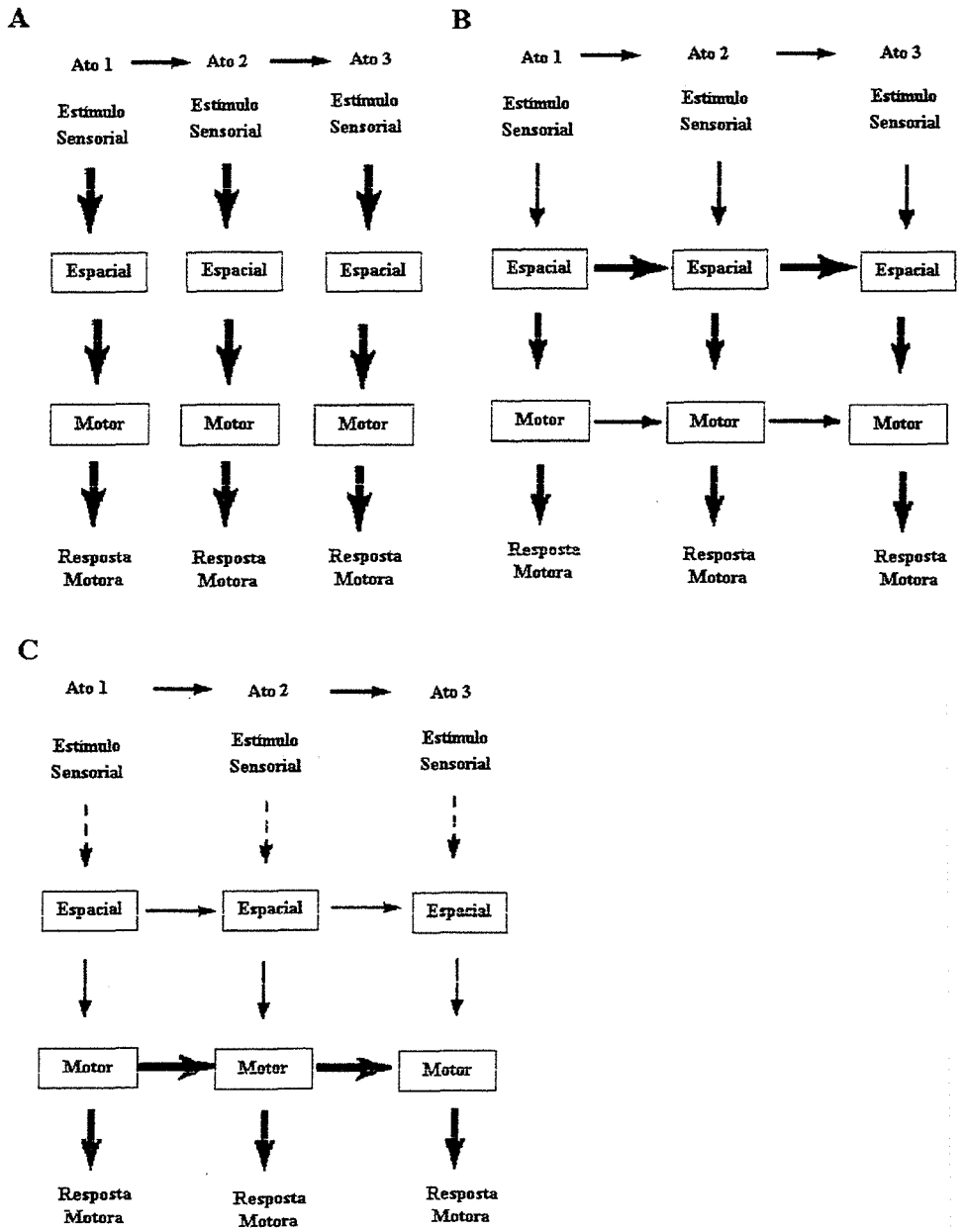

Figura 1 - Alterações hipotéticas do processamento da informação durante o aprendizado de procedimento sequencial. Para maiores detalhes, ver Hikosaka et al., 1999. (A) Representa o estágio anterior à aprendizagem, no qual o sujeito executa três ações (Atos 1-3) passo a passo, procedendo a transformações sensório-motoras a cada ação (conexão vertical). Ao executar as ações continuamente na mesma ordem, (B) e (C), processos seqüenciais são formados entre as ações (conexões horizontais) ao nível de cada sistema de coordenadas, espacial (SCE) ou motor (SCM). O tamanho das setas indica, em termos relativos, a intensidade das conexões [ou a "densidade" do engrama]. Adaptado de Hikosaka et al., 1999.

1.3.2.2 Na Esquiva Inibitória e no Aprendizado Discriminativo de Esquiva (ADE)

Lesões bilaterais da formação hipocampal provocam tanto amnésias anterógradas, que impossibilitam a formação de novas memórias, como amnésias retrógradas, que impossibilitam a evocação de antigas memórias. Por sinal, as amnésias retrógradas provocadas por lesões na formação hipocampal estão sempre relacionadas com acontecimentos mais recentes do que as amnésias provocadas por lesões isocorticais, o que gerou a hipótese de que as memórias declarativas migrem da formação hipocampal às regiões isocorticais (Squire, 1992).

No caso da memória da tarefa de **esquiva inibitória**, observou-se que a mesma é sensível a infusões de muscimol, um agonista do receptores gabaérgicos do tipo A, e de AP5, um antagonista dos receptores NMDA, quando administrados: no hipocampo, imediatamente pós-treino; no córtex entorrinal, de 30 a 180 minutos pós-treino; e no córtex parietal posterior, de 60 a 180 minutos pós-treino (Izquierdo *et al.*, 1997; Zanatta *et al.*, 1997). Além disto, a administração pré-teste de CNQX, um antagonista não específico dos receptores glutamatérgicos do tipo AMPA, é amnésico quando infundido no hipocampo 1 dia após o treino; no córtex entorrinal, de 1 a 31 dias; e no córtex parietal posterior, de 1 a 90 dias (Izquierdo e Medina, 1997; Izquierdo *et al.*, 1997; Roesler *et al.*, 1997). Estes resultados podem sugerir, a princípio, que a memória da esquiva inibitória (tanto pela análise de sua consolidação como a de sua evocação) migra do hipocampo para o córtex entorrinal, e deste para o córtex parietal posterior.

No *aprendizado discriminativo de esquiva* em coelhos (ADE), o animal aprende a se esquivar de um choque através de uma determinada resposta comportamental (no caso, caminhar em uma esteira). Tal choque é pareado e deve ser associado com um entre dois tons sonoros apresentados. Tal aprendizado é feito através de várias apresentações, onde o animal vai continuamente melhorando o desempenho (Gabriel *et al.*, 1988; Vogt e Gabriel, 1993). No circuito mostrado na Figura 2, o núcleo talâmico ântero-dorsal (AD) projeta-se ao córtex cingulado posterior que, por sua vez, tem conexões recíprocas com o núcleo talâmico ântero-ventral (AV) (Shibata, 1993). Durante o ADE, o AD é ativado logo após a novidade, enquanto o AV é ativado em estágios avançados do aprendizado, possivelmente porque AD suprime AV através da área 29c/d do cingulado posterior. Por sinal, esta área

aumenta gradativamente de atividade durante os estágios do aprendizado. Ocorre, portanto, a migração do engrama ao longo dos diferentes componentes do circuito: de AD para AV e área 29 c/d (Gabriel *et al.*, 1988; Vogt e Gabriel, 1993). Após extensivo treinamento, alterações do engrama migram para outra região do cérebro, pois a atividade diferencial neste circuito devida ao treinamento desaparece (Vogt e Gabriel, 1993), assim como lesões combinadas dos núcleos médio-dorsais e anteriores não afetam o desempenho de animais extensivamente treinados (Hart *et al.*, 1997).

1.3.2.3 Consolidação: Um Processo Duradouro

O armazenamento definitivo da memória de longa duração não é executado de imediato, mesmo que para tarefas pontuais que exijam apenas uma rápida sessão de treino (*v.g.*, esquiva inibitória) (McGaugh, 2000). Conforme proposto inicialmente por Müller e Pilzecker em 1900 (ver McGaugh, 2000), o processo de consolidação requer algumas horas, e sua ruptura gera amnésia retrógrada. Conforme postulado por McGaugh (2000), tal característica não se deve a limitações da maquinaria molecular e celular do cérebro, pois as memórias transitórias (memória de trabalho e de curta duração) são quase que imediatamente criadas. Provavelmente, este longo período ocorre pela necessidade adaptativa de modulação da intensidade do traço mnemônico; isto é, durante o processo de consolidação, processos endógenos seriam ativados tornando possível a análise da relevância da informação e, conforme a prioridade de seu conteúdo, seu armazenamento com maior ou menor robustez.

Portanto, devido ao grande dinamismo do engrama, o processo de consolidação seria melhor denominado de *fase de processamento da informação*, pois o termo consolidação implica que o traço já esteja estabelecido e apenas precisa ser estabilizado. Por sinal, Bloch (1970) já defendia esta idéia que, porém, não vingou, pelo menos em termos de nomenclatura.

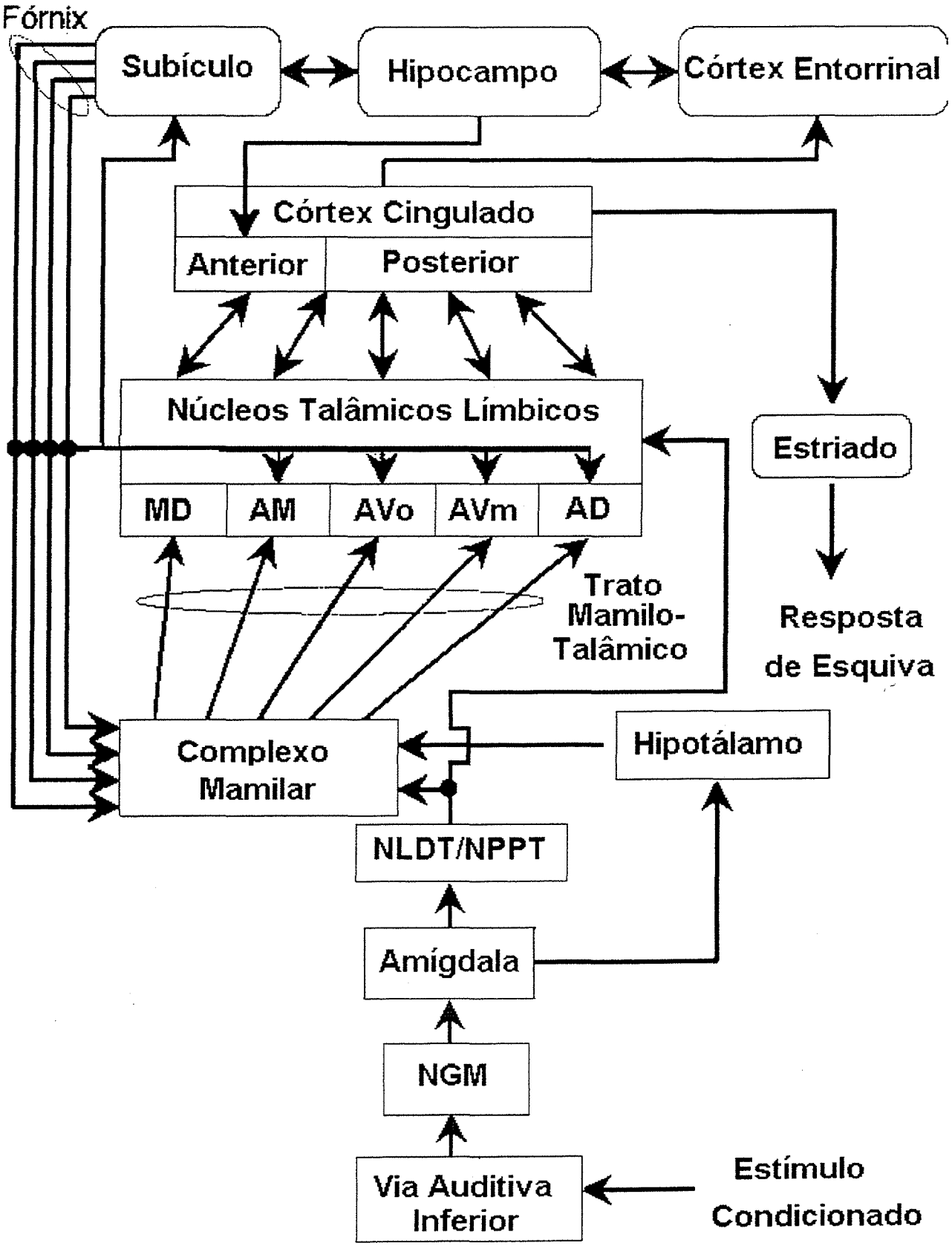

Figura 2 - Diagrama esquemático da circuitaria neuronal e do fluxo de informação ao qual o córtex cingulado está inserido e está envolvido no aprendizado do aprendizado discriminativo de esquiva. Adaptado de Kubota e Gabriel, 1995.

1.4 O Córtex Telencefálico

Ao longo do desenvolvimento embrionário dos vertebrados, três vesículas básicas (*rombencéfalo*, *mesencéfalo* e *prosencéfalo*) surgem a partir da parte anterior do tubo neural, que formará o encéfalo. Vesículas secundárias surgem, ao pares, a partir do prosencéfalo (ou encéfalo anterior): o *diencéfalo* (que dará origem ao tálamo e ao hipotálamo) e o *telencéfalo*. O telencéfalo é caracterizado por um substância cinzenta em sua superfície exterior, o **córtex**, e uma substância branca, composta por fibras e preenchida por estruturas subcorticais, tais como os núcleos da base e a amígdala (Bear *et al.*, 1995).

O córtex do telencéfalo (ou simplesmente córtex, embora este não seja o único) é formado por diversas camadas de neurônios (no máximo, seis) e tem espessura ínfima em relação à sua superfície. O tamanho do córtex acompanha, em termos alométricos, as dimensões corporais (Finlay e Darlington, 1995). Existe uma grande variação em sua superfície ao longo das diferentes espécies: por exemplo, de 3 a 5 cm^2 em pequenos insentívoros e roedores, a 1100 cm^2 em seres humanos (Northcutt e Kaas, 1995). Entretanto, ocorre apenas uma pequena variação em termos de espessura, que apenas dobra entre as espécies de menor e maior dimensão (Rakic, 1995). Tais aspectos são uma conseqüência de como o córtex é formado em sua ontogenia, bem como é organizado em termos funcionais.

Em termos funcionais, observa-se que o córtex tem uma *organização modular*, onde agrupamentos de neurônios altamente conectados entre si exibem propriedades coletivas e desempenham uma certa função elementar. Estes agrupamentos, dispostos em "colunas", são compostos por aproximadamente cem neurônios (Rakic, 1988) cujas interações formariam unidades ainda maiores, que por sua vez formariam as **áreas corticais** (Mountcastle, 1978). Ambos, o número de módulos e a área cortical total, estão diretamente relacionados ao número de células que a zona ventricular é capaz de produzir pois a grande maioria dos neurônios de uma coluna tem origem restrita à mesma região da zona ventricular e o número de neurônios por coluna é sempre aproximadamente o mesmo, mesmo que haja variações durante o processo ontogenético (Rakic, 1995). Portanto, o aumento do número de neurônios é acompanhado pelo aumento da superfície cortical, e não por sua espessura, bem como pelo aumento de módulos corticais, que, mais adiante,

poderão vir a formar, inclusive, novas áreas corticais, como abordaremos a seguir.

1.4.1 Áreas Corticais: Classificação e Evolução

Em todos os mamíferos, o córtex pode ser dividido, com base na laminação, em *isocórtex* e *alocórtex*. O isocórtex corresponde à região cortical que apresenta seis camadas pelo menos em algum estágio de sua ontogenia, enquanto o alocórtex apresenta, no máximo, três camadas. Em acréscimo, existe uma zona de transição entre o isocórtex e o alocórtex. Nela, o padrão de laminação varia em um gradiente de proximidade entre o isocórtex e o alocórtex. O giro do cíngulo, em seres humanos, e grande parte da região cingulada, em ratos, estão situados nesta região de transição (Zilles e Wree, 1995) (Figura 3).

Considerando equivocados aspectos evolucionários aceitos no séc. XIX, consagrou-se o termo *neocórtex* para denominar o isocórtex e *paleocórtex* e *arquicórtex* (de implicações funcionais olfatória e límbica, respectivamente) para denominar subdivisões do alocórtex (Northcutt e Kaas, 1995; Preuss e Kaas, 1999). Por sinal, analisando o cérebro de peixes, observa-se que o pálio, região homóloga ao córtex dos mamíferos, é caracterizado por três divisões básicas: lateral, dorsal e medial. Não há nenhuma evidência de que a partir de um paleopálio olfativo tenha emergido um arquipálio ou um neopálio em anfíbios ou répteis (Northcutt e Kaas, 1995) [os termos paleopálio e arquipálio estão aqui empregados de acordo com a referência, embora normalmente o prefixo *arqui* esteja associado a eventos anteriores aos associados ao prefixo *paleo*]. Entretanto, não podemos negligenciar que o isocórtex tem como característica inovativa a presença de seis camadas, inexistente em répteis ou aves. Acredita-se que o pálio dorsal dos répteis tenha originado o isocórtex, embora ainda haja dúvidas sobre uma possível contribuição da crista dorsal ventricular dos répteis, situada logo abaixo do pálio dorsal (Kaas e Reiner, 1999; Northcutt e Kaas, 1995; Preuss e Kaas, 1999). Por sinal, as células precursoras dos gânglios da base, uma estrutura subcortical, geram também a maioria (senão todos) os neurônios inibitórios isocorticais (Anderson *et al.*, 1997).

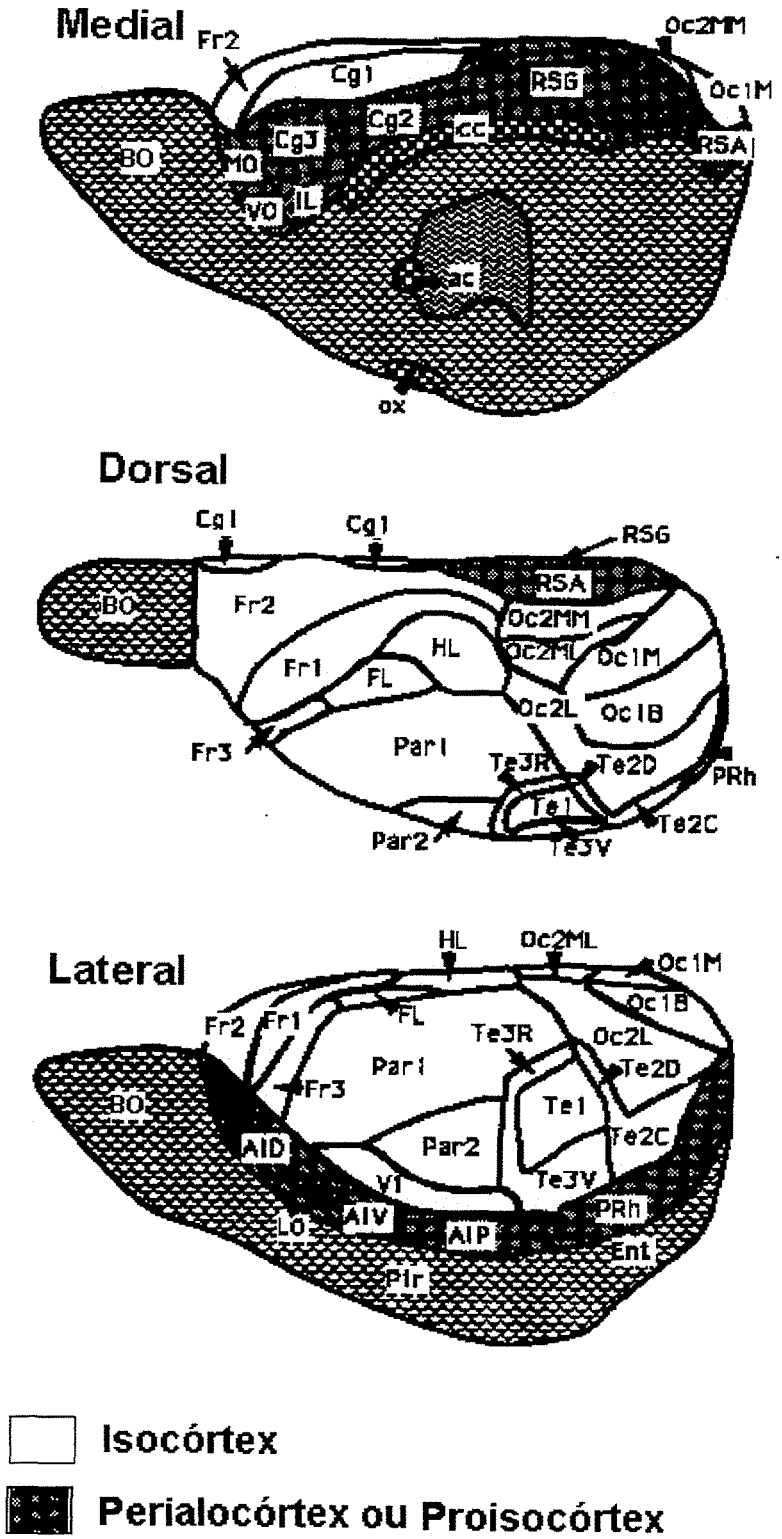

Figura 3 - Desenho esquemático do mapa cortical do cérebro do rato adulto em diferentes cortes sagitais. RSG e RSA (siglas do inglês referentes aos córtices retrospleniais granular e agranular, respectivamente) pertencem ao córtex cingulado posterior (ou córtex retrosplenial, em ratos). O Perialocórtex e o Proisocórtex formam a zona de transição entre o isocórtex e o alocórtex. Adaptado de Zilles e Wree, 1995.

A formação hipocampal e o isocórtex no rato têm, respectivamente, 1,2 e 1,5 cm^2 de superfície externa (Zilles e Wree, 1995), enquanto que em seres humanos, o isocórtex é várias vezes maior (Bear *et al.*, 1995). Esta crescente proeminência estrutural e funcional do isocórtex em relação ao alocórtex ao longo da evolução é possivelmente fruto de diferenças no padrão de neurogênese de ambos os córtices, e não do fato do isocórtex ser uma estrutura sem homólogos, em termos evolutivos. Alometricamente, o expoente de crescimento do isocórtex em relação ao tamanho corporal é maior e, como conseqüência, detém o potencial de atingir proporcionalmente maior área nos animais maiores. Assim, por apresentar maior suporte físico, deteve maior potencial de realizar as funções recentemente adquiridas no processo evolutivo (Finlay e Darlington, 1995). Entretanto, o processo mais avançado executado pelo cérebro, a consciência, emerge da atividade combinada de inúmeras regiões cerebrais, filogeneticamente recentes ou não. Das regiões corticais responsáveis por amplas associações, podemos citar tanto a **região pré-frontal** e o córtex parietal posterior, situados, no homem, no isocórtex (Gross e Graziano, 1995; Tononi e Edelman, 1998), e o hipocampo (Eichenbaum, 1993), situado no alocórtex.

Mamíferos com maiores cérebros não apenas têm seu isocórtex expandido, mas, também, mais diferenciados em termos anátomo-funcionais, com a adição de novas áreas. Somadas, ambas as características permitem um maior refinamento do processamento da informação, ao extrair-lhe maior número de elementos (Krubitzer *et al.*, 1995; Northcutt e Kaas, 1995). A evolução do córtex pré-frontal (descrito a seguir), é um claro exemplo deste fenômeno (Preuss e Kaas, 1999).

1.4.2 Córtex Pré-Frontal

Conforme apontado por Zilles e Wree (1995), diversos estudos têm mapeado, de forma discrepante, o córtex segundo diversas classificações e nomenclaturas.

De todo o córtex, a região pré-frontal foi a que mais se desenvolveu durante o processo de hominização (Deacon, 1997; Preuss e Kaas, 1999). Ela é diretamente responsável por nossa alta capacidade de planejamento e de verbalização, bem como de socialização (Damásio, 1996). Não é de se surpreender, então, que haja ainda maiores diferenças entre a região pré-frontal humana e a de ratos, cuja distância evolutiva é ainda maior.

Em seres humanos, o córtex pré-frontal é definido como a região do isocórtex que possui conexões recíprocas com o núcleo médio-dorsal do tálamo (Zilles e Wree, 1995). Em ratos, porém, esta definição em termos anatômicos aplica-se a apenas parte do **córtex cingulado anterior** (especificamente, área Cg1, que corresponde à área de Brodmann 24), ao **córtex insular agranular** e a áreas orbitofrontais (Zilles e Wree, 1995). Entretanto, existem regiões corticais no rato que desempenham funções similares às da região pré-frontal de seres humanos e que, por tal motivo, são também incluídas em sua classificação. Estas regiões, por sinal, podem ser, no ponto de vista filogenético, homólogas às áreas do córtex pré-frontal humano ao qual tenham correspondência funcional (Seamans *et al.*, 1995). Como exemplos, temos a **área pré-central medial (Fr2)**, as demais regiões do córtex cingulado anterior, Cg2 e Cg3, e a região infralímbica (Caviness, 1975; Caviness e Frost, 1980; Hicks e Huerta, 1991; Seamans *et al.*, 1995; Van Eden, 1992; Zilles e Wree, 1995).

Fr2 pertence ao isocórtex dos ratos e corresponde, em termos funcionais, às áreas de Brodmann (AB) 6, que pertence ao córtex pré-motor, e 6 a, 8 e 10, que pertencem ao córtex pré-frontal associativo (Caviness, 1975; Caviness e Frost, 1980; Hicks e Huerta, 1991; Van Eden, 1992). Cg3 pertence à zona de transição entre o isocórtex e o alocórtex, é conhecida com região pré-límbica (PrL), corresponde à AB 32 e possivelmente seja a origem filogenética da região dorsolateral dos seres humanos (AB 46), pois desempenha funções similares (Seamans *et al.*, 1995). O córtex insular agranular, por sua vez, corresponde, nos primatas, provavelmente ao córtex orbitofrontal (ou região pré-frontal ventromedial, conforme Damásio, 1996) (Groenewegen, 1988; Leonard, 1969; Preuss, 1995).

1.4.3 Córtex Cingulado Posterior

Além do córtex cingulado anterior, que pertence às regiões frontais do córtex, temos o **córtex cingulado posterior**, com características anátomo-funcionais bem distintas. Em ratos, o córtex cingulado posterior apresenta sempre cinco camadas e possui cinco subdivisões, *29 a, b, c, d, e*, de acordo com o padrão de laminação e aferências (Vogt e Peters, 1981). A área do córtex cingulado posterior humano que pertence ao isocórtex (AB 23) talvez corresponda às subdivisões 29 c e 29 d do cingulado posterior dos ratos (Vogt e

Peters, 1981).

A Figura 2 mostra esquematicamente o Circuito de Papez, postulado como o "sistema da emoção" (Papez, 1937), de participação indiscutível nos processos mnemônicos. Esta proposição é, por sinal, ainda hoje por muitos aceita, pois o dano em algumas de suas áreas promove grandes déficits no comportamento emocional e poucas alterações na percepção ou na inteligência (Bear *et al.*, 1995). Conforme se observa nesta figura, as projeções talâmicas ao córtex cingulado diferenciam-se em relação ao eixo ântero-posterior (Kubota e Gabriel, 1995). Este recebe informações sensoriais através dos núcleos anteriores do tálamo (Caviness e Frost, 1980; Hedberg e Stanton, 1995; Horikawa *et al.*, 1988; Vogt *et al.*, 1981) e também é inervado por uma via que parte da formação hipocampal, através do subículo. Por sinal, os neurônios do córtex cingulado posterior podem entrar em ressonância com os neurônios do hipocampo, apresentando ritmo teta sincronizado (Hedberg *et al.*, 1993; Leung e Borst, 1987). Esta via subículo-cingulada apresenta PLD e DLD, e os dendritos de suas sinapses, situadas nas camadas mais profundas do córtex cingulado posterior, estão intimamente associados aos terminais talâmicos (Greengard *et al.*, 1991; Hedberg e Stanton, 1995; Vogt *et al.*, 1981). Acredita-se que a informação que passa por ambas as vias é comparada e filtrada, sendo apenas os sinais coincidentes seriam reforçados e armazenados através da PLD e da DLD da via subículo-cingulada (Hedberg e Stanton, 1995). A posição estratégica do córtex cingulado posterior no circuito de Papez (Figura 2), intermediando a sinalização entre o hipocampo e o isocórtex, deve conferir a esta região grande importância nos processos mnemônicos. Por sinal, o isocórtex como um todo recebe informações do hipocampo apenas por dois locais, o córtex pré-frontal ou o córtex cingulado posterior (Bear *et al.*, 1995).

1.5 Neurofarmacologia da Memória

Nesta Introdução, não nos deteremos nos aspectos da neurofarmacologia da memória da tarefa de esquiva inibitória (sistemas de neurotransmissores e neuromoduladores, cascatas enzimáticas *etc.*), pois foram abordados nos artigos desta Tese (Capítulos 3, 4, 5 e 6). Alguns importantes aspectos, porém, já foram abordados em 1.3.2.2.

2. Objetivos

1) Verificar a participação do **córtex cingulado** na consolidação da memória da tarefa de esquiva inibitória;
2) Verificar a participação da **área pré-central medial** na consolidação da memória da tarefa de esquiva inibitória;
3) Verificar a participação do **córtex cingulado anterior** na evocação da memória da tarefa de esquiva inibitória;
4) Verificar o envolvimento do **córtex insular agranular** na consolidação da memória da tarefa de esquiva inibitória.

3. O Córtex Cingulado e a Consolidação da Memória da Tarefa de Esquiva Inibitória

Neurobiology of Learning and Memory **72,** 118–127 (1999)
Article ID nlme.1998.3892, available online at http://www.idealibrary.com on IDEAL®

Differential Effects of Post-training Muscimol and AP5 Infusions into Different Regions of the Cingulate Cortex on Retention for Inhibitory Avoidance in Rats

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Adult male Wistar rats were bilaterally implanted with indwelling cannulae in four different coordinates of the cingulate cortex: (1) the anterior cingulate (AC), (2) the rostral region of the posterior cingulate (RC), (3) the upper portion of the caudal region of the posterior cingulate (UC), and (4) the lower portion of the caudal region of the posterior cingulate (LC). After recovery, animals were trained in a step-down inhibitory avoidance task (3.0-s, 0.4-mA foot shock). Either immediately, or 90 or 180 min after training, animals received a 0.5-μl infusion of vehicle (phosphate buffer, pH 7.4), of muscimol (0.5 μg), or of AP5 (5.0 μg). Retention testing was carried out 24 h after training. Muscimol was amnestic when given into any of the three coordinates of the posterior cingulate cortex 90 min after training, and when given into LC immediately post-training. In addition, AP5 was amnestic when given into UC 90 min post-training, but not when given into any other region and/or at any other time. None of the treatments had any effect when given into AC. The results suggest that memory processing of the inhibitory avoidance task is regulated by the posterior but not by the anterior cingulate cortex, through muscimol-sensitive synapses, relatively late after training. AP5-sensitive synapses appear to play a very limited role in these processes, restricted to UC.

Key Words: cingulate cortex; $GABA_A$ receptors; NMDA receptors; memory; rat.

INTRODUCTION

The hippocampus, amygdala, entorhinal cortex, and parietal cortex are sequentially involved in the consolidation of memory for a step-down inhibitory avoidance task in rats through mechanisms sensitive to the *N*-methyl-D-aspartate (NMDA) receptor antagonist aminophosphonopentanoic acid (AP5) and to the γ-aminobutyric acid type A ($GABA_A$) receptor agonist muscimol (Izquierdo, Da Cunha, Rosat, Jerusalinsky, Ferreira, & Medina, 1992; Izquierdo, Quillfeldt, Zanatta, Quevedo, Schaeffer, Schmitz, & Medina, 1997; Izqui-

This study was supported by Conselho Nacional de Pesquisa (CNPq) and PRONEX, Brazil. We are grateful to J. A. Quillfeldt and Beatriz Aparecida de Almeida Moleta for their contribution in the present work.

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1074-7427/99 $30.00

erdo & Medina, 1997). The results indicate that the hippocampus and amygdala participate early on, and the entorhinal and parietal cortex play a role in memory formation 0.5–3 h later (Izquierdo *et al.*, 1997; Izquierdo & Medina, 1997).

The cingulate cortex is a synaptic target of hippocampal output and thalamic sensory information, and has a strategic position in the Papez circuit of emotion processing. It intermediates signals between the hippocampal formation and associative neocortex (Bear, Connors, & Paradiso, 1995; Martin, 1996; Zilles & Wree, 1995). The anterior thalamic nuclei receive a number of direct and indirect projections from the hippocampus, and project differently to both the anterior cingulate cortex (AC) and the posterior cingulate cortex, with the projections of the anterior thalamic nuclei to the posterior cingulate being especially dense (Horikawa, Kinjo, Stanley, & Powell, 1988; Shibata, 1993; Vogt, Rosene, & Peters, 1981). It has been postulated that this circuit may have a comparator function, where incoming data are compared with mnemonic data and, if they are in accordance, already planned behavioral programs are executed; if not, outputs are generated that heighten attention and inhibit planned actions (Kubota & Gabriel, 1995).

The posterior cingulate, also known as the retrosplenial cortex (Zilles & Wree, 1995), exhibits theta frequency highly coherent with that of the hippocampus (Hedberg, Simpson, & Stanton, 1993; Leung & Borst, 1987), and both homosynaptic long-term potentiation and associative long-term depression have been described in the posterior cingulate (Hedberg & Stanton, 1995). AC presents decremental nonassociative NMDA-dependent long-term potentiation (Sah & Nicoll, 1991) and belongs to the medial prefrontal cortex, because of its reciprocal connections with the medial dorsal thalamic nucleus (Zilles & Wree, 1995).

The posterior cingulate mediates spatial memory (Sutherland & Hoesing, 1993) and the development of water maze spatial navigation strategies in rats (Riekkinen, Kuitunen, & Riekkinen, 1995). Evidence of the involvement of AC and the medial prefrontal cortex in working and short-term memory in rats has been growing (Broersen, Heinsbroek, de Bruin, Joosten, van Hest, & Olivier, 1994; Broersen, Heinsbroek, de Bruin, Uylings, van Hest, & Olivier, 1995; Freeman, Cuppernell, Flannery, & Gabriel, 1996; Granon, Vidal, Thinus-Blanc, Changeaux, & Poucet, 1994; Seamans Floresco, & Philips, 1995). However, the role of AC and medial prefrontal cortex in long-term memory remains controversial. Immediate post-training infusions of scopolamine into AC impair memory consolidation of an inhibitory avoidance task in rats (Riekkinen *et al.*, 1995).

Here we investigate the participation of the posterior and the anterior cingulate cortices in the consolidation of memory for an inhibitory avoidance task in rats.

METHODS

Subjects

A total of 528 male Wistar rats (age, 60 to 90 days) from our breeding colony was used. The animals were housed five to a cage with food and water ad libitum. The animal house was on a 12-h light/dark cycle (lights on at 7:00 AM) at a temperature of 23°C.

Surgery and Behavioral Procedures

The animals were bilaterally implanted under thionembutal anesthesia (30 mg/kg, ip) with a 27-gauge guide cannulae. After at least 48 h, all animals were trained in a step-down inhibitory avoidance task (Izquierdo *et al.*, 1992, 1997). The rats were placed on a 2.5 cm high by 7.0 cm wide formica platform at the left of a 50 × 25 × 25-cm apparatus, whose floor was a series of parallel 0.1-cm caliber stainless-steel bars spaced 1.0 cm apart. Latency to step down placing the four paws on the grid was measured with an automatic device. In the training session, immediately upon stepping down, the animals received a 3.0-s, 0.4-mA foot shock. A retention test was carried out 24 h after training. The test session was procedurally identical except that no foot shock was given and the step-down latency was cut off at 180 s; i.e., test session values higher than 180 s were counted as 180 s. Retention test performance was taken as a measure of retention.

Infusion Procedure and Control of Cannula Placements

At the time of infusion, 30-g cannulae were fitted into the guide cannulae (Izquierdo *et al.*, 1992, 1997). Animals received a bilateral infusion of 0.5 μl of vehicle (phosphate buffer, pH 7.4), of muscimol (0.5 μg), or of AP5 (5.0 μg) immediately, 90, or 180 min after training. The sites of infusion were chosen using coordinates (from bregma and dura) obtained from the atlas of Paxinos and Watson (1986), as follows (units in cm): (I) AC, A 0.22, L ±0.10, V −0.26; (II) rostral region of posterior cingulate (RC), A −0.38, L ±0.05, V −0.19; (III) upper portion of the caudal region of posterior cingulate (UC), A −0.58, L ±0.06, V −0.19; and (IV) lower portion of the caudal region of posterior cingulate (LC), A −0.58, L ±0.10, V −0.28 (Fig. 1). RC and UC cover the Area 29c in different positions of the anterior-posterior axis, while LC covers the Area 29b and also a small part of the Area 29c at the same coronal plane as UC (Vogt *et al.*, 1981; Zilles & Wree; 1995). The coordinate of AC was selected taking into account the study of Riekkinen *et al.* (1995).

Two to 24 h after the end of the behavioral procedure all animals received an infusion of 0.5 μl of 4% methylene blue through the infusion cannulae and were killed by decapitation. Their brains were removed and stored in formalin for histological localization of infusion sites as explained elsewhere (Izquierdo *et al.*, 1992, 1997). Infusion placements were correct in 122 animals implanted in AC, and in 116, 123, and 109 animals implanted in RC, UC, and LC, respectively. Only animals with correct cannula locations (Fig.1) were included in the final statistical analysis.

Statistics

Data are reported as means (standard error of mean) of the retention test performance. Differences from the saline control group of the same time of infusion were analyzed by the independent-samples *t* test, two-tailed. Comparisons between training and test session performances in each group were analyzed by the paired-samples *t* test. Training session differences among all groups of the same site and time of infusion were evaluated by one-way analyses of variance. $p < .05$ was considered to indicate statistical significance.

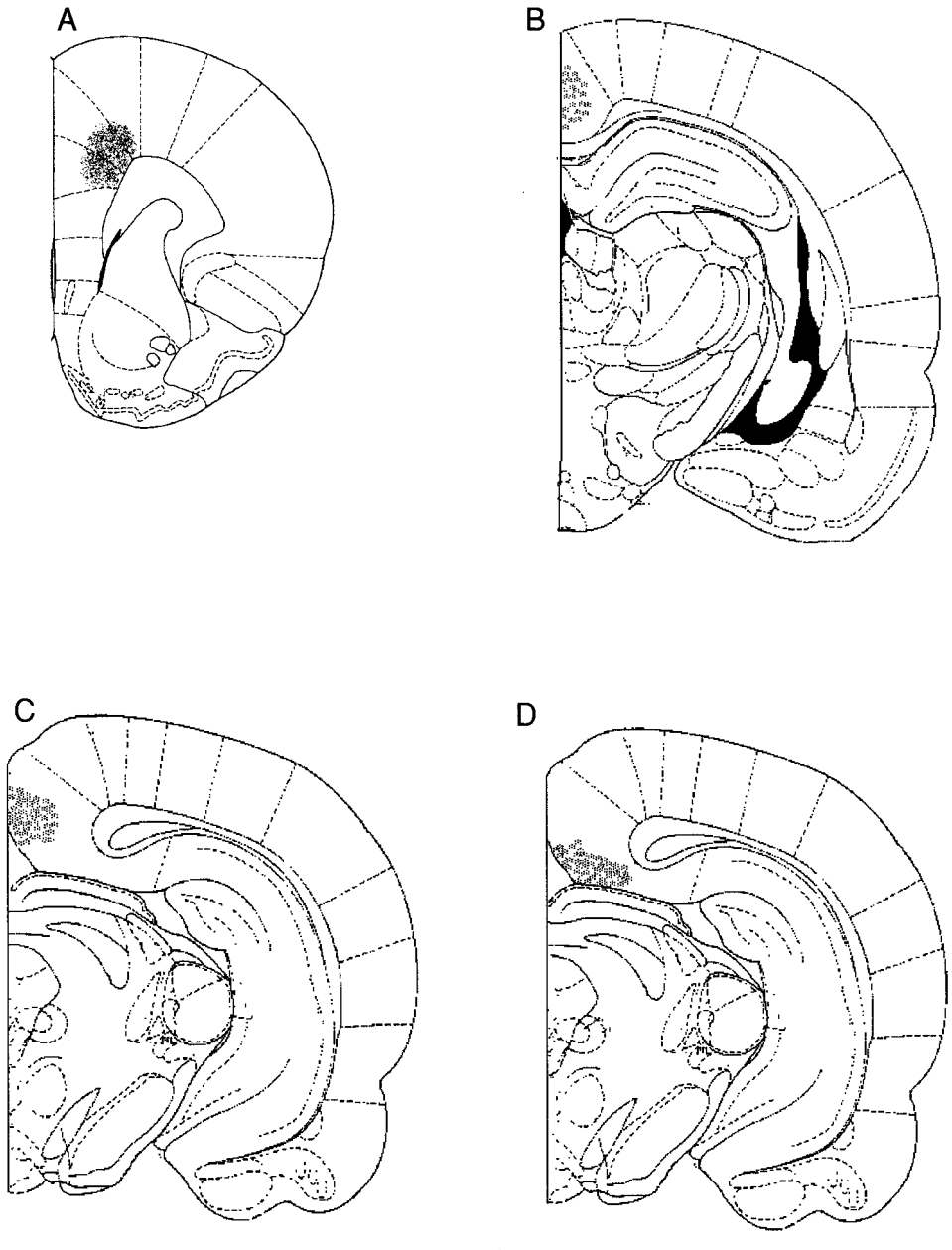

FIG. 1. A, B, C, and D, schematic drawings of rat brain sections at planes A +0.22, −0.38, −0.58, and −0.58, respectively, from the atlas of Paxinos and Watson (1986) showing (stippled) the extension of the areas reached by infusions into the anterior cingulate cortex, and into RC, UC, and LC. The figures illustrate a composite of all infusions given on both sides in the structures above. The maximum extension of the site(s) reached by the infusions was less than 1 mm^3 in the animals with correct infusion placements.

RESULTS

Results are shown in Fig. 2. There were no differences among groups in training session performance (overall mean, 6.5 s; standard error of mean,

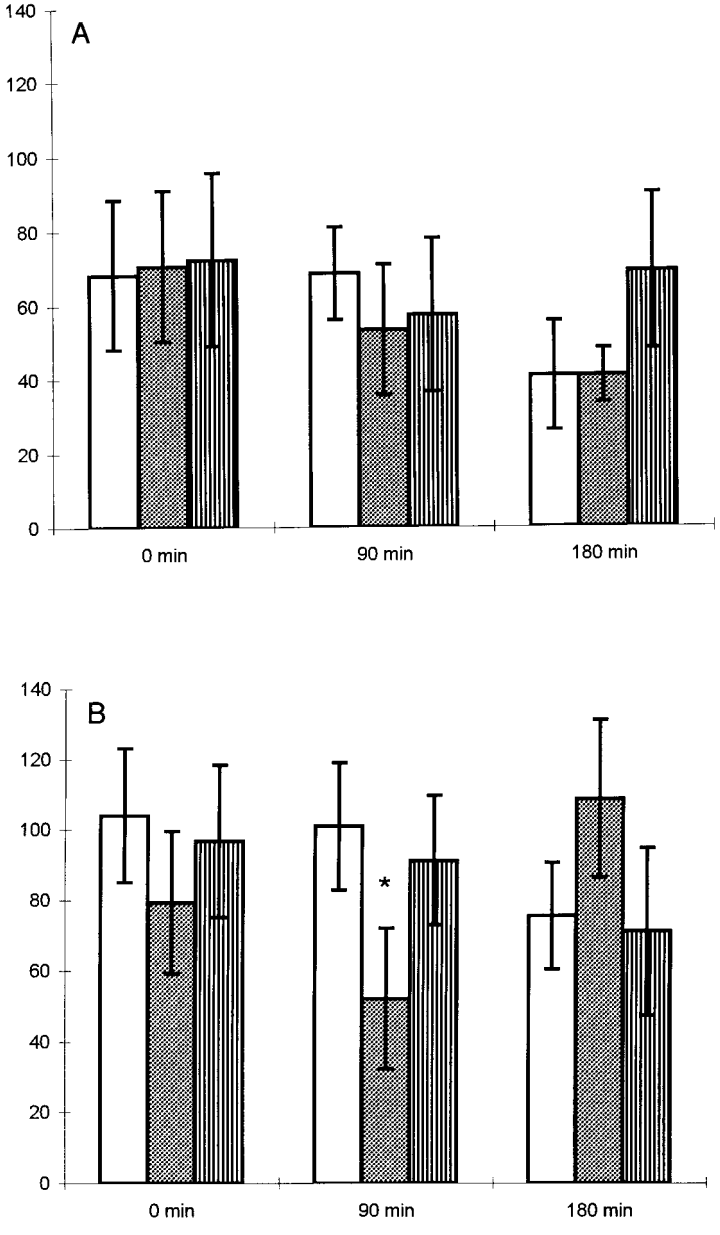

FIG. 2. A, B, C, and D, median (interquartile range) test session latency in groups infused bilaterally into the anterior cingulate cortex and into RC, UC, and LC, respectively, with saline (0.5 μl, pH 7.4) (white columns), muscimol (0.5 μg) (dark columns), or AP5 (5.0 μg) (hatched columns), 0, 90, or 180 min post-training. Asterisk indicates statistical significance in Mann-Whitney *U* tests, two-tailed, at $p < .05$ from the saline control group of the same time of infusion. *N* per group was 12–16 in AC. *N* per group was 10–15, 10–16, and 10–16 in RC, UC, and LC, respectively. Muscimol infusions given into RC, UC, and LC 90 min post-training were amnestic. Ninety-minute post-training AP5 and immediate post-training muscimol infusions were amnestic into UC and LC, respectively.

0.24 s; $p > .10$). All groups showed a significant difference between training and test session performance ($p < .05$). Muscimol and AP5 hindered retention test performance when given into UC 90 min after training ($p < .05$), but not

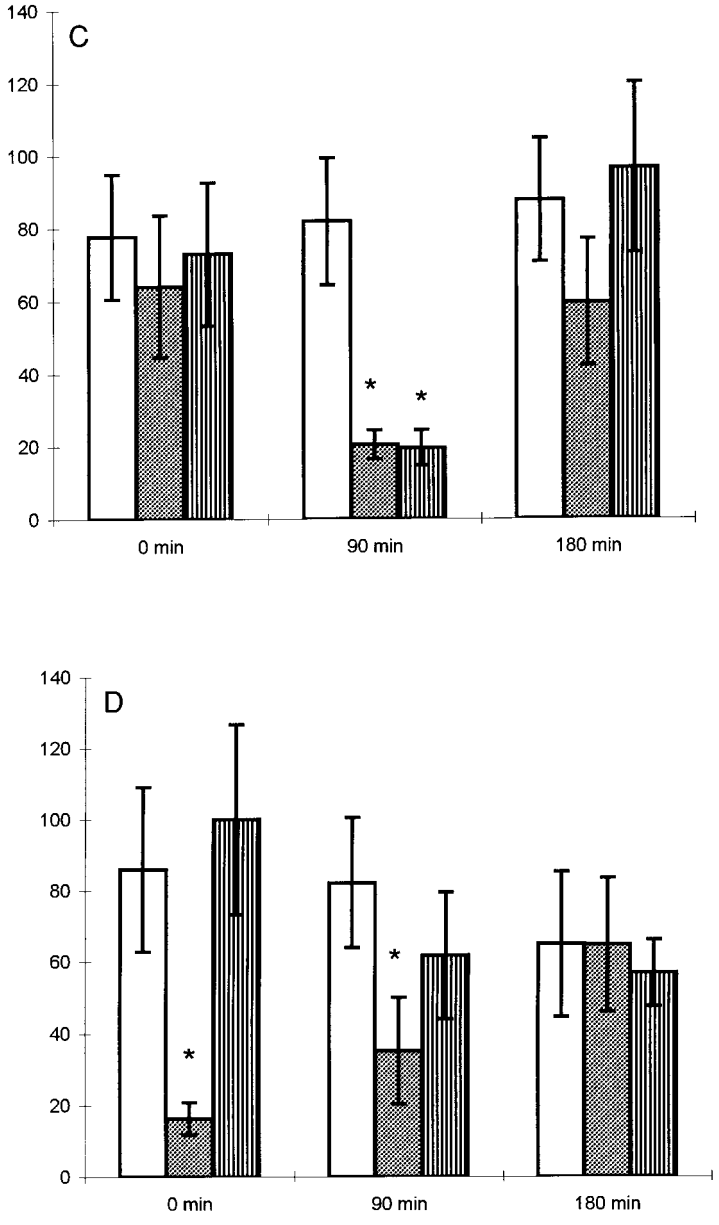

FIG. 2—*Continued*

immediately or 180 min after training ($p > .10$). Muscimol hindered retention test performance when given into LC immediately or 90 min after training ($p < .05$), but not 180 min after training ($p > .10$). Muscimol hindered retention test performance when given into RC 90 min after training ($p < .05$), but not immediately or 180 min after training ($p > .10$). AP5 infusions into LC and RC were not effective ($p > .10$). Infusions of muscimol and AP5 immediately, 90, or 180 min after training did not affect retention when given into AC ($p > .10$). *N* per group was 12–16 in AC. *N* per group was 10–15, 10–16, and 10–16 in RC, UC, and LC, respectively.

DISCUSSION

Previous studies from our laboratory showed that the hippocampus, amygdala, entorhinal cortex, and parietal cortex are sequentially involved in the consolidation of memory for a step-down inhibitory avoidance task (Izquierdo *et al.*, 1992, 1997; Izquierdo & Medina, 1997). The present results show that consolidation of memory for inhibitory avoidance is sensitive to muscimol infusions given 90 min after training into posterior cingulate, but not into AC, and that there are specific regions of the posterior cingulate into which infusions of muscimol or AP5 immediately or 90 min after training, respectively, disrupt consolidation for such a memory.

The presence of an amnestic effect of the drugs when administered into a given coordinate of the posterior cingulate at specific post-training times suggests that (1) the area corresponding to this coordinate is involved in the consolidation of memory for inhibitory avoidance by mechanisms sensitive to muscimol or AP5; or (2) merely that alterations in the activity of $GABA_A$ or NMDA receptors in this area affect the transactional processes involved in consolidation, even though these processes are mediated by structures other than the posterior cingulate. We think that these alternatives should be addressed having in mind that 1.0-μl infusions of muscimol, twice larger in volume than our infusions, maximally reduce glucose uptake in a restricted region of 1 mm^3 (Martin, 1991), indicating that there is a gradient of effectiveness from the center of infusion that may justify differences of effects between infusion sites 1.0 mm apart.

The amnestic effect of muscimol when given 90 min after training into any of the three coordinates of posterior cingulate is similar to previously described effects observed for this drug when infused into the entorhinal and posterior parietal cortices (Izquierdo *et al.*, 1997). The lack of effect at 180 min indicates that the posterior cingulate may end its muscimol-sensitive participation in consolidation before the entorhinal and parietal cortices do, or merely that an incorrect balance between excitation and inhibition in the posterior cingulate after 180 min from training does not disrupt the transactional processes of memory consolidation. Since immediate post-training infusions of muscimol were amnestic when given into LC, the balance of activity of at least part of the posterior cingulate may be fundamental for memory consolidation early in consolidation.

The strategic position of the posterior cingulate in the Papez circuit, intermediating signals between the hippocampal formation and the associative neocortex (Bear *et al.*, 1995; Martin, 1996; Zilles & Wree, 1995), may be related to its time window in consolidating memory (90 min as a whole; 0–90 min in LC), inasmuch as this interval occurs between that of the hippocampus (0 min) and those of the entorhinal (30–180 min) and parietal cortex (60–180 min) (Izquierdo *et al.*, 1997). The activity-dependent synaptic changes long-term potentiation and long-term depression have been described in the posterior cingulate and might be involved in filtering the hippocampal output to the associative cortex, mediating a putative comparator function (Hedberg *et al.*, 1993; Hedberg & Stanton, 1995). Therefore, it is possible that the posterior cingulate is involved in consolidation by processing the flow of information from the hippocampus to other cortical areas.

Time- and drug-dependent differences between the effects of infusions into UC and LC might be related to the different electrophysiological properties between Area 29b, covered by LC, and Area 29c, covered by UC and LC (Kubota & Gabriel, 1995). In the "comparator" circuit, the anterodorsal thalamic nucleus (AD) projects to the posterior cingulate, which has reciprocal connections with the anteroventral thalamic nucleus (AV) (see Shibata, 1993). During discriminative avoidance learning in rabbits, AD is activated early by novel events, while AV is activated in later stages with decrease of AD activity, probably because of AD suppression of AV activity through the posterior cingulate. In addition, activity in Area 29c/d increases massively during avoidance learning, relative to the preliminary training session (Gabriel *et al.*, 1988; Vogt & Gabriel, 1993). After considerable overtraining of intact rabbits, discriminative neuronal training-induced activity (TIA) in this circuit disappears (Vogt & Gabriel, 1993). In addition, combined medial dorsal and anterior thalamic lesions do not affect performance if the rabbits were previously submitted to extensive overtraining (Hart *et al.*, 1997). The effect of immediate post-training infusions of muscimol into LC might be related to the involvement of AD in the early stages of discriminative avoidance learning (Gabriel *et al.*, 1988), where lesions increase 29b activity but disrupt TIA mediated by 29c/d (Kubota & Gabriel, 1995). As memory consolidation for inhibitory avoidance evolves, other regions of the posterior cingulate become involved, as suggested by our muscimol infusions given into UC and RC 90 min after training.

The amnestic effect of AP5 when given into UC at 90 min post-training, but not when given into LC and RC, indicates that there are regional differences in the posterior cingulate regarding information processing mediated by NMDA receptors. UC, but not LC and RC, might process memory consolidation similarly to the hippocampus and entorhinal and parietal cortices (Izquierdo & Medina, 1997). However, we may not discard the involvement in consolidation of NMDA receptors in LC and RC, despite the lack of effect of AP5 infusions when given into these regions, since functional NMDA receptors are seen along the granular posterior cingulate cortex (Näkki, Koistinaho, Sharp, & Sagar, 1995; Olney, Labruyere, & Price, 1989; Sharp, Jasper, Hall, Noble, & Sagar, 1992).

The lack of effect of muscimol and AP5 infusions in AC suggests that normal activity of this area is not essential for the consolidation of memory for inhibitory avoidance up to 180 min from training. However, immediate post-training infusions of scopolamine into AC, but not the posterior cingulate, impair retention of a scotophobic inhibitory avoidance task in rats (Riekkinen *et al.*, 1995). It is possible that the cholinergic synapses in AC are more important than GABAergic or glutamatergic transmission in memory modulation in this area. Otherwise, our results are in accordance with the hypothesis that AC is not involved in long-term memory, but mediates working or recent memory, while the posterior cingulate is involved in primacy or reference memory in rats, rabbits (Aggleton *et al.*, 1995; Broersen *et al.*, 1994, 1995; Freeman *et al.*, 1996; Granon *et al.*, 1994; Kubota & Gabriel, 1995; Seamans *et al.*, 1995; Shibata, 1993), or humans (Valenstein, Bowers, Verfaellie, Heilman, Day, & Watson, 1987).

Further studies are necessary to clarify the functions of the anterior and the posterior cingulate cortices in memory. The present contribution focuses on the

time window in which memory consolidation for inhibitory avoidance in rats is sensitive to post-training infusions of muscimol and AP5 into different regions of the posterior cingulate cortex, but not in the anterior cingulate cortex.

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3.1 Atualização

Uma versão mais atualizada do Atlas de Paxinos e Watson (4ª edição, de 1998) renomeia Cg3 (região cingulada 3) para córtex pré-límbico (PrL).

4. A Área Pré-Central Medial e a Consolidação da Memória da Tarefa de Esquiva Inibitória

Pharmacology Biochemistry and Behavior, Vol. 66, No. 3, pp. 615–622, 2000

0091-3057/00/$–see front matter

ELSEVIER

PII S0091-3057(00)00277-X

Involvement of the Medial Precentral Prefrontal Cortex in Memory Consolidation for Inhibitory Avoidance Learning in Rats

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Received 28 August 1999; Revised 6 January 2000; Accepted 18 January 2000

MELLO E SOUZA, T., M. R. M. VIANNA, C. RODRIGUES, J. QUEVEDO, B. A. MOLETA AND I. IZQUIERDO. *Involvement of the medial precentral prefrontal cortex in memory consolidation for inhibitory avoidance learning in rats.* PHARMACOL BIOCHEM BEHAV **66**(3) 615–622, 2000.—Adult male Wistar rats were trained in a step-down inhibitory avoidance learning task (3.0-s, 0.4-mA foot shock), received a 0.5-μl infusion of muscimol (0.02, 0.1, or 0.5 μg), AP5 (0.16, 0.34, 0.5, 1.6, or 5.0 μg), SCH 23390 (0.05, 0.34, 0.5, or 1.75 μg), saline, or vehicle (DMSO 20%) into the anterior medial precentral area (Fr2) (CI) immediately after training, and were tested 24 h later. Muscimol (0.02, 0.1, or 0.5 μg), AP5 (0.34 or 0.5 μg), or SCH (0.5 or 1.75 μg) were amnesic. Then, animals were infused with muscimol (0.1 or 0.5 μg), AP5 (0.34, 0.5, or 5.0 μg), or SCH (0.5 μg) at other posttraining times and/or into the junction of Fr1–Fr2 (CII). Muscimol (0.1 and 0.5 μg) or SCH into CI were amnesic when given 90 or 180 min after training, but not when given 270 min after training. Muscimol (0.5 μg, but not 0.1 μg) or SCH into CII were amnesic when given 90 min after training, but not when given 0 or 180 min after training. AP5 (0.5, but not 5.0 μg) was amnesic when given into CI, but not into CII, at 0 or 180 min posttraining, and a trend toward an amnesic effect was seen at 90 min posttraining. The results suggest that 1) the glutamatergic, GABAergic, and dopaminergic systems in Fr2 are involved in the consolidation of memory for inhibitory avoidance learning, either directly or as parts of modulatory systems; and 2) timing of involvement of anterior Fr2 (CI) is different from that of posterior Fr2 (CII). © 2000 Elsevier Science Inc.

Prefrontal cortex Dopamine NMDA receptors AMPA receptors GABA Memory consolidation

THE hippocampus, the amygdala, and the entorhinal, parietal, and posterior cingulate cortices are involved in the consolidation of memory for a step-down inhibitory avoidance learning task in rats through mechanisms sensitive to the *N*-methyl-D-aspartate (NMDA) glutamatergic receptor antagonist aminophosphonopentanoic acid (AP5) and to the γ-aminobutyric acid type A ($GABA_A$) receptor agonist muscimol. Infusion of muscimol or AP5 is amnesic when given immediately after training into the hippocampus and amygdala, 30–180 min after training into the entorhinal cortex, or 60–180 min after training into the parietal cortex (16–18). Infusion of muscimol is amnesic when given 90 min after training into the posterior cingulate (27). Taking into account that AP5 and muscimol interfere with the signaling of the most important excitatory and inhibitory neurotransmitters of the central nervous system, respectively, these results indicate that 1) the hippocampus and amygdala participate early on, and 2) the entorhinal, parietal, and cingulate cortices play a role in memory formation at least 0.5 h later (16–18,27). Otherwise, timing of involvement of other neurotransmitter systems in the hippocampus, such as the cholinergic, dopaminergic, and adrenergic systems, is different from that of the glutamatergic and gabaergic systems, probably because these three systems are involved in modulatory rather than in core mechanisms [see review in (18)].

The search for the involvement of other areas, such as the prefrontal cortex (in the case of this work, its precentral area—Fr2), in the consolidation of memory for inhibitory avoidance learning is one of our main interest. The identification of the prefrontal cortex is based on reciprocal connections between this region and the medial dorsal nucleus of the thalamus (43),

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despite the inclusion of the rat medial precentral (Fr2) and the infralimbic (IL) areas as parts of the rat medial prefrontal cortex (12,38,39). Fr2 comprises an area equivalent to the sum of human Brodmann's areas (BA) 8 and 10 (5,6), which corresponds to part of the prefrontal association cortex.

The prefrontal cortex and other brain structures mediate working memory (10,32), which is defined as a system that processes and holds information for very short periods (seconds), and by which the animal perform cognitive tasks, such as comprehension, thinking, and planning (1,10). The prefrontal cortex is also fundamental for delayed responses (11,31,35) and, in the case of BA 8, for visual conditional learning (30). In humans, BA 10 is involved in autobiographical episodic memory (7).

Fr2 also seems to comprise an area corresponding to the primate premotor and supplementary motor areas (BA 6) (5,6,13,39), which receive converging axons from the prefrontal and parietal cortex and convert signals encoding desired actions into how the actions will be carried out (2). The primate premotor area is also involved in working memory and delayed responses (8,22,37,41), as well as in motor memory consolidation (36) and in visuomotor sequence learning (14). Neurons in a specific region of the primate supplementary area are activated particularly when subjects encounter a new context that requires motor plans to be updated (15).

Increased levels of dopamine in the prefrontal and premotor cortex were seen in primates performing a task related to working memory (41). Evidence has shown that D_1 dopaminergic receptors are involved in working memory (10,34,41,42). In a previous study, we showed that immediately posttraining infusion of the type 1 dopaminergic (D_1) receptor antagonist SCH23390 (0.5 μg) or of muscimol into the medial precentral area (Fr2, CI) was amnesic for long-term memory (test at 24 h), but not for short-term memory (test at 1.5 h), in a one-trial step-down inhibitory avoidance learning task in rats. In addition, infusion of these drugs 6 min prior to training impaired immediate memory for this task (19).

The objective of the present work is to analyze the involvement of the anterior medial precentral area (Fr2) in the consolidation of memory for a step-down inhibitory avoidance learning task in rats. To analyze the involvement of the two main neurotransmitters of the central nervous system, glutamate and GABA, infusion of AP5 or muscimol was given immediately after training in different concentrations, range of which included those concentrations used in previous works (16,17,27). Some of these concentrations, mainly those that were effective, were also infused 1) at other posttraining times, to see how long these drugs may affect consolidation of memory for this task, and/or 2) into a more posterior region of Fr2, because the rat Fr2 corresponds to different regions of the human brain (5,6,13,39) and might have functional differences along the anterior-posterior axis.

Because the dopaminergic system in the prefrontal cortex is involved in working memory (10,34,41,42) and might be involved in other functions, such as memory consolidation for inhibitory avoidance learning, we also intend to analyze the involvement of this system in Fr2. Therefore, different concentrations of SCH were infused into anterior Fr2 (CI) immediately after training, and the most effective concentration was also infused at other posttraining times and/or into CII.

METHOD

Subjects

Five hundred thirteen male Wistar rats (age, 60–90 days) were obtained from our breeding colony. The animals were housed five to a cage with food and water ad lib under a 12 L:12 D cycle (lights on at 0700 h) at a temperature of 23°C.

Surgery and Behavioral Procedures

The animals were bilaterally implanted under thionembutal anesthesia (30 mg/kg, IP) with 27-gauge guide cannulae. At least 48 h later, all animals were trained in a step-down inhibitory avoidance learning task (16,17,19,27). The rats were placed on a 2.5-cm high by 7.0-cm wide formica platform at the left of a 50 × 25 × 25-cm apparatus, the floor of which was a series of parallel 0.1-cm caliber stainless steel bars spaced 1.0 cm apart. Latency to step down placing the four paws on the grid was measured. In the training session, immediately upon stepping down, the animals received a 3.0-s, 0.4-mA foot shock. A retention test was carried out 24 h after training. The test session was procedurally identical except that no foot shock was given, and the step-down latency was cut off at 180 s; i.e.., test session values higher than 180 s were counted as 180 s. Retention test performance was taken as a measure of retention.

Infusion Procedure and Control of Cannula Placements

At the time of infusion, 30-gauge cannulae were fit into the guide cannulae (16,17,19,27). Animals received a bilateral infusion, which was given into anterior Fr2 (CI) immediately after training, of 0.5 μl of the $GABA_A$ agonist receptor muscimol HBr (0.02, 0.1, or 0.5 μg), of the glutamate NMDA antagonist 2-amino-S-phosphonopentanoic acid (AP5) (0.16, 0.34, 0.5, 1.6, or 5.0 μg), of the D_1 receptor antagonist R(+)SCH 23390 HCl (0.05, 0.34, 0.5, or 1.75 μg), of saline (phosphate buffer, pH 7.4), or of vehicle (solution of DMSO 20% in saline). To verify the involvement of Fr2 at other posttraining times, muscimol (0.1 and 0.5 μg), AP5 (0.5 and 5.0 μg), or SCH (0.5 μg) were also infused 90, 180, or 270 min after training (muscimol and AP5 were infused only in the higher concentration at 270 min posttraining). These posttraining time points were chosen because drugs presumably diffuse away within 90 min (26). To verify whether these drugs may also disrupt memory consolidation when infused into a more posterior region of Fr2, muscimol (0.5 μg), AP5 (5.0 μg), or SCH (0.5 μg) were also infused into the junction of Fr1–Fr2 (CII) immediately, 90, or 180 min after training. In addition, infusion of muscimol (0.1 μg) or AP5 (0.34 or 0.5 μg) was also administered 90 min after training into CII. All drugs were purchased from Research Biochemicals International (RBI), Natick, MA. The sites of infusion were chosen using coordinates (from bregma and dura) obtained from (29), as follows (units in cm): most anterior part of Fr2 (CI), A +0.47, L ±0.28, V −0.10 (Fig. 1A); and Fr1–Fr2 junction (CII), A +0.20, L ±0.20, V −0.01 (Fig. 1B).

Two to 24 h after the end of the behavioral procedure all animals received an infusion of 0.5 μl of 4% methylene blue through the infusion cannulae, and were killed by decapitation. Their brains were removed and stored in formalin for histological localization of infusion sites as explained elsewhere (16,17,19). Infusion placements were correct in 337 and 170 animals implanted in coordinates I and II, respectively. Only animals with correct cannula locations (Fig. 1) were included in the final statistical analysis.

Statistics

Data are reported as median (interquartile range) of the retention test performance. Training session performances of groups of the same site and time of infusion were compared

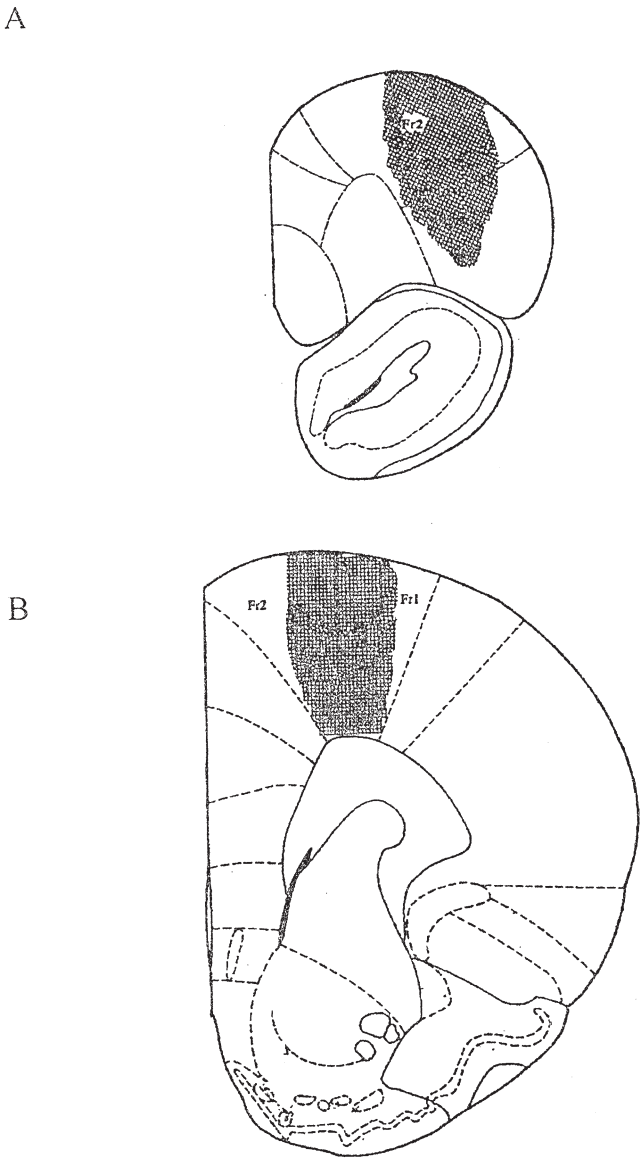

FIG. 1. (A, B) Schematic drawings of rat brain sections at planes +0.47 and +0.22, respectively, from (29) showing (stippled) the extension of the areas reached by infusions into coordinates I and II. In each animal, maximum extension of the site(s) reached by the infusions was less than 1.5 mm^3, as ascertained by the spread of a 0.5 μl infusion of 4% methylene blue into each of the structures, 24 h after the last behavioral manipulation.

by the Kruskal–Wallis analyzes of variance. Differences between training and test session performances in each group were evaluated by a Wilcoxon test. Differences from the control group of the same time of infusion in test session performances were evaluated by Mann–Whitney *U*-test, two-tailed. $p < 0.05$ was considered to indicate statistical significance.

RESULTS

Training vs. Test Session Performances

There is no difference among groups regarding the training session performances (overall median, 4.9 s; overall interquartile range, 3.3/7.6 s; Kruskal–Wallis ANOVA, $p > 0.10$; data not shown).

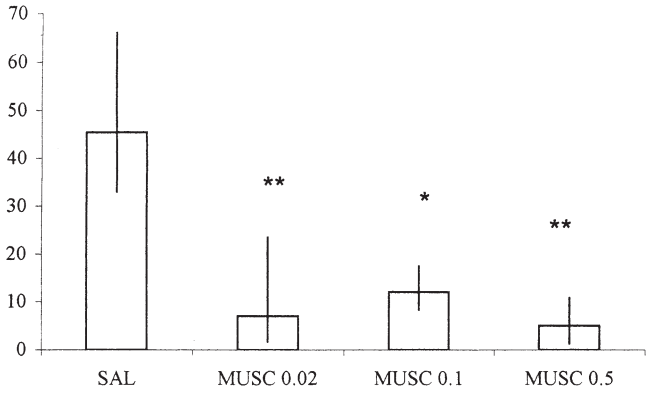

FIG. 2. Median (interquartile range) test session latency in groups infused bilaterally into coordinate I of Fr2 with saline (SAL) or muscimol (0.02, 0.1, or 0.5 μg) immediately after training. Double and single asterisks indicates statistical significance in Mann–Whitney *U*-tests, two tailed, at $p < 0.01$ and at $p < 0.05$, respectively, from the respective control group. *N* per group was 10.

Animals infused into CI with AP5 (0.34 μg) immediately after training, SCH (0.5 μg) 0, 90, or 180 min after training, or the higher dose of muscimol 0 or 180 min after training did not show a difference between training and test session performance (Wilcoxon test, $p > 0.10$). Animals infused into CII with the higher dose of muscimol 90 min after training did not show a difference between both sessions (Wilcoxon test, $p > 0.10$). The other groups showed a difference between both sessions (Wilcoxon test, $p < 0.05$).

Effects of Infusion Into Anterior Fr2 (CI) Given Immediately After Training

Three dose–response curves were obtained from infusions into CI immediately after training (Figs. 2, 3 and 4). Muscimol (0.02, 0.1, or 0.5 μg), SCH (0.5 or 1.75 μg, but not 0.05 or 0.34 μg), or AP5 (0.34 or 0.5 μg, but not 0.16, 1.6, or 5.0 μg) were amnesic when given into CI immediately after training

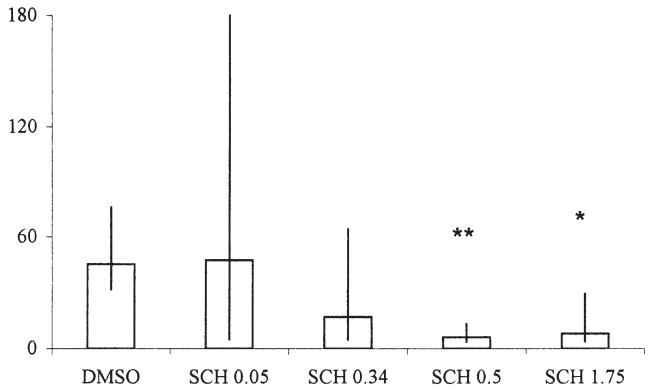

FIG. 3. Same of the previous figure but for infusions of SCH (0.05, 0.34, 0.5, or 1.75 μg). *N* per group was 9–13.

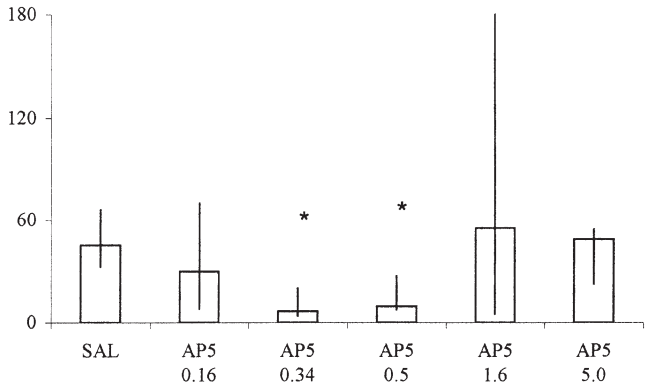

FIG. 4. Same of the previous figure but for infusions of AP5 (0.16, 0.34, 0.5, 1.6, or 5.0 μg). *N* per group was 10.

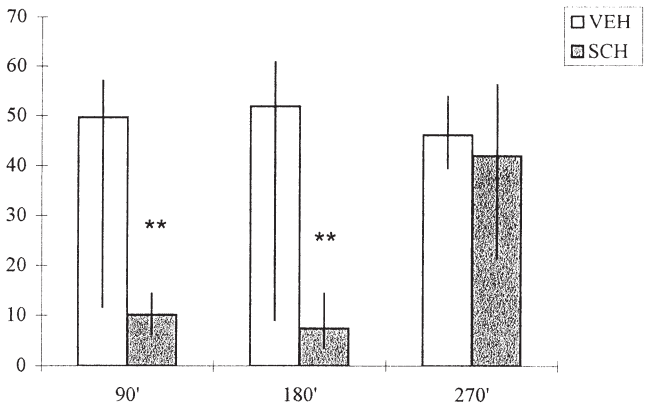

FIG. 6. Same of the previous figure but for infusions of SCH (0.5 μg). *N* per group was 9–11.

(Mann–Whitney *U*-test, two-tailed, $p < 0.05$; $p > 0.10$ for groups not different from controls). *N* per group was 10, 9–13, and 10, respectively.

Effects of Infusion Into Anterior Fr2 (CI) Given 90–270 min After Training

Muscimol (0.1 or 0.5 μg) was amnesic when given into CI 180 min after training (Mann–Whitney *U*-test, two tailed, $p < 0.05$), but it was amnesic when given 90 min after training only at the higher dose (Mann–Whitney *U*-test, two tailed, $p < 0.05$) and was not effective when given 270 min after training (Mann–Whitney *U*-test, two tailed, $p > 0.10$) (Fig. 5). A trend toward an amnesic effect was seen when muscimol at 0.1 μg was given at 90 min posttraining (Mann–Whitney *U*-test, two tailed, $p < 0.10$). *N* per group was 8–11. SCH (0.5 μg) was amnesic when given 90 or 180 min (Mann–Whitney *U*-test, two tailed, $p < 0.05$), but not 270 min after training (Mann–Whitney *U*-test, two tailed, $p > 0.10$) (Fig. 6). A trend toward an amnesic effect was seen when AP5 (0.5, but not 0.34 or 5.0 μg) was given at 90 min posttraining (Mann–Whitney *U*-test, two tailed, $p < 0.10$) (Fig. 7a). AP5 (0.5, but not 5.0 μg) was amnesic when given 180 min (Mann–Whitney *U*-test, two tailed, $p < 0.05$), but not when given 270 min after training (Mann–Whitney *U*-test, two tailed, $p > 0.10$) (Fig. 7b). *N* per group was 9–11.

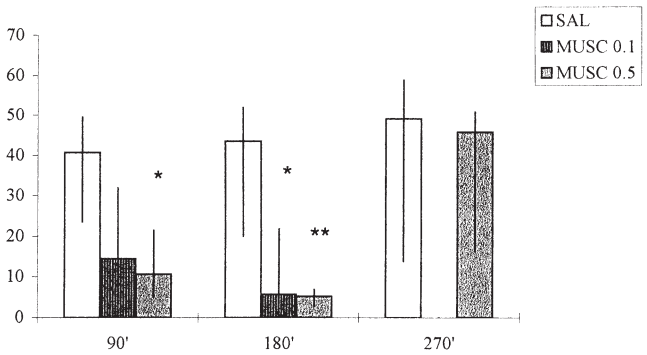

FIG. 5. Median (interquartile range) test session latency in groups infused bilaterally into coordinate I of Fr2 with saline (0.5 μl, pH 7.4) or muscimol (0.1 or 0.5 μg) (see legend), 90, 180, or 270 min posttraining. Double and single asterisk indicates statistical significance in Mann–Whitney *U*-tests, two tailed, at $p < 0.01$ and at $p < 0.05$, respectively, from the control group. *N* per group was 8–11.

Effects of Infusion Into the Junction of Fr1–Fr2 (CII) Given 0–180 min After Training

Muscimol was amnesic at the higher concentration (Mann–Whitney *U*-test, two tailed, $p < 0.05$), but not at the lower concentration (Mann–Whitney *U*-test, two tailed, $p > 0.10$), when given 90 min after training into CII. Muscimol was not effective when given 0 or 180 min after training into CII (Mann–Whitney *U*-test, two tailed, $p > 0.10$) (Fig. 8). *N* per group was 10–11.

AP5 (0.5 or 5.0 μg) was not effective when given into CII 0, 90, and 180 min after training (Mann–Whitney *U*-test, two tailed, $p > 0.10$) (Fig. 9). *N* per group was 8–11.

Infusion of SCH into CII was amnesic when given 90 min after training (Mann–Whitney *U*-test, two tailed, $p < 0.05$), but not when given immediately or 180 min after training (Mann–Whitney *U*-test, two tailed, $p > 0.10$) (Fig. 10). *N* per group was 10–11.

DISCUSSION

The present results suggest that 1) the glutamatergic, GABAergic, and dopaminergic systems in Fr2 are involved in the consolidation of memory for inhibitory avoidance learning, either directly or as parts of modulatory systems; and 2) the most anterior part of Fr2 is involved in this for a longer period than the most posterior part.

Previous studies from our laboratory showed that the hippocampus, the amygdala, the entorhinal, parietal, and posterior cingulate cortices are involved in the consolidation of memory for a step-down inhibitory avoidance learning task by muscimol-dependent mechanisms (16–18,27). The amnesic effect of muscimol (0.5 μg) into coordinate I when given 0–180 min after training suggests two explanations: 1) the most anterior part of Fr2 is involved in the consolidation of memory for inhibitory avoidance learning by mechanisms sensitive to muscimol during a period of time equivalent to that of the hippocampus (0 min) and the entorhinal (30–180 min), parietal (60–180 min), and posterior cingulate (90 min) cortices combined; or (2) alterations in the activity of the most anterior part of

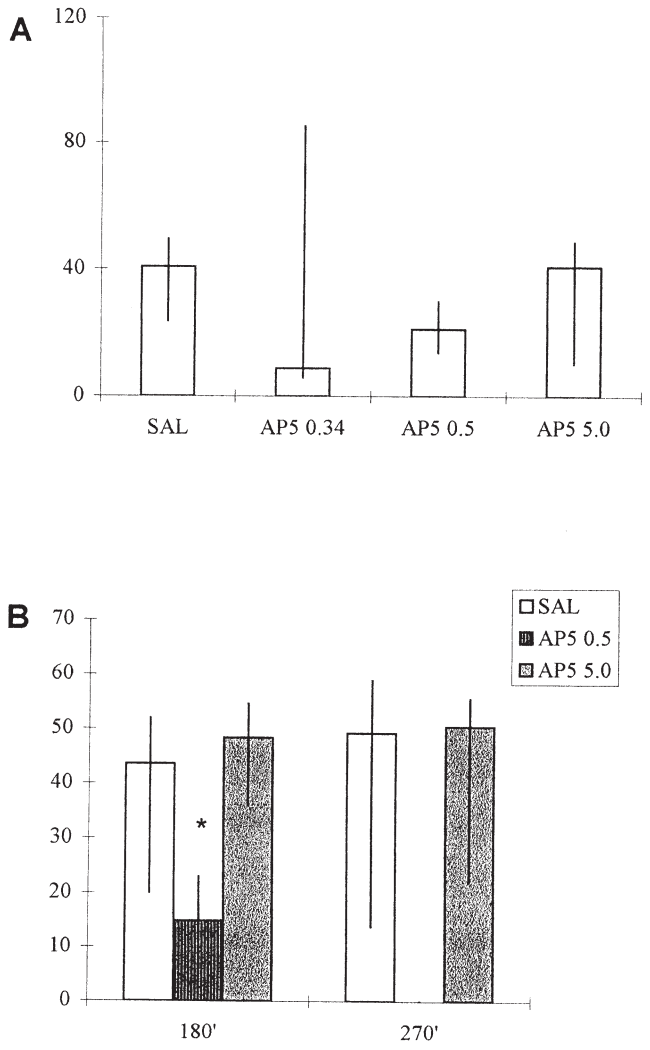

FIG. 7. (A) Median (interquartile range) test session latency in groups infused bilaterally into coordinate I of Fr2 with saline (SAL) or AP5 (0.34, 0.5, or 5.0 μg) 90 min after training. (B) Same of A but for infusions of saline (0.5 μl, pH 7.4) or AP5 (0.5 or 5.0 μg) (see legend) 180 or 270 min posttraining. Single asterisk indicates statistical significance in Mann–Whitney *U*-tests, two tailed, at $p < 0.05$, from the control group. *N* per group was 9–11.

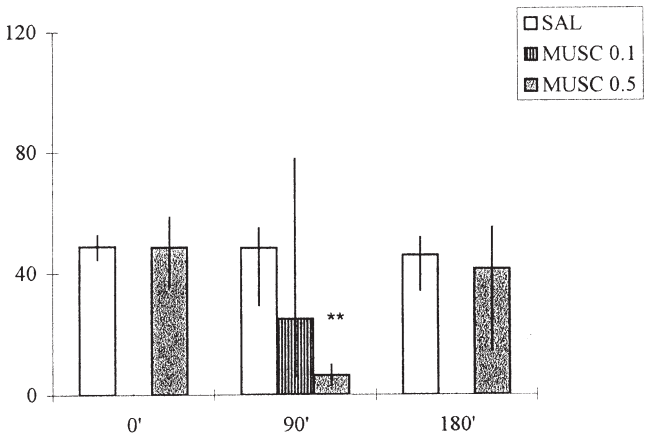

FIG. 8. Median (interquartile range) test session latency in groups infused bilaterally into coordinate II with saline (SAL) or muscimol (0.1 or 0.5 μg) immediately, or 90 or 180 min after training. Double asterisks indicates statistical significance in Mann–Whitney *U*-tests, two tailed, at $p < 0.01$ and at $p < 0.05$, respectively, from the respective control group. *N* per group was 10–11.

Fr2 merely affect the transactional processes involved in consolidation 0–180 min posttraining. In the case of the most posterior part of Fr2, its time window seems to occur between that of the hippocampus (0 min), and those of the entorhinal (30–180 min) and parietal cortex (60–180 min) (19), because muscimol (0.5 μg) infusion was effective only at 90 min posttraining.

The differentiation of Brodmann's areas 6 (premotor area) and 8, which belong to the posterior dorsolateral frontal cortex, and area 10 (frontopolar cortex) in humans, but not in rats (5,6,13,39), may be related to further evolutionary advances of the human brain, prominent in the neocortex. Therefore, we think that the different intervals of involvement between the most anterior and the most posterior parts of Fr2 by mechanisms sensitive to muscimol might be related to functional rather than anatomical differences of Fr2 along the anterior–posterior axis.

The fact that muscimol was amnesic when given into CI 90 min after training only at the higher concentration suggests that Fr2 is less sensitive to muscimol infusion at 90 min posttraining relative to other posttraining times, 0 and 180 min; i.e., muscimol might have two peaks of action, at 0 and 180 min. The reasons for this are unknown. Increasing evidence of simultaneous and coordinated activity of different brain regions in the posttraining period suggests a "multiple consolidation of memory" (4). We might speculate that the processes of consolidation are widely distributed at this time, being mediated by several cortical areas, such as the entorhinal, parietal, and cingulate cortices (17,18,27), and the precentral area. Therefore, the network would be less sensitive to alterations in the precentral area.

The amnesic effect of SCH infused into both coordinates might be related to the role of dopamine in modulating pre-

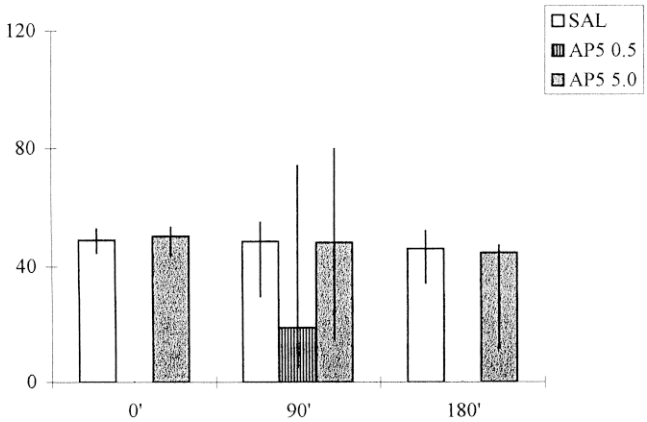

FIG. 9. Same of the previous figure but for infusions of AP5 (0.5 or 5.0 μg). *N* per group was 8–11.

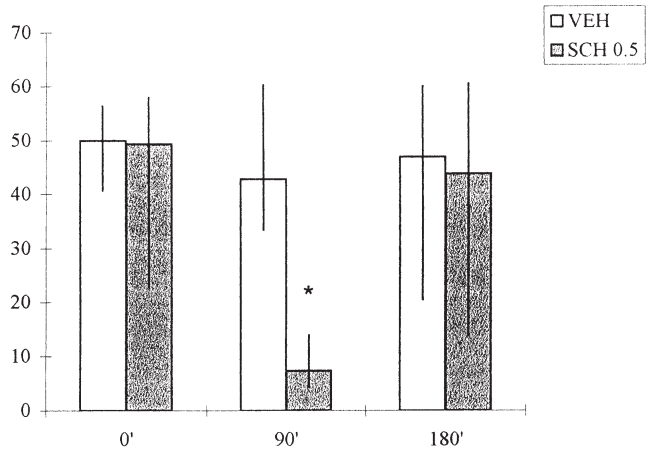

FIG. 10. Same of the previous figure but for infusions of SCH (5.0 μg). *N* per group was 10–11.

frontal pyramidal cell excitability, which is, for instance, essential for working memory for a oculomotor task, which requires memory-guided saccades (10,34). Primates have increased levels of extracellular dopamine in the prefrontal cortex during performance of a delayed alternation task, and in the premotor area, during performance of both this task and a sensory-guided paradigm (41). In addition, spatial working memory performance exhibits an inverted U dose–response curve to D_1 receptor stimulation levels in the prefrontal cortex; i.e., intermediate levels of D_1 receptor stimulation optimize working memory performance (42). A previous study showed that SCH (0.5 μg) impaired immediate memory for inhibitory avoidance learning when applied prior to training, and impaired consolidation when applied immediately after training (19). In this study, the dose–response curve shows that SCH is amnesic when infused immediately after training at doses higher than a threshold, value of which is higher than 0.34 μg. It is worth pointing out that 1) the highest dose almost reachs the saturation point of SCH in vehicle (DMSO 20% in saline), and 2) the dose of 0.5 μg is enough to significantly bind SCH to D_1 receptors, but not to serotonergic receptors (3,9,25). Therefore, low levels of D_1 receptor stimulation in Fr2 may disrupt consolidation of memory for inhibitory avoidance learning.

Because AP5 was amnesic when given into anterior CI immediately after training only at intermediate doses, this drug presents a U dose–response curve. In addition, AP5 is effective in a small range of concentrations. The reasons for this are unknown, and deserve further studies. Some cues raises from other studies: 1) a presynaptic modulation of dopamine release from NMDA and non-NMDA glutamate receptors, as occurs in the striatum (40); 2) a stimulation of GABA release by NMDA receptor activation, which might inhibit the release of dopamine, as probably occurs in the medial prefrontal cortex (21); 3) glutamatergic neurotransmission at non-NMDA receptors in the prefrontal cortex is increased or decreased by low or high doses, respectively, of the NMDA receptor antagonist ketamine, being intermediate doses ineffective (28); 4) in the striatum, high levels of NMDA receptor activation increase dopamine release, an effect that has been attributed to general excitation (24). However, it is worth pointing out that the amnesic effect of AP5 when given into anterior Fr2 (CI) immediately and 180 min after training suggests that NMDA receptors in this area are essential for memory consolidation in inhibitory avoidance learning at these times. In addition, our data do not definitively rule out the possibility that NMDA receptors are involved in this process at 90-min posttraining, because a trend toward an amnesic effect of AP5 (0.5 μg) was found at this time. It is also worth pointing out that muscimol is amnesic when infused immediately after training in a larger range of concentrations than SCH or AP5, even though it has not been necessary to cover the whole dose–response curve of this drug.

The timing of involvement of NMDA, $GABA_A$, and D_1 receptors in anterior Fr2 is probably the same (0–180 min), despite the decrease of sensitivity for muscimol and AP5 at 90 min posttraining. This similarity might be interpreted as an indirect evidence for either an interaction of these systems in Fr2, as occurs in the striatum and in the medial prefrontal cortex (21,28,40), or an interaction that emerges from the recycling of information in the network formed by the prefrontal cortex, the motor and premotor areas, the basal ganglia, and the ventral tegmental area: the motor loop (2). For instance, a contralateral infusion of bicuculline, a $GABA_A$ antagonist, into the medial prefrontal cortex increases the release of dopamine in the striatum, an effect that is reverted by an infusion of an excitatory amino acid receptor antagonist into the ventral tegmental area, but not directly into the striatum, which receives afferents from both the medial prefrontal cortex and the ventral tegmental area (23). Alterations in the glutamatergic system in Fr2 might indirectly interfere with its dopaminergic system via other structures. The fact that anterior Fr2 is less sensitive to muscimol and AP5 infusion at 90 min posttraining might also be explained by one of these putative interactions among the neurotransmitter systems in Fr2. For instance, different mechanisms may be responsible for the activation of dopamine release in the striatum following potent stressors and in the prefrontal cortex following relatively lower stressors (20). The set of mechanisms involved in consolidation at 90 min posttraining might differ from that at 0 or 180 min posttraining. Further studies may clarify these issues and their relevance to memory for inhibitory avoidance learning.

The lack of effect or even a trend toward an effect when AP5 at both concentrations was given into CII suggests that NMDA receptors in this region are not essential for memory consolidation in inhibitory avoidance learning. However, our results suggest that D_1 receptors, not only in anterior Fr2, but also in posterior Fr2, may be involved in consolidation of memory for inhibitory avoidance learning. However, D_1 receptors in posterior Fr2 may be relevant only at 90 min posttraining.

We should point out that another explanation of our results might be that other structures than Fr2 were also reached by our infusions; i.e., each infusion might reach a larger area than showed by our histological procedure. In the case of infusions into CI, our histology showed it was restricted to Fr2 in its majority, but some of them reached the sulcal cortex, an area that corresponds to the primate orbitofrontal cortex (Fig. 1). Therefore, infusions into CI might reach the sulcal cortex and, less probably, the medial prefrontal cortex, which are involved in memory for inhibitory avoidance learning (33). In the case of CII, infusions might reach the dorsal and, less probably, the ventral medial prefrontal cortex. However, the possibility of this explanation is unlikely, because 1) 1.0-μl infusions of muscimol, twice larger in volume than our infusions, maximally reduce glucose uptake in a restricted region of 1 mm^3 (26); and 2) muscimol infusion

into ventral medial prefrontal cortex at the coronal plane + 2.2 cm was not effective in altering retention for inhibitory avoidance learning measured 24 h after training (27).

In conclusion, further studies are necessary to clarify the function of the medial precentral area (Fr2) in memory and the interaction of its neurotransmitter systems. The present contribution focuses on the time window in which memory consolidation for inhibitory avoidance learning in rats is sensitive to posttraining infusion of muscimol, AP5, or SCH, at different doses, into the medial precentral area (Fr2).

ACKNOWLEDGEMENTS

This study was supported by CNPq and PRONEX, Brazil. We are grateful to Silvia Alejandra Minetti, Marcelo Madruga, and Márcia Kauer Sant'Anna for their contribution in the present work.

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4.1 Atualização

Uma versão mais atualizada do Atlas de Paxinos e Watson (4ª edição, de 1998) diferencia em termos estruturais as duas regiões do **Fr2** aqui estudadas. A parte mais anterior (de + 0,52 a + 0,47 cm do bregma) recebeu a denominação de **área frontal associativa (FrA)**, enquanto a mais posterior recebeu a denominação de **córtex motor secundário (M2)**. Portanto, há concordância mais recente sobre a diferenciação funcional por nós defendida.

5. Participação do Córtex Cingulado Anterior na Evocação da Memória da Tarefa de Esquiva Inibitória

Embora o artigo aqui apresentado mostre a participação de diversas estruturas na evocação da memória de longa duração, destacaremos apenas a participação do córtex cingulado anterior (Cg1 e Cg3), regiões com homologia ao córtex cingulado anterior e ao córtex pré-frontal dorsolateral medial de humanos, respectivamente.

O estudo da participação do córtex cingulado anterior na evocação da tarefa de esquiva inibitória foi motivado pelo trabalho de Seamans *et al.* (1995), que demonstra que esta estrutura participa da evocação da memória do labirinto radial.

6. Envolvimento dos Receptores Serotonérgicos do Córtex Insular Agranular na Consolidação da Memória da Tarefa de Esquiva Inibitória

Este estudo visou iniciar o entendimento da participação do córtex insular agranular na memória da tarefa de esquiva inibitória. Começamos, então, pela análise da participação de seus receptores serotonérgicos 1A, uma vez que esta região é alvo massivo deste sistema neuromodulador (Zilles e Wree, 1995).

Referido artigo foi submetido à revista "Behavioural Pharmacology".

ELSEVIER

Behavioural Brain Research 114 (2000) 183–192

BEHAVIOURAL BRAIN RESEARCH

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Research report

Molecular signalling pathways in the cerebral cortex are required for retrieval of one-trial avoidance learning in rats

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Received 24 January 2000; received in revised form 10 April 2000; accepted 10 April 2000

Abstract

Rats were implanted bilaterally with cannulae in the CA1 region of the dorsal hippocampus, the entorhinal cortex, anterior cingulate cortex, posterior parietal cortex, or the basolateral complex of the amygdala. The animals were trained in one-trial step-down inhibitory avoidance and tested 24 h later. Prior (10 min) to the retention test, through the cannulae, they received 0.5 μl infusions of a vehicle (2% dimethylsulfoxide in saline), or of the following drugs dissolved in the vehicle: the glutamate NMDA receptor blocker, aminophosphonopentanoic acid (AP5, 2.0 or 5.0 μg), the AMPA receptor blocker, 6,7-dinitroquinoxaline-2,3 (1H,4H)dione (DNQX, 0.4 or 1.0 μg), the metabotropic receptor antagonist, methylcarboxyphenylglycine (MCPG, 0.5 or 2.5 μg), the inhibitor of cAMP-dependent protein kinase (PKA), Rp-cAMPs (0.1 or 0.5 μg), the PKA stimulant, Sp-cAMPs (0.5 μg), or the inhibitor of the mitogen-activated protein kinase (MAPK), PD098059 (10 or 50 μM). All these drugs, at the same doses, had been previously found to alter long-term memory formation of this task. Here, retrieval test performance was blocked by DNQX, MCPG, Rp-cAMPs and PD098059 and enhanced by Sp-cAMPs infused into CA1 or the entorhinal cortex. The drugs had similar effects when infused into the parietal or anterior cingulate cortex, except that in these two areas AP5 also blocked retrieval, and in the cingulate cortex DNQX had no effect. Infusions into the basolateral amygdala were ineffective except for DNQX, which hindered retrieval. None of the treatments that affected retrieval had any influence on performance in an open field or in a plus maze; therefore, their effect on retention testing can not be attributed to an influence on locomotion, exploration or anxiety. The results indicate that the four cortical regions studied participate actively in, and are necessary for, retrieval of the one-trial avoidance task. They require metabotropic and/or NMDA glutamate receptors and PKA and MAPK activity. In contrast, the basolateral amygdala appears to participate only through a maintenance of its regular excitatory transmission mediated by glutamate AMPA receptors.

Keywords: Retrieval; AMPA glutamate receptors; Metabotropic glutamate receptors; NMDA glutamate receptors; cAMP-dependent protein kinase; Mitogen-activated protein kinase; CA1; Entorhinal cortex; Posterior parietal cortex; Anterior cingulate cortex; Basolateral amygdala

1. Introduction

Retrieval of one-trial step-down avoidance is inhibited by the bilateral infusion into the dorsal CA1 area of the rat hippocampus by antagonists of AMPA/kainate or metabotropic glutamate receptors, or by inhibitors of the cAMP-dependent protein kinase (PKA), mitogen-activated protein kinase (MAPK) or protein kinase C (PKC) signalling pathways [20,22,44]. The effect of the drugs is not as a result of influences on locomotion, exploration or anxiety, because the treatments had no effect on behaviour in an open-field or a plus-maze [22,44]. To our knowledge, these are the first data that provide hints on the molecular basis of re-

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PII: S0166-4328(00)00226-6

trieval. The involvement of the enzymatic pathways mentioned in CA1 is important because they are known to be crucial for consolidation of this task [2,3,17,19,20,29,31,43–45] and others [2,17,29], as well as of long-term potentiation [17,29]. Further, there is ample cross-talk between these signalling pathways [24,29,37], particularly PKA and MAPK [24,29]. Glutamate receptors stimulate PKA and MAPK activity (see below) [17,29]. AMPA, NMDA and metabotropic glutamate receptors in CA1 [6,8,15,17,19] are necessary for memory formation of this or other tasks.

The CA1 area of the hippocampus is part of a circuit that includes the subiculum, entorhinal cortex, dentate gyrus and CA3 [42]. This circuit has been recently demonstrated to be functionally active and to be capable of reverberation [12]. By virtue of the longitudinal spread of the connections of its various components [11,20], it probably involves a large portion of the hippocampus every time it is activated. Recently it was calculated that 40% of the hippocampus is needed for encoding and 70% of it is needed for retrieval of a spatial learning task [30]. Measurement of the activity of PKA [3], PKC [31] or other enzymes [17] at various times after one-trial inhibitory avoidance training reveals marked changes measurable in the entire rat hippocampus.

The circuit described above is connected through the entorhinal region to many areas of the cortex, including the posterior parietal and cingulate cortex, and to the basolateral nuclear complex of the amygdala as well [11,42,47]. All these areas participate in the encoding and consolidation of one-trial inhibitory avoidance, each in a different manner [1,5,7,17,19,21,27]. The entorhinal, parietal and cingulate cortex are involved in consolidation through NMDA receptor-mediated, muscimol-sensitive mechanisms operating in a timed fashion in the first few hours of consolidation in parallel to hippocampal processing [21,27]. The anterior cingulate seems to play a lesser role in consolidation of the one-trial avoidance task [27]; but there is some evidence that it might be relevant to at least some forms of retrieval in other tasks [39]. The basolateral amygdala is critical for the early modulation of consolidation of aversively-motivated experiences [7,26,42] and may be important for the expression of emotional components at the time of retrieval [14,18,23,28,47]. The entorhinal and parietal cortex are involved in the modulation of consolidation hours after training [1,17]. Various studies point to the need of an intertwined activity of all these brain region, as well as others, in the consolidation of one-trial avoidance and related tasks [1,5,15,17,18,20,21,42].

Here we study the effect on retrieval of one-trial avoidance of the bilateral infusion into CA1, entorhinal cortex, posterior parietal cortex, anterior cingulate cortex, and basolateral amygdala of drugs acting on specific molecular mechanisms known to be critical for encoding and consolidation of this task. The drugs used are: the glutamate AMPA receptor antagonist, 6,7-dinitroquinoxaline-2,3 (1H,4H)dione (DNQX); the glutamate NMDA receptor antagonist, DL-amino-5-phosphonopentanoic acid (AP5); the generic glutamate metabotropic receptor antagonist, α-methyl-(4-carboxyphenyl)glycine (MCPG); the inhibitor (Sp-cAMPs) and the stimulant (Sp-cAMPs) of the cAMP-dependent protein kinase (PKA); and the inhibitor of the mitogen-activated protein kinase (MAPK) pathway, PD098059. The choice of these particular drugs obeys to the following reasons. AMPA receptors mediate most of the regular excitatory synaptic transmission in the brain. NMDA and metabotropic receptors lead to an increase in cellular Ca^{2+}; the former because of Ca^{2+} entry, and the latter through the release of endogenous Ca^{2+}. The increased Ca^{2+} in turn stimulates a variety of metabolic processes, including the activity of the protein kinases mentioned [17,29,43–45]. Metabotropic receptors, in addition, can stimulate PKA indirectly via activation of adenylyl cyclase [41]. In CA1, PKA and MAPK are crucial to memory formation [1,3,17,22,44–46] and, as shown recently [20,22], also for the retrieval of long-term memory.

2. Material and methods

Five experiments were carried out. In all of them, rats were trained in one-trial inhibitory avoidance and tested for retention 24 h later, a time at which long-term memory (LTM) is well established [17,19]. Drugs were bilaterally infused through indwelling cannulae into different brain structures 10 min prior to retention testing. Experiment 1 studied the CA1 region. Experiment 2 investigated the entorhinal cortex. Experiment 3 examined the posterior parietal cortex. Experiment 4 studied the anterior cingulate cortex. In experiment 5 the infusions were into the basolateral amygdala nuclear complex. In each experiment, in addition, we examined the influence of the drugs that had any effect on retrieval on open-field behaviour and on performance in an elevated plus-maze [22,33,43].

One-trial inhibitory avoidance was as follows [1,3,4,6,8,15,16,21,22,27,44–46]. Wistar rats (2.5–3 months-old, 230–300 g) were placed on a 2.5-cm high, 8-cm wide platform facing a 43 × 25-cm grid of bronze bars in a 25-cm high yellow acrylic box. Latency to step down onto the grid with all four paws was measured; upon stepping down the animals received a 0.4 mA, 2-s scrambled footshock and were immediately withdrawn from the training apparatus. Retention testing was 24 h after training. The test session was similar in all respects to the training session except that the footshock was

omitted. A 180 s ceiling was imposed on retention test latencies. This required the use of non-parametric statistics: a Kruskal-Wallis analyses of variance followed by individual Mann–Whitney *U*-tests, two-tailed.

A total of 575 rats were used 3–5 days prior to training the animals were implanted bilaterally under deep sodium thiopental anaesthesia (40 mg kg^{-1}) with 27-gauge cannulae aimed 1.0 mm above each of the desired structures (coordinates according to the atlas of Paxinos and Watson [32]): (1) the pyramidal cell layer of the CA1 subarea of the dorsal hippocampus, coordinates A: −4.16, L: ±3.0, V: +1.3; (2) the entorhinal cortex, coordinates A: −6.7, L: ±5.0, V: 7.8; (3) the posterior parietal cortex, coordinates A: −2.8, L: ±5.0, V: 4.8; (4) the anterior cingulate cortex [27,36], coordinates A: +2.2, L: ±1.0, V: 2.0; (5) the basolateral complex of the amygdaloid nucleus, coordinates A: −2.3, L: ±4.8, V: 7.7. The cannulae were fixed to the skull with dental acrylic [1,15,16,22,27,44,46].

Prior (10 min) to the retention test, the following treatments were infused through the cannulae, first on the left side and then on the right side: vehicle (2% dimethylsulfoxide in saline); AP5, 2.0 or 5.0 μg/side; DNQX, 0.4 or 1.0 μg/side; MCPG, 0.5 or 2.5 μg/side; Rp-cAMPs (10.1 or 0.5 μg/side; Sp-cAMPs, 0.5 μg/side; or PD098059, 10 or 50 μM. All these drugs, at the same doses, had been found to have strong effects on memory consolidation of this task [6,15,19,20,22]. All of them except DNQX were recently reported to affect retrieval of the one-trial avoidance task when given into CA1 prior to a test session carried out 31 days after training [22]. Infusion volume was 0.5 μl in all cases. In numerous previous papers it was found that the infusion of 0.5 μl of the vehicle has no effect on short- or long-term memory formation or retrieval or other behaviours [1,8,14–17,19–22]. Infusions were performed at a rate of 1 μl per min first on the left side and then on the right side. After the infusions were completed, the cannula was left in place for an additional 15 s. Thus, the entire bilateral infusion procedure took 90 s [1,15,43,44].

The treatments that were found to alter retrieval were studied, in addition, on exploratory activity in an open-field [43] and on pro- or anti-conflict behaviour in an elevated plus-maze [33]. After (10 min) the infusions into each of the structures mentioned above, rats were placed either in a 50 cm wide, 42 cm deep, 60 cm high plywood open-field over 2 min, or an elevated plus-maze as described by Pellow and her coworkers [33] for 5 min. Crossings of black lines dividing 12 equal rectangles in the floor and rearings were counted in the open-field [22,43]. Total number of entries into the four arms, number of entries into the open arms, percentage of time spent in the open arms, and rearings were counted in the plus maze [22,33,43]. The subjects of these experiments were animals chosen at random among those that had been previously studied in the inhibitory avoidance task. An interval of 1–3 days was allowed between inhibitory avoidance testing and exposure to the open field and/or the plus-maze.

Cannula placements were verified as described [1,6,15,16,21]. Briefly, 24 h after their last behavioural manipulation (inhibitory avoidance, plus maze or open field) the animals received a 0.5 μl infusion of 4% methylene blue in saline through the infusion cannulae. The spread of the dye was taken to represent the probable spread of the solution(s) that had been infused before through exactly the same cannula location, and was examined histologically. The spread was in all cases less than 1 mm^3, which coincides with previous work from our group [1,15,16,21] and, importantly, with that of Martin [25] who infused the same volume (0.5 μl) of radioactive lidocaine or muscimol and measured both the radioactivity and the inhibitory effect of the two substances on 2-D-glucose uptake by brain tissue. In the present experiments, cannula placements were found to be correct (i.e. within 1 mm^3 of the intended site) in 119 of the 120 animals implanted in CA1, in 123 of the 125 animals implanted in the entorhinal cortex, in 108 of the 110 rats implanted in the parietal cortex, in 107 of the 110 animals implanted in the anterior cingulate, and in all of the 110 animals implanted in the basolateral amygdala. The maximum extension of infusion sites in each brain region is shown in Fig. 1, which is a composite of all data from animals with correct cannula placements in each brain region studied.

Statistics were non-parametric for the test session inhibitory avoidance data, and for their comparison with the corresponding training session value for each group, as a result of the fact that there was a ceiling of 180 s in the test session latency measurements. A Kruskal-Wallis ANOVA was followed by individual Mann-Whitney tests, two-tailed. Parametric statistics (super ANOVA followed by post-hoc Duncan multiple range tests) were applied to all other measures: comparisons among groups in training session latencies, plus-maze measures, and open field crossing or rearing values.

3. Results

3.1. Experiment 1: effect on retrieval of drugs infused bilaterally into CA1

Training session latencies were not significantly different among groups in a super ANOVA (overall mean, 4.7 s; median, 4.5 s; range, 1.0–11.0 s) ($N = 119$).

Retention test performance was significantly impaired by DNQX at the two dose levels studied, by MCPG at the higher dose, and by Rp-cAMPs and

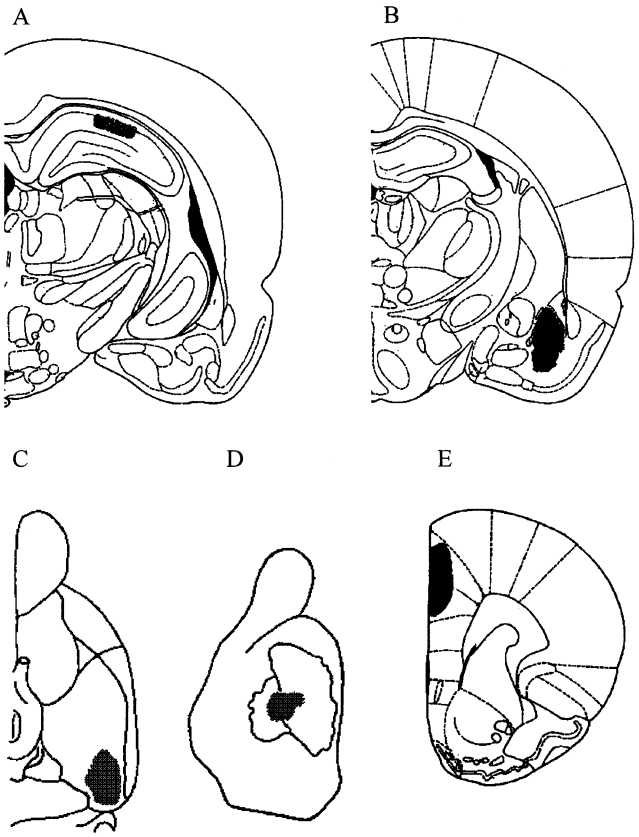

Fig. 1. Maximum area reached by the infusions in (A) dorsal hippocampal CA1; (B) basolateral amygdala; (C) entorhinal cortex; (D) posterior parietal cortex; and (E) anterior cingulate cortex. A, B and E are drawings based on antero-posterior planes −4.16, −2.3 and +2.2 of the atlas by Paxinos and Watson [32], and C and D are drawings based on the atlas by Zilles [49].

PD098059 at the two doses studied. In all these groups, training-test latency differences were not significant in Mann–Whitney *U*-tests at $P = 0.1$ level. Retention test performance was significantly enhanced by Sp-cAMPs, and unaffected by AP5 (Fig. 2).

The effect on plus maze and open field performance of the higher dose of the drugs that influenced retrieval was studied. None of them had any effect on these two behaviours (Table 1).

3.2. Experiment 2: effect on retrieval of drugs infused bilaterally into the entorhinal cortex

Training session latencies were not significantly different among groups (overall mean, 5.5 s; median, 5.2; range, 1.1–17.5 s) ($N = 123$).

Retention test performance was significantly impaired by DNQX, MCPG, Rp-cAMPs and PD098059 at the two dose levels studied. In all these groups, training-test latency differences were not significant in Mann–Whitney *U*-tests at $P = 0.1$ level. Retention test performance was significantly enhanced by Sp-cAMPs, and unaffected by AP5 (Fig. 3).

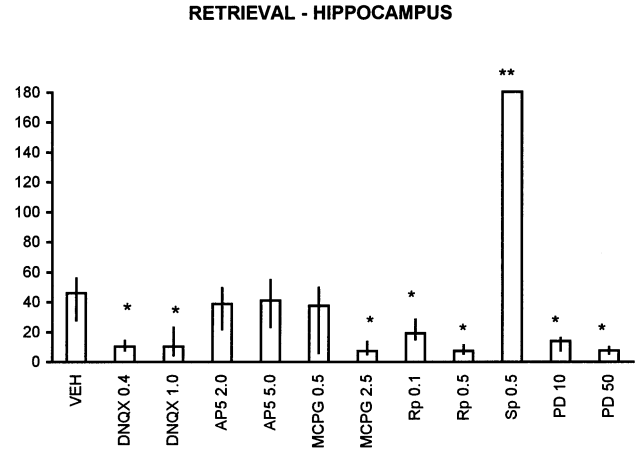

Fig. 2. In this and following figures, ordinates express median (interquartile range) test session latency, in s. $N = 9–11$ per group. Here, treatments were given by bilateral 0.5 μl infusions into the dorsal CA1 region of the hippocampus 10 min prior to retention testing. Testing was at 24 h from training. The treatments were a vehicle (2% dimethylsulfoxide in saline), AP5 (0.5 μg/side), DNQX (0.4 or 1.0 μg/side), MCPG (0.5 or 2.5 μg/side), Rp-cAMPs (0.1 or 0.5 μg/side), Sp-cAMPs (0.5 μg/side), or PD098059 (PD, 10 or 50 μM). All drugs were dissolved in the vehicle to a 0.5 μl volume. Training-test session latency differences were significant at a $P < 0.02$ level in the control group, in the groups treated with AP5, in the group treated with the lower dose of MCPG, and in the group treated with Sp-cAMPs. Asterisks indicate significant differences in retention test performance from control group at $P < 0.02$ level in Mann–Whitney *U*-tests, two-tailed. Retention test performance was hindered by the two doses of DNQX, Rp-cAMPs and PD 098059, and by the higher dose tested of MCPG. Sp-cAMPs enhanced retention test performance and AP5 had no effect.

The effect on plus maze and open field performance of the higher dose of the drugs that influenced retrieval was studied. None of them had any effect on behaviour in these two paradigms (Table 2).

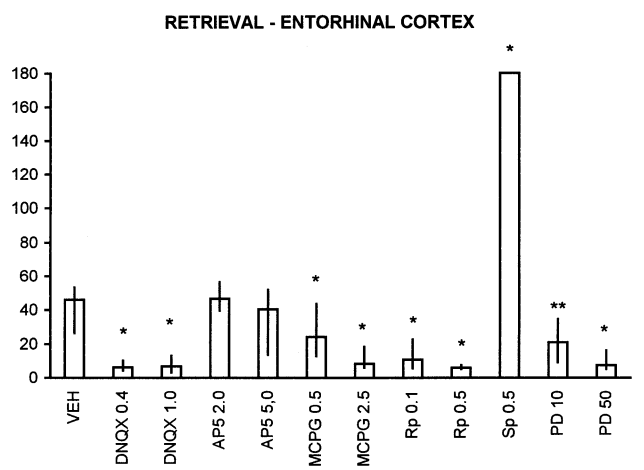

Fig. 3. Same as in Fig. 2, but for treatments given into the entorhinal cortex. $N = 9–11$ per group. Training-test session latency differences were significant at a $P < 0.02$ level in the control group, in the groups treated with AP5, and in the group treated with Sp-cAMPs. Retention test performance was inhibited by the two doses of DNQX, MCPG, Rp-cAMPs and PD, and enhanced by Sp-cAMPs. AP5 had no effect.

Table 1
(A) Effect of drugs infused into the hippocampus 10 min before trial on plus maze behaviour[a]

Drug	Total entries	Entries into open arms	% time in open arms	Rearings
Vehicle	16.1 ± 1.2	10.3 ± 1.2	56.7 ± 5.7	10.6 ± 0.9
DNQX 1.0	15.1 ± 2.2	8.3 ± 1.5	50.1 ± 7.3	8.9 ± 1.7
MCPG 2.5	13.3 ± 2.2	8.1 ± 1.4	56.9 ± 9.6	9.7 ± 1.9
Rp 0.5	16.7 ± 2.2	10.7 ± 1.5	61.1 ± 7.1	6.6 ± 1.3
Sp 0.5	10.0 ± 1.4	4.6 ± 0.7	34.9 ± 8.8	5.9 ± 1.0
PD 50	12.3 ± 2.5	7.9 ± 2.4	42.9 ± 9.6	6.6 ± 1.3

(B) Effect of drugs infused into the hippocampus 10 min before trial on open field behaviour

Drug	Rearings	Crossings
Vehicle	9.1 ± 1.3	41.0 ± 4.7
DNQX 1.0	6.6 ± 1.6	40.0 ± 9.1
MCPG 2.5	7.7 ± 1.8	33.6 ± 7.8
Rp 0.5	7.7 ± 2.6	43.6 ± 9.4
Sp 0.5	10.4 ± 1.8	29.7 ± 11.0
PD 50	3.9 ± 0.8	20.9 ± 6.1

[a] $n = 7$ per group; differences among groups were not significant in one-way super ANOVA or in Duncan multiple range tests ($P = 0.1$ or less).

3.3. Experiment 3: effect on retrieval of drugs infused bilaterally into posterior parietal cortex

Training session latencies were not significantly different among groups (overall mean, 5.3 s; median, 4.0 s; range, 1.0–22.0 s) ($N = 108$).

Retention test performance was significantly impaired by DNQX, MCPG, AP5, Rp-cAMPs and PD098059 at the two doses studied; in all these groups, training-test latency differences were not significant in Mann–Whitney U-tests at $P = 0.1$ level. In contrast, retention performance was enhanced by Sp-cAMPs (Fig. 4).

None of these drugs, at the higher dose level, affected plus maze or open field performance (Table 3).

3.4. Experiment 4: effect on retrieval of drugs infused bilaterally into anterior cingulate cortex

Training session latencies were not significantly different among groups (overall mean, 5.1 s; median, 4.3 s; range, 1.0–21.7 s) ($N = 107$).

Retention test performance was significantly impaired by MCPG, AP5, Rp-cAMPs and PD098059 at the two doses studied. In all these groups, training-test latency differences were not significant in Mann–Whit-

Table 2
(A) Effect of drugs infused into the entorhinal cortex 10 min before trial on plus maze behaviour[a]

Drug	Total entries	Entries into open arms	% time in open arms	Rearings
Vehicle	11.3 ± 1.5	6.1 ± 1.0	44.0 ± 5.7	4.9 ± 1.0
DNQX 1.0	6.0 ± 1.5	2.9 ± 1.0	23.5 ± 8.6	4.4 ± 0.9
MCPG 2.5	11.3 ± 2.0	7.3 ± 1.9	48.7 ± 8.8	7.6 ± 2.2
Rp 0.5	8.3 ± 1.2	4.6 ± 0.8	35.9 ± 7.3	10.3 ± 1.2
Sp 0.5	11.5 ± 1.1	6.6 ± 0.7	35.1 ± 6.0	11.4 ± 1.3
PD 50	15.4 ± 1.2	9.8 ± 1.0	55.3 ± 6.4	9.1 ± 1.2

(B) Effect of drugs infused into the entorhinal cortex 10 min before trial on open field behaviour

Drug (μg/side)	Rearings	Crossings
Vehicle	6.4 ± 1.6	24.4 ± 7.6
DNQX 1.0	8.3 ± 2.7	23.0 ± 7.1
MCPG 2.5	5.6 ± 1.4	18.8 ± 5.2
Rp 0.5	4.6 ± 0.8	22.1 ± 3.9
Sp 0.5	9.3 ± 1.6	28.0 ± 4.1
PD 50	4.8 ± 1.0	23.6 ± 4.2

[a] $n = 8$ per group; differences among groups were not significant in one-way super ANOVA or in Duncan multiple range tests ($P = 0.1$ or less).

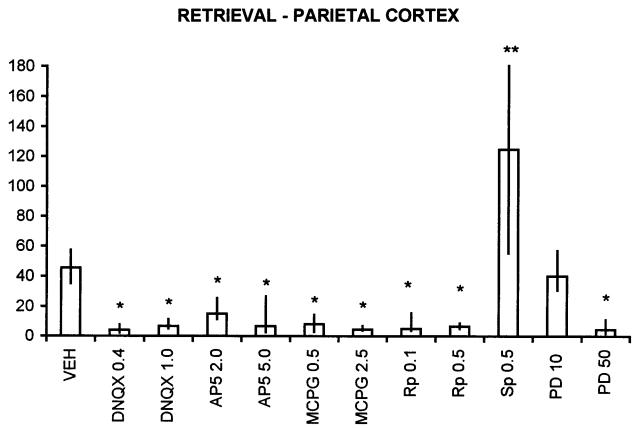

Fig. 4. Same as in preceding figures, but for treatments given into the posterior parietal cortex. $N = 8–11$ per group. Training-test session latency differences were significant at a $P < 0.02$ level in the control group, in the animals treated with the lower dose of PD, and in the animals treated with Sp-cAMPs. Retention test performance was inhibited by the two doses of AP5, DNQX, MCPG and Rp-cAMPs, and by the higher dose of PD. Sp-cAMPs enhanced retention test performance.

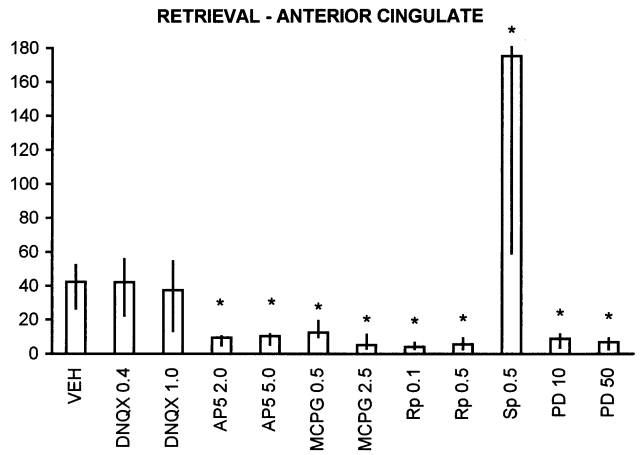

Fig. 5. Same as in preceding figures, but for treatments given into the anterior cingulate cortex. $N = 8–11$ per group. Training-test session latency differences were significant at a $P < 0.02$ level in the control group, in the animals treated with DNQX, and in the group treated with Sp-cAMPs. Retention test performance was unaffected by DNQX, this being the only brain area among those studied in which this happened. Retention test performance was inhibited by the two doses of AP5, MCPG, Rp-cAMPs and PD, and enhanced by Sp-cAMPs.

ney U-tests at $P = 0.1$ level. Retention test performance was enhanced by Sp-cAMPs, and it was unaffected by DNQX (Fig. 5).

None of the drugs that affected retrieval, at the higher dose level, had any influence on plus maze or open field performance (Table 4).

3.5. Experiment 5: effect on retrieval of drugs infused bilaterally into the basolateral nuclear complex of the amygdala

Training session latencies were not significantly different among groups (overall mean, 4.7 s; median, 4.5 s; range, 1.3–18.2 s) ($N = 110$).

Retention test performance was significantly impaired by DNQX at the two dose levels studied. In this group, training-test latency differences were not significant in Mann–Whitney U-tests at $P = 0.1$ level. Retention test performance was unaffected by any of the other treatments given into the baso-lateral amygdala (Fig. 6).

DNQX (1.0 μg) had no effect on plus maze or open field performance (Table 5).

Table 3
(A) Effect of drugs infused into the parietal cortex 10 min before trial on plus maze behaviour[a]

Drug (μg/side)	Total entries	Entries into open arms	% time in open arms	Rearings
Vehicle	14.3 ± 2.1	1.9 ± 1.5	38.5 ± 9.8	7.5 ± 1.0
AP5	9.6 ± 2.2	1.7 ± 0.4	34.0 ± 8.1	7.1 ± 1.9
DNQX 1.0	10.1 ± 1.8	1.4 ± 0.5	27.6 ± 9.2	8.0 ± 1.7
MCPG 2.5	14.0 ± 2.6	2.6 ± 0.5	51.4 ± 9.4	7.8 ± 2.1
Rp 0.5	10.9 ± 2.0	1.6 ± 0.6	31.3 ± 11.3	8.6 ± 1.6
Sp 0.5	9.4 ± 1.6	2.1 ± 0.6	41.0 ± 11.5	5.6 ± 1.8
PD 50	11.6 ± 2.2	2.0 ± 0.2	39.4 ± 3.6	7.1 ± 1.5

(B) Effect of drugs infused into the parietal cortex 10 min before trial on open field behaviour

Drug (μg/side)	Rearings	Crossings
Vehicle	5.8 ± 1.6	35.4 ± 4.3
AP5	6.0 ± 0.9	41.6 ± 4.4
DNQX 1.0	6.5 ± 1.6	34.4 ± 8.5
MCPG 2.5	7.0 ± 1.9	16.4 ± 6.5
Rp 0.5	4.3 ± 1.8	45.6 ± 4.5
Sp 0.5	8.3 ± 1.8	52.1 ± 4.1
PD 50	5.5 ± 0.6	39.3 ± 2.9

[a] $n = 8$ per group; differences among groups were not significant in one-way super ANOVA or in Duncan multiple range tests ($P = 0.1$ or less).

Table 4
(A) Effect of drugs infused into the anterior cingulate cortex 10 min before trial on plus maze behaviour[a]

Drug (μg/side)	Total entries	Entries into open arms	% time in open arms	Rearings
Vehicle	16.6 ± 1.2	10.0 ± 1.0	15.7 ± 2.1	6.3 ± 1.1
AP5	15.6 ± 1.9	9.0 ± 1.2	11.9 ± 1.5	10.6 ± 1.5
DNQX 1.0	14.0 ± 1.5	8.0 ± 1.2	15.6 ± 2.6	8.2 ± 0.8
MCPG 2.5	13.8 ± 3.3	9.5 ± 2.6	20.1 ± 5.2	5.8 ± 1.1
Rp 0.5	13.0 ± 1.9	6.8 ± 1.4	16.8 ± 2.5	5.0 ± 0.8
Sp 0.5	8.5 ± 1.9	5.0 ± 1.6	14.6 ± 5.0	9.2 ± 1.2
PD 50	12.2 ± 1.4	7.2 ± 0.7	27.7 ± 9.7	2.2 ± 0.7

(B) Effect of drugs infused into the anterior cingulate cortex 10 min before trial on open field behaviour

Drug (μg/side)	Rearings	Crossings
Vehicle	5.5 ± 0.5	37.0 ± 5.7
AP5	8.8 ± 2.0	26.2 ± 3.8
DNQX 1.0	5.5 ± 0.9	31.3 ± 5.9
MCPG 2.5	8.8 ± 1.8	36.2 ± 4.1
Rp 0.5	7.2 ± 1.1	34.8 ± 2.3
Sp 0.5	11.0 ± 2.6	51.8 ± 8.9
PD 50	7.7 ± 2.1	31.2 ± 6.6

[a] $n = 6$ per group; differences among groups were not significant in one-way super ANOVA or in Duncan multiple range tests ($P = 0.1$ or less).

4. Discussion

The results are clear and their interpretation is simple. The effect of the drugs studied is not explainable by alterations in locomotor or exploratory activity or anxiety levels. They may therefore be viewed as effects on retrieval itself. By the way, it may be noted that the variability of the effects of the various treatments on plus maze or open field behaviour appeared to be larger than that observed in the retention test of inhibitory avoidance. This further attests to the specificity of the effects observed on the latter.

All the cortical regions studied (CA1, entorhinal, posterior parietal and anterior cingulate) participate actively in retrieval of the one-trial task. By 'actively' we mean that it does not simply involve regular excitatory glutamatergic transmission mediated by AMPA receptors; it involves in addition, glutamate receptors that enhance intracellular Ca^{2+} levels, and, importantly, activity of the PKA and the MAPK pathways. CA1 and the entorhinal cortex require metabotropic glutamate receptors, and the parietal cortex and the anterior cingulate area require in addition NMDA receptors in order for retrieval to occur. Recently we reported that hippocampal PKC is also necessary for retrieval [43]. All cortical regions studied except the anterior cingulate require intact AMPA receptors as well. This suggests that CA1, entorhinal and parietal cortex require regular ionophore-mediated excitatory transmission in order to participate in retrieval, whereas the cingulate cortex appears to rely only on metabotropic receptors.

In contrast, in the basolateral amygdala, receptors that regulate Ca^{2+} levels (NMDA, metabotropic) or the cAMP-PKA and MAPK signalling pathways are not required for retrieval. Regular AMPA receptor-

Table 5
(A) Effect of DNQX infused into the amygdala 10 min before trial on plus maze behaviour[a]

Drug (μg/side)	Total entries	Entries into open arms	% time in open arms	Rearings
Vehicle	15.3 ± 3.1	8.3 ± 2.1	49.8 ± 8.5	10.6 ± 0.9
DNQX 1.0	13.4 ± 0.6	7.3 ± 0.4	54.7 ± 3.1	10.8 ± 1.4

(B) Effect of DNQX infused into the amygdala 10 min before trial on open field behaviour

Drug (μg/side)	Rearings	Crossings
Vehicle	9.5 ± 1.4	42.1 ± 5.2
DNQX 1.0	8.5 ± 1.0	45.4 ± 2.7

[a] $n = 8$ per group; differences among groups were not significant in one-way super ANOVA or in Duncan multiple range tests ($P = 0.1$ or less).

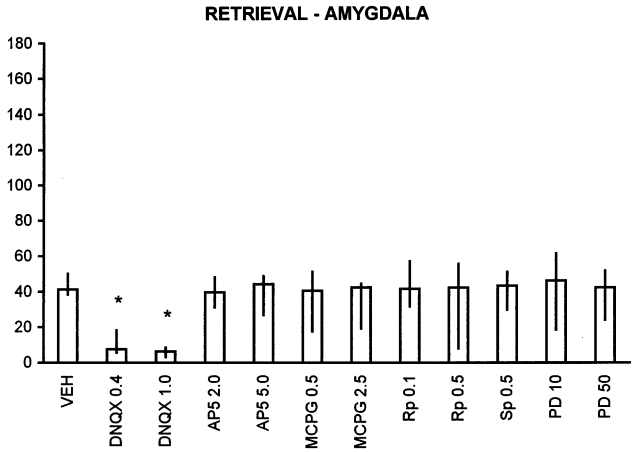

Fig. 6. Same as in preceding figures, but for treatments given into the basolateral amygdala $N = 9–11$ per group. Training-test session latency differences were significant at a $P < 0.02$ level in all groups except the two that were treated with DNQX. Intra-amygdala DNQC hindered retention test performance. All other drugs were ineffective when given into the basolateral amygdala.

mediated excitatory transmission in this structure is apparently needed, as has been shown before in experiments using the less specific blocker, CNQX, both in this [14,21] and in closely related tasks [23,28]. Unlike that of DNQX, the effect of intra-amygdala (or intrahippocampal) CNQX on retrieval is only partial, even at high doses [14,28]. In addition, also unlike DNQX, CNQX has a mild anxiolytic (anti-conflict) effect when tested in the plus maze [28]. At any rate, the finding that only DNQX but not the other drugs was able to affect retention when given into the amygdala indicates that this structure does no play such an 'active' role in retrieval as the cortical regions do. As suggested by many others [7,15,28,43], the amygdala may be involved in the processing and expression of emotional rather than cognitive (associative, spatial, etc.) components of memories. One-trial inhibitory avoidance includes both types of components [10,17,20,34,38]. As shown in experiments 1–4, its retrieval is affected by a variety of drugs that do not influence anxiety levels. As shown in many other papers, neither is its encoding or consolidation [18,20].

In conclusion, retrieval of one-trial inhibitory avoidance involves the active participation of a large network of cortical connections that include the hippocampus, the entorhinal, posterior parietal and anterior cingulate cortex. The network is the same that is required for consolidation [21,27]. Also, the relatively simple molecular picture of the involvement of the CA1 region of the hippocampus in retrieval delineated in previous papers [20,22] is now extended to several other cortical regions. These differ in their requirement of the diverse glutamate receptor types, but all require intact PKA and MAPK are necessary for retrieval.

Also like in consolidation [7,26,43], the role of the amygdala in retrieval appears to be mostly modulatory. It is possible that the basolateral amygdala may be the link through which hormonal and neuromodulatory systems influence retrieval [9,13,35,48].

Some but not all of the molecular mechanisms involved in LTM consolidation are also crucial for retrieval. This is the case of some of the glutamate receptors studied, PKA and MAPK. However, in the hippocampus at least, the activation of NMDA receptors [15], calcium-calmodulin dependent protein kinase II CaMKII activity [8], and the inhibition of ectonucleotidase activity [4] that are so characteristic of the early phase of consolidation of this task, are not needed for its retrieval [4,20,22]. Therefore, the widely held notion that retrieval is or should be a function of consolidation [13,35,40,50] is true only in part. Some mechanisms are common to both, but others are not; and there is no assurance that those mechanisms subserve the same physiological functions in consolidation and retrieval, or that they do so in the different cortical regions. For example, as happens with short- and long-term memory [44,45] the same protein kinases may phosphorylate different substrates in each process. This remains to be studied.

Acknowledgements

Supported by PRONEX, Brazil. We thank C. Rodrigues, T. de David, C. Madche, A. Aguzzoli, D.M. Cardoso and B. Moletta for their technical help in the experiments.

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Involvement of serotonergic type 1A (5-HT1A) receptor in the Agranular Insular Cortex in the Consolidation of Memory for Inhibitory Avoidance in Rats

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Abstract

Adult male Wistar rats were bilaterally implanted with indwelling cannulae in the agranular insular cortex of the prefrontal cortex. After recovery, animals were trained in a step-down inhibitory avoidance task (3.0-s, 0.4-mA foot-shock) and received, right after training, a 0.5-µl infusion of vehicle (20% DMSO), of the serotonergic type 1A (5-HT_{1A}) receptor agonist DPAT (0.0125, 0.125, or 1.25 µg), or of the 5- HT_{1A} receptor antagonist NAN (0.125 or 1.250 µg). Retention testing was carried out 24 h after training. DPAT (1.25 µg but not 0.0125 or 0.125 µg) was amnesic ($p < 0.05$). NAN was not effective at any dose ($p > 0.10$). These results show that an overactivation of 5-HT_{1A} receptors in the agranular insular cortex impairs memory consolidation of inhibitory avoidance, in rats, right after training. This suggests that a negative feedback loop between the insular cortex and the raphe nuclei modulates memory consolidation.

***Key words*:** memory, insular cortex, serotonergic receptor

INTRODUCTION

The rat agranular insular cortex (IC) lies deep in the rhinal sulcus and neighbors the dorsally following neocortex in the prefrontal cortex (Zilles and Wree, 1995). This region has been referred to as the visceral cortex because it receives and integrates taste and visceral information from the thalamus in order to mediate visceral and related cognitive reactions (Bermúdez-Rattoni et al., 1995; Cechetto and Saper, 1987; Krushel and Van der Kooy, 1988; Morgan and LeDoux, 1999).

Among others, IC receives visceral information concerned to the effects of stomach-irritating toxins, which is very important to the acquisition of conditioned taste aversion (CTA) (Bermúdez-Rattoni and McGaugh, 1991). In addition, IC receives convergent inputs from the limbic and the primary sensory systems as any other sensory area in the cortex (Krushel and Van der Kooy, 1988). Indeed, IC is involved in memory processes, such as CTA, as mentioned before, aversive classical conditioning (Hankins et al., 1974), water maze spatial learning (Bermúdez-Rattoni et al., 1991), and conditioned fear (Morgan and LeDoux, 1999), as well as inhibitory avoidance (Bermúdez-Rattoni et al., 1991; Santos-Anderson and Routtenberg, 1976). Specifically in the case of (step-through) inhibitory avoidance, it seems that IC is involved in acquisition, consolidation, and retrieval, but not in storage (Bermúdez-Rattoni et al., 1995).

IC sends outputs to the solitary tract (Groenewegen et al., 1990), brain stem, and spinal cord autonomic regions (Groenewegen, 1988). IC has been likened to primate orbital cortex for several reasons: First, both are implicated in emotional processes, such as emotionality (Nonneman et al., 1974) and blood pressure and heart rate control (Hardy and Holmes, 1988; Powell et al., 1985). Second, both mediate the extinction of appetitivelly motivated bar-press responses (Butter, 1969; Kolb et al., 1974), suggesting that IC mediates response strategies changes in order that animals adapt to new situations. Third, their reciprocal connections with the mediodorsal thalamus and the amygdala are similar (Groenewegen, 1988; Groenewegen et al., 1990; Sarter and Markowitsch, 1984).

Serotonergic type 1A (5-HT1A) receptors are classically characterized by their high affinity for DPAT and for being coupled to protein G, by which they may either inhibit or stimulate adenylyl cyclase activity (Frazer and Hensler, 1994). However, 5-HT1A

receptors that are not coupled to protein G seem to occur in the hippocampus and neocortex (Nénonéné et al., 1994). 5-HT1A receptors are located both pre and postsynaptically. Although presynaptic 5-HT1A receptors control 5-HT neuronal activity, recent evidence indicates an additional role of postsynaptic cortical 5-HT1A receptors as part of a negative feedback loop (De Vry, 1995).

5-HT1A receptor agonists have been clinically used as antidepressant or anxiolytic drugs, and may desensitize behavioral and electrophysiological responses, with antiagressive, anticataleptic, antiemetic, and possibly anticraving effects (De Vry, 1995; Frazer and Hensler, 1994).

In rats, DPAT impairs consolidation of memory for inhibitory avoidance when infused into the hippocampus 3 to 6 h, but not immediately after training (Bevilaqua et al., 1997), or into the entorhinal cortex immediately or 3 to 6 h after training (Ardenghi et al., 1997). DPAT is amnesic in these cases probably because of its effects in inhibiting adenylyl cyclase activity via protein G (Ardenghi et al., 1997; Bevilaqua et al., 1997; for review, see Izquierdo and Medina, 1997).

Since the involvement of IC via its 5-HT1A receptors in memory consolidation of step-down inhibitory avoidance has not yet been studied, the aim of the present work was to see whether infusion of its agonist, DPAT, and antagonist, NAN may influence retrieval of this memory at 24 h post-training.

METHODS

Subjects

A total of 85 male Wistar rats (age, 60 to 90 days) from our breeding colony was used. Animals were housed five to a cage with food and water *ad libitum.* The animal house was on a 12-h light/dark cycle (lights on at 7:00 AM) at a temperature of 23^0 C.

Surgery and Behavioral Procedures

Animals were bilaterally implanted under thionembutal anesthesia (30 mg/kg, i.p.) with 27-gauge guide cannulae. After at least 48 h, all animals were trained in a step-down inhibitory avoidance task (Izquierdo *et al.*, 1992; Izquierdo *et al.*, 1997). Animals were placed on a 2.5-cm high by 7.0-cm wide formica platform at the left of a 50 x 25 x 25-cm

apparatus, floor of which was a series of parallel 0.1-cm caliber stainless-steel bars spaced 1.0 cm apart. Latency to step down placing the four paws on the grid was measured. In the training session, immediately upon stepping down, the animals received a 3.0-s, 0.4-mA foot-shock. Animals were tested 24 h after training. Test session was procedurally identical to the training session except that no foot shock was given and the step-down latency was cut off at 180 s; i.e., test session values higher than 180 s were counted as 180 s. Retention test performance was taken as a measure of retention.

Infusion Procedure and Control of Cannulae Placements

At the time of infusion, 30-g cannulae were fit into the guide cannulae (Izquierdo *et al.*, 1992 and 1997). Animals received, right after training, a 0.5-µl infusion of vehicle (20% DMSO), of the serotonergic type 1A (5-HT_{1A}) receptor agonist (±)-2-Dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide (8-OH-DPAT, 0.0125, 0.125, or 1.25 µg), or of the 5-HT_{1A} receptor antagonist 1-(2-Methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine hydrobromide (NAN, 0.125 or 1.25 µg). Infusion site was chosen using coordinates from bregma and dura obtained from the Atlas of Paxinos and Watson (1986), as follows (units in cm): A +0.35, L ± 0.30, V 0.36 (Fig. 1)

Two to 24 h after the end of the behavioral procedure all animals received an infusion of 0.5 µl of 4% methylene blue through the infusion cannulae and were killed by decapitation. Their brains were removed and stored in formalin for histological localization of infusion sites as explained elsewhere (Izquierdo *et al.*, 1992 and 1997). Only animals with correct cannulae locations (Fig.1) were included in the final statistical analysis.

Statistics

Parametric statistics were used. Data are reported as means ± standard errors of means of retention scores of both test sessions. However, before performing the parametric tests, data were transformed to their Neperian logarithms in order to normalize their distribution. Differences from the control group were evaluated by the one-way analyses of variance (ANOVA), in the training session, and by the independent-samples t test, two-tailed, in the test session. The paired-samples t test evaluated the differences between training and test session performance in each group. $P < 0.05$ was considered to indicate

statistical significance.

RESULTS

There was no difference among groups in training session performance [ANOVA: overall mean, 3.86 s; standard error of mean, 0.37 s; $F(5,68) = 1.048$; $p = 0.397$; data not shown].

Results are shown in Fig. 2. Controls learned (paired-samples t test: $N = 15$, $p = 0.001$), as well as animals treated with DPAT or NAN ($p < 0.01$). DPAT (1.25 μg but not 0.0125 or 0.125 μg) was amnesic relative to controls when infused into IC right after training (independent-samples t test, $p = 0.035$, 0.302, and 0.515, respectively). N per group was 12, 13, and 11, respectively. NAN (0.125 or 1.25 μg) was not effective in altering retention of inhibitory avoidance 24 h after training (independent-samples t test, $p = 0.592$ and 0.584, respectively). N per group was 11 and 12, respectively.

DISCUSSION

In the present study, we showed that infusion into the insular cortex of a 5-HT1A agonist receptor, DPAT, disrupts memory consolidation for step-down inhibitory avoidance when infused right after training.

It is well established that IC is involved in CTA, an associative learning model in which animals become aversive, in a single trial, to a specific taste experienced as a conditioned stimulus followed by a gastric irritation as an unconditioned stimulus (Bermúdez-Rattoni et al., 1995). However, hedonic responses to taste remain intact in IC-lesioned or even in decerebrated rats (Kiefer, 1985). Previous studies also showed that the same structure is involved in memory for water maze spatial learning and inhibitory avoidance (Bermúdez-Rattoni et al., 1991; Santos-Anderson and Routtemberg, 1976). Pretraining and post-training infusions into IC of tetrodoxin were effective in impairing retention for both tasks, indicating that IC mediates both acquisition and consolidation (Bermúdez-Rattoni et al., 1991). In addition, reversion by homotopic fetal IC grafts of the disruptive effects of lesions induced by NMDA infusions on the ability to recall showed

that IC is also involved in retrieval of inhibitory avoidance but probably not in its long-term storage (Bermúdez-Rattoni et al., 1995).

The amnesic effect of DPAT on memory consolidation for inhibitory avoidance suggests that 5-HT1A receptors in IC are physiologically less active during memory consolidation and/or their hyperactivation triggers a negative feedback mechanism which disrupts memory consolidation. However, the former alternative may, at first, be ruled out, since NAN was not effective in facilitating memory; if the latter is correct, a question remains: does this negative feedback mechanism perform a physiological function in the intact brain or is it merely activated pharmacologically?

Evidence has shown that a feedback loop in the frontal cortex may account for part of an inhibition of 5-HT neuronal activity due to systemic administration of 5-HT1A receptor agonists: First, DPAT increases the firing rate of fronto-cortical neurons (Ceci et al., 1992) at the same doses that inhibits the firing rate of dorsal raphe serotonergic neurons (Blier and deMontigny, 1987; Blier et al., 1987). Second, this effect on dorsal raphe serotonergic neurons is reduced by fronto-cortical deafferentiation (Ceci et al. 1994). So, DPAT may activate the afferent projections from the frontal cortex -- including those from the medial precentral area and the anterior cingulate cortex (Sesack et al., 1989), and those from the insular cortex (Peyron et al., 1998; Reep and Winans, 1982) -- to the dorsal raphe, which, in turn, would decrease the activity of its 5-HT neurons. It is worth to point out that 5-HT1A receptor agonists inhibit a variety of behavioral responses, being powerful tools in the treatments of depression, anxiety and, possibly, other psychological disorders (De Vry, 1995; Frazer and Hensler, 1994). Therefore, it is reasonable to postulate that the amnesic effect of DPAT (1.25 μg) is due to an involvement of 5-HT1A receptors in a negative feedback mechanism by which IC mediates or simply modulates memory processes, at least those related to inhibitory avoidance. In addition, this amnesic effect might also contribute to the understanding of side effects of treatments against depression which affect the serotonergic system.

5-HT1A receptors in the hippocampus and the entorhinal cortex have been implicated in consolidation of memory for inhibitory avoidance by means of its interaction of the cAMP signaling pathway (Bevilaqua et al., 1997; Izquierdo and Medina, 1997). Compelling evidence shows that this signaling pathway is very important in mediating

memory consolidation (Bach et al., 1999; Bevilaqua et al., 1997; Bourtchuladze et al., 1998; Izquierdo and Medina, 1997; Schafe et al., 1999). Therefore, DPAT might also act upon this signaling pathway in the neurons of IC and, as a consequence, impair memory consolidation.

There may be functional differences of IC along the anterior-posterior axis, as ascertained by the differential effects on water maze and CTA performance of permanent lesions produced by bilateral microinjections of NMDA into different coordinates of IC (Bermúdez-Rattoni et al., 1991). Our infusions combined reached an area equivalent to the anterior IC of Nerad et al. (1996), lesions of which did not disrupt water maze or CTA performance. Therefore, anterior IC may be involved in the consolidation of memory of inhibitory avoidance but not of water maze or CTA.

The present study focused on the effect of altering the serotonergic transmission in the insular cortex on the consolidation of memory for inhibitory avoidance. In addition, this study might also have some clinical relevance, since such an approach of interfering the serotonergic transmission has currently been used in the therapeutics of some mood disorders, especially depression, in which several prefrontal areas are affected.

ACKNOWLEDGMENTS

This study was supported by Conselho Nacional de Pesquisa (CNPq) and PRONEX, Brazil. We are grateful to Maria Beatriz Moleta for her contribution in the present work.

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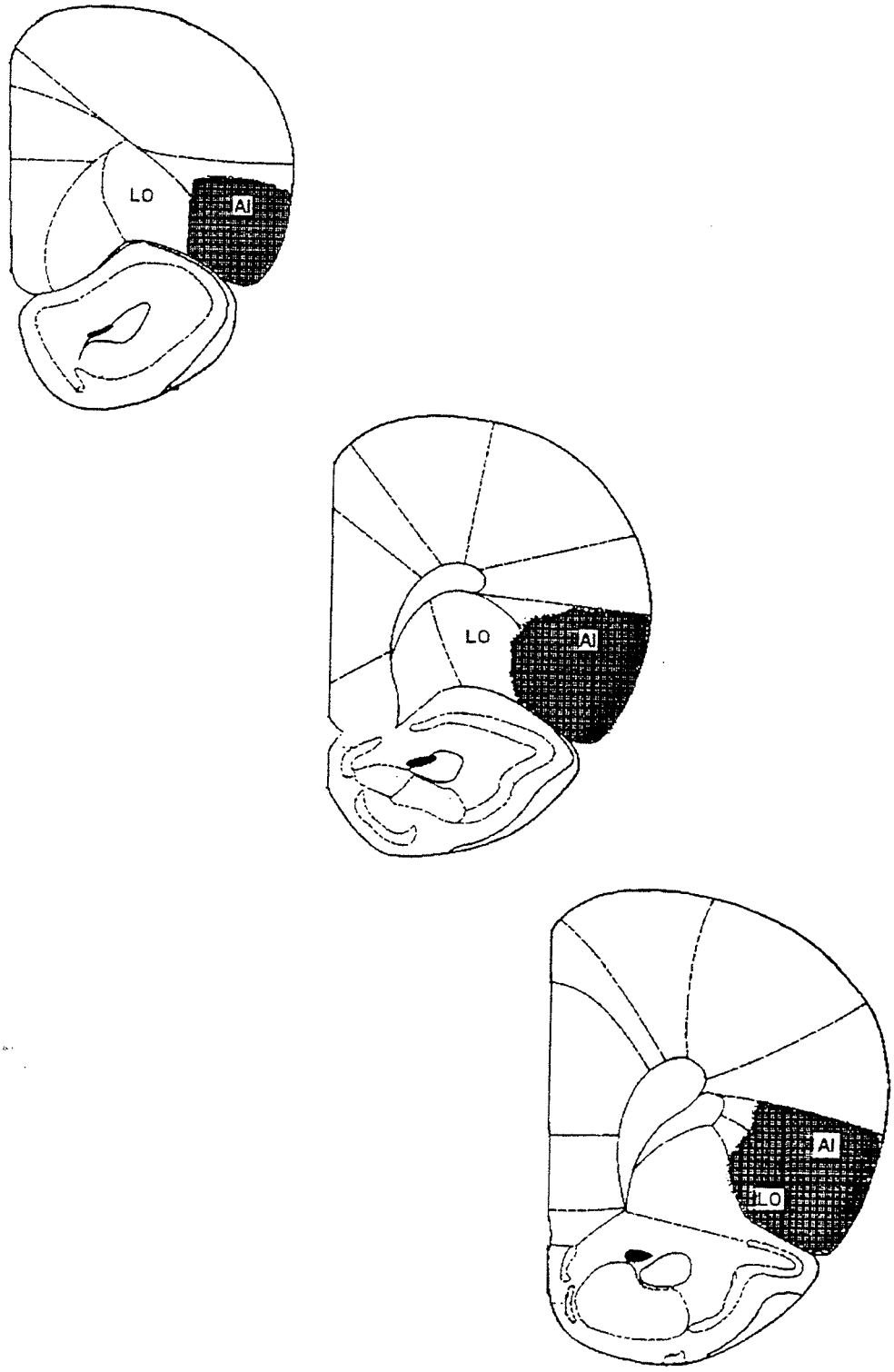

FIG. 1. Schematic drawing of rat brain sections at planes + 0.42, 0.37, and 0.32 cm of the anterior-posterior axis, from the Atlas of Paxinos and Watson (1986), showing (stippled) a composite of the extension of infusions of 4% methylene blue given into both sides of the ventrolateral prefrontal cortex (details in METHODS). The figure illustrates a composite of all infusions given on both sides. AI -- agranular insular cortex; LO -- lateral orbital cortex.

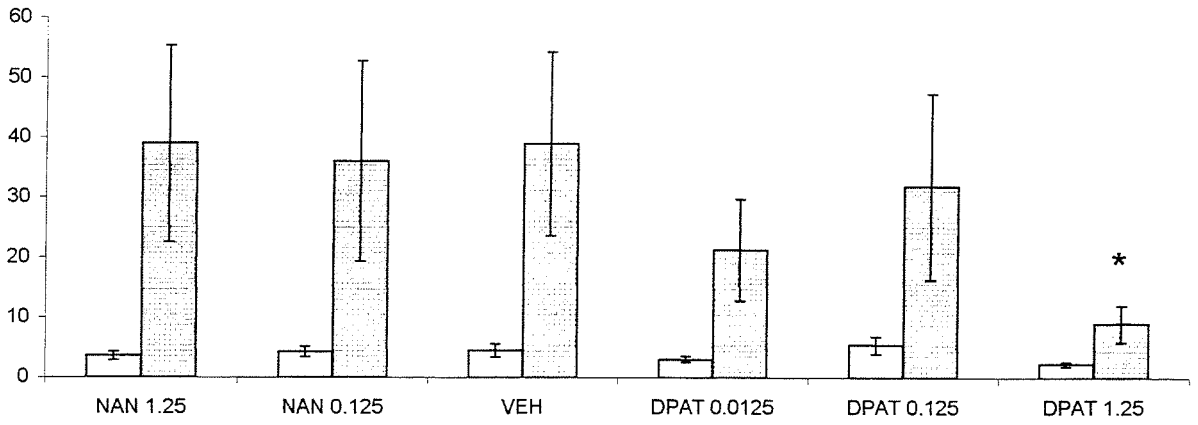

FIG. 2. Means (± SEM) of retention scores of the training and test sessions (white and gray bars, respectively) in groups infused bilaterally into the ventrolateral prefrontal cortex right after training with NAN (0.125 or 1.250 μg) or DPAT (0.0125, 0.125, or 1.25 μg). Horizontal axis is oriented towards an increasing 5-HT_{1A} receptor activity. Asterisk indicates statistical significance at $p < 0.05$ (independent-samples t test, two-tailed; for more details, see *Statistics* under METHODS). N per group was 11-15.

7. Discussão

Nesta Discussão, não nos deteremos nos aspectos da neurofarmacologia da memória da tarefa de esquiva inibitória, pois estes foram extensivamente discutidos nos artigos dos Capítulos 3, 4, 5 e 6. Procuraremos, sim, posicionar os referidos trabalhos diante do atual conhecimento sobre memória e sobre a função das diversas estruturas corticais aqui estudadas. Questionaremos, inclusive, os próprios paradigmas que nortearam os referidos trabalhos.

7.1 Estão os Nossos Paradigmas Corretos?

Os estudos aqui apresentados sobre o envolvimento do **córtex cingulado** e do **Fr2** na memória da tarefa de esquiva inibitória foram realizados (e interpretados) segundo alguns paradigmas:

1. A classificação dos tipos de memória em sistemas de memórias, onde as memórias teriam localização específica (ver 1.3.1).

2. A PLD como fenômeno subjacente à memória. Portanto, os mecanismos centrais da memória estariam relacionados com a transmissão glutamatérgica.

3. A hipótese da migração das memórias declarativas da formação hipocampal ao isocórtex (1.3.2.2). Combinando com a abordagem de sistemas de memórias (item 1), as memórias teriam localização espaço-temporal específica.

4. Evocação mede memória (abordagem fenomenológica).

7.1.1 A Topologia do Engrama

Como abordado em 1.3.2.2, postula-se que as memórias declarativas migrem da formação hipocampal às demais regiões corticais. Às evidências já mencionadas, poderíamos ainda adicionar as seguintes: (1) as alterações metabólicas provenientes da consolidação da memória de labirinto radial ocorrem primeiramente nas regiões do alocórtex (*v.g.*, hipocampo), para só depois ocorrerem na **região frontal**, no **córtex cingulado** e em regiões isocorticais temporais (Sif *et al.*, 1991); e, durante o sono, neurônios ativos durante um aprendizado voltam a se ativar de forma correlacionada

(Wilson e McNaughton, 1994), o que poderia indicar uma transferência do traço mnemônico. Por sinal, o sono é de conhecida importância no reforço do traço da memória de algumas tarefas (Karni *et al.*, 1994; Smith, 1985).

Entretanto, a questão da localização intermediária das memórias declarativas no hipocampo ou mesmo em outra área que não o isocórtex (*v.g.*, **córtex cingulado posterior**) é controversa. Dois resultados experimentais mostram-se contrários à hipótese da migração da memória: (1) a correlação de atividade dos neurônios do hipocampo e do córtex parietal durante a aprendizagem e durante o sono só é respeitada ao se observar as estruturas isoladamente; quando se observa a atividade conjunta de ambas as estruturas, o hipocampo é primeiramente ativado apenas durante o aprendizado, mas não no sono (Qin *et al.*, 1997); e (2) a lesão bilateral na amígdala reverte a piora do desempenho no labirinto aquático provocada por lesão bilateral no hipocampo (Roozendaal *et al.*, 1999).

É possível que uma memória sensorial complexa seja uma propriedade emergente da atividade de vários sistemas em interação, conforme defendido por Fuster (1997). Neste caso, a memória não necessariamente estaria localizada em algum de seus componentes (*v.g.*, **córtex cingulado** ou **Fr2**), mas resulte [emerja] da atividade global da rede neuronal. Entretanto, **não** podemos descartar a relevância das mudanças físicas localizadas, ou mesmo, o deslocamento físico em cadeia destas mudanças. Por sinal, pequenas e bem localizadas alterações poderiam promover alteração de atividade em toda uma rede, conforme defendido em modelo computacional (em Shaw *et al.*, 1988), o que desencadearia mudança comportamental. Também não podemos negligenciar a existência das "mother-cells", tampouco descartar a localização de funções específicas em áreas cerebrais (estas últimas, abordadas em 1.4).

Em 1.3.2.1 mostramos a hipótese na qual dois sistemas interagem para a execução de uma tarefa sensório-motora. Portanto, mesmo que o hipocampo, o **córtex cingulado posterior**, o **Fr2** ou outra estrutura cortical qualquer não seja **o sítio** de armazenamento da memória em dado instante, certamente tal estrutura (ou parte dela) será um dos componentes de um sistema ainda mais amplo. Como conseqüência, as alterações plásticas de cada estrutura vão interferir nas complexas interações do sistema, tendo como resultado final a memória.

Os fenômenos de plasticidade que podem intermediar estas alterações vêm sendo

intensamente estudados nas últimas décadas, de onde se destacam a PLD e a DLD, abordadas em 1.2. Devemos ressaltar, porém, que a PLD e a DLD são respostas padrões de sistemas biológicos manipulados sobre certas condições artificiais, não necessariamente fisiológicas: os protocolos de indução. Portanto, é mais provável que estas respostas apenas indiquem algumas características dos fenômenos de plasticidade sináptica que realmente sejam fisiológicos.

A abordagem de estudar o envolvimento de estruturas ao longo da consolidação da memória através da infusão de muscimol e de AP5, drogas que afetam a PLD (Bliss e Collingridge, 1993), tem o potencial de proporcionar importantes informações a respeito do envolvimento das diversas estruturas corticais **(mais precisamente, de seus diversos sistemas de neurotransmissão e de suas diversas cascatas enzimáticas,** cujos alguns detalhes foram extensivamente discutidos nos Capítulos 3, 4, 5 e 6). Vale ressaltar, porém, que suas conclusões podem não ser sempre idênticas às dos fenômenos de plasticidade sináptica naturalmente ocorrentes, pois as propriedades de um nível de organização são conseqüência das propriedades de níveis de organização inferiores, embora são sejam necessariamente semelhantes ou mesmo totalmente explicáveis por estas (Blomberg, 1994; Roland, 1994). Entretanto, esta ressalva, quem nem sempre é aplicável, não diminui a importância da Neurofarmacologia Comportamental, uma importante abordagem que auxilia o entendimento integrado de uma série de fenômenos associados a diferentes níveis de organização: sináptico, circuital e comportamental. O conhecimento advindo desta integração pode, inclusive, ter aplicação clínica futura.

Voltando à questão da migração da memória, podemos fazer algumas considerações finais a seu respeito. Pode-se especular que a expressão da memória da esquiva inibitória esteja **sempre** condicionada à atividade neuronal do isocórtex (*v.g.*, córtex parietal associativo). As alterações de atividade neuronal nesta região decorrentes da expressão da memória poderiam ocorrer, então, não porque nela haja alterações plásticas no início da consolidação, mas porque seus neurônios estariam, direta ou indiretamente, conectados com os neurônios de outras partes do sistema que têm sua conexões alteradas neste momento (*v.g.*, hipocampo e **córtex cingulado posterior**). Assim, a hipótese da migração da memória não estaria correta, mesmo que os sítios de plasticidade se desloquem em direção ao seu sítio de armazenamento, como evidenciado por este e trabalhos

anteriores (Izquierdo *et al.*, 1997; Zanatta *et al.*, 1997).

A memória pode, também, em seu prolongado dinamismo, ativar cada vez menos regiões cerebrais (*v.g.* em Haglund *et al.*, 1994, e em Haier *et al.*, 1992) e ser menos sensível a determinados tratamentos farmacológicos (*v.g.*, CNQX é amnésico para a esquiva inibitória quando dado pré-teste no hipocampo 1 dia, mas não 31 dias após o treino) (Izquierdo *et al.*, 1997). A memória da esquiva inibitória "sairia" das sinapses glutamatérgicas do hipocampo, embora nunca tenha estado apenas nelas. Por sinal, como demonstramos, várias estruturas são importantes para a evocação na tarefa da esquiva inibitória no 1° dia pós-treino, como o **córtex cingulado anterior**, que mobiliza seus receptores glutamatérgicos metabotrópicos e do tipo NMDA, mas não do tipo AMPA, ou suas enzimas PKA e MAPK (Capítulo 5).

7.1.2 Papel das Estruturas na Memória

O hipocampo serviria só para formar memórias? E o **córtex cingulado posterior**, seria um mero auxiliar do hipocampo, filtrando as informações que por ele passam no diálogo entre o hipocampo e o isocórtex? Qual o papel do **córtex cingulado anterior** e do **Fr2** na memória da tarefa de esquiva inibitória? E do **córtex insular agranular**?

A formação hipocampal seria uma "região formadora de memória" por dois aspectos: (1) é capaz de executar associações amplas (Eichenbaum, 1993), pois o córtex entorrinal, estrutura que tem grande contato com o hipocampo, é um centro convergente de informações de diferentes modalidades sensoriais (conforme abordado em Iijima *et al.*, 1996); (2) é capaz de grande plasticidade (Bliss e Collingridge, 1993; Tsumoto, 1992), o que permite uma maior maleabilidade entre as interações dos diversos sistemas (Eichenbaum, 1993). Com estas características, unificaria diferentes traços de memória, muitas vezes transitórios dentro de cada (sub)sistema operacional, a ponto de serem fixados com maior robustez, em um todo. Este todo não necessariamente deve persistir com o tempo, pois a evocação posterior da memória não necessariamente deva envolver a ativação de todos os sub-traços primordiais (Spear, 1978). A propósito, apenas as representações mediadas pelo hipocampo permitem associações de diversos itens, envolvendo amplo armazenamento no cérebro (Eichenbaum *et al.*, 1996). Porém, outros aspectos do processamento da informação precisam ser relevados, o que é feito pelas demais estruturas

do Circuito de Papez.

Postula-se que existem dois aspectos básicos relacionados com a emoção que são processados por diferentes sistemas: a avaliação de seu conteúdo e a resposta comportamental associada (Damásio, 1996; Ellsworth, 1991; LeDoux, 1995). O hipocampo pode mediar componentes de uma memória sem qualquer conteúdo emocional (*v.g.*, localização espacial na tarefa do labirinto radial). Entretanto, como observado por Maddock (1999), o **córtex cingulado posterior** é a segunda estrutura que mais freqüentemente se mostra metabolicamente alterada em estudos que envolvem experiências com conteúdo emocional em seres humanos, enquanto que não há evidência consistente de sua participação em tarefas sem conteúdo emocional [embora, a princípio, não devemos descartar esta possibilidade]. Maddock, então, propõe que o **córtex cingulado posterior** está envolvido com a interação entre emoção e memória episódicas. Esta interação estaria, a princípio, associada à avaliação dos conteúdos emocionais e não à geração de respostas emocionais, bem como o **córtex cingulado posterior** poderia estar envolvido com a implementação de memórias episódicas nos seus primeiros estágios de consolidação, por avaliar o seu conteúdo emocional (Maddock, 1999). Já Jung *et al.* (1998) mostraram que, quando ratos estão apreendendo um labirinto radial, o córtex pré-frontal medial, da qual o **córtex cingulado anterior** faz parte, ativa-se. Esta ativação, porém, limita-se a aspectos relacionados com a memória de trabalho e tomada de decisão, e não ao componente espacial da tarefa (Jung *et al.*, 1988). Em acréscimo, Kubota e Gabriel (1995) postulam que o circuito no qual os **córtices cingulados anterior e posterior** participam tem uma função comparativa, onde informações recentes são comparadas com as informações já memorizadas na memória de longa duração. Caso elas se coadunem, "programas" anteriormente formulados são executados; caso contrário, o **córtex cingulado** aumenta a atenção ao problema e inibe ações estereotipadas. Propomos, inclusive, que a inibição da descida à plataforma no teste da tarefa de esquiva inibitória possa ser uma destas ações estereotipadas a serem inibidas por tal sistema comparador. Vale salientar que ato de explorar ambientes novos (ou relativamente novos) é um comportamento natural do rato, a menos que algo que seja tomado como uma ameaça esteja envolvido (*v.g.*, a presença de um predador, um comportamento inato, ou a possibilidade de choque, que é aprendido).

Assim, a princípio, o componente associado à avaliação do conteúdo emocional da

memória parece ser mediado pelo **córtex cingulado posterior**. Entretanto, o componente associado à elaboração de respostas seria tanto o **córtex cingulado anterior,** como, possivelmente, o **córtex cingulado posterior**. **Fr2**, que pertence ao SCM (em 1.3.2.1) e tem correspondentes homólogos com o **córtex pré-motor** (em 1.4.2), também deve estar participando do circuito responsável pela elaboração de respostas. Nossos resultados não contradizem e, a princípio, confirmam algumas destas hipóteses, estendendo-as a mais uma espécie, o rato, e a mais uma tarefa comportamental, a esquiva inibitória.

Verificamos que o **córtex cingulado posterior** do rato participa do processo de consolidação da tarefa de esquiva inibitória tanto por mecanismos sensíveis ao muscimol (até 90 min pós-treino), quanto ao AP5 (a 90 minutos pós-treino) (Capítulo 3). Por sinal, a memória da tarefa de esquiva inibitória é episódica, com alto conteúdo emocional (aversivo), além de não ser mais sensível ao muscimol e ao AP5 nesta estrutura em estágios mais avançados do processo de consolidação (a 180 min pós-treino).

O conteúdo emocional de uma memória pode reforçar-lhe a evocação (Bradley *et al.*, 1992; Cahill e McGaugh, 1998). Este efeito não é sensível a lesões hipocampais, mas a lesões da amígdala (Cahill *et al.*, 1995; Hamann *et al.*, 1997), podendo ter o **córtex cingulado posterior** participação no processo (Maddock, 1999). Logo, embora esta estrutura possa mediar o trânsito de informações entre o hipocampo e o isocórtex, ela pode não ter função completamente subsidiária à do hipocampo, uma questão que merece estudos posteriores.

Como por nós demonstrado, o **córtex cingulado anterior** (Cg1 e Cg3) é necessário para a evocação da tarefa de esquiva inibitória (Capítulo 5). Por sinal, esta estrutura é também importante para a evocação de respostas ao labirinto radial, possivelmente devido ao fato de participar de um circuito comparativo (Seamans *et al.*, 1995).

Bontempi *et al.* (1999) encontraram que o **córtex cingulado anterior** e **regiões frontais** passam a ter maior atividade metabólica pela evocação de uma memória de 25 dias, enquanto que o hipocampo passa a ter menor atividade. O circuito comparador do qual o **córtex cingulado anterior** faz parte poderia inibir o hipocampo (Bontempi *et al.*, 1999), gerando maior flexibilidade comportamental. Além disto, Izquierdo *et al.* (2000) encontraram que o hipocampo é, sim, importante para a evocação da esquiva inibitória a 31

dias pós-treino, mas através de seus receptores glutamatérgicos metabotrópicos e de suas enzimas PKA e MAPK. Assim, a visão abordada em 1.3.2.2 de que a memória "sairia" do hipocampo após alguns dias é, possivelmente, equivocada (pelo menos em relação à estrutura como um todo, e, não, particularmente, em relação aos seus receptores AMPA, como abordado anteriormente).

Curiosamente, dentro das estruturas que possivelmente participam do processamento da elaboração das respostas, **Fr2**, mas não o **córtex cingulado anterior** (pelo menos na coordenada por nós utilizada), mostrou-se sensível ao muscimol durante a consolidação (por sinal, de 0 a 180 min pós-treino). O **Fr2** em sua parte mais anterior, atualmente denominada de **FrA**, revelou-se essencial à consolidação da memória da esquiva inibitória através de mecanismos sensíveis ao muscimol e ao SCH 23390 (este, um antagonista dos receptores dopaminérgicos D1), tanto durante seus estágio iniciais, como em estágios mais avançados. Por ser o **Fr2** ainda pouco conhecido, tanto em suas propriedades ao nível sináptico como ao nível circuital, ainda não nos é possível avaliar com profundidade o motivo pelo qual isto acontece. Portanto, ainda não podemos afirmar se o **Fr2** é um sítio de armazenamento da memória ou apenas está envolvido com os mecanismos subjacentes à consolidação. Porém, se levarmos em conta que existe PLD na região pré-frontal que é induzido pela estimulação da formação hipocampal (Hirsch e Crepel, 1990; Laroche *et al.*, 1990), podemos especular que certos componentes importantes da memória relacionados com, por exemplo, planos de ação, poderiam ser guardados nesta região, mesmo que não necessária e especificamente nestas sinapses com a PLD acima descrita (*i.e.*, ainda há a possibilidade de que estas potenciações apenas induzam outras mudanças, permanentes, como ocorre no diálogo entre o SCE e o SCM, abordado em 1.3.2.1). Por sinal, **Fr2** pertence ao SCM hipotetizado por Hikosaka *et al.* (1999); logo, este poderia, a princípio, apresentar mudanças plásticas permanentes. Entretanto, o **córtex cingulado anterior** pode não ser necessário à consolidação da memória da esquiva inibitória de uma via, como também demonstrado em gatos (McCleary, 1961), embora o seja em memórias aversivas com outros componentes cognitivos envolvidos (conforme abordado no Capítulo 6). Por sinal, a PLD do **córtex cingulado anterior** dura menos de uma hora (Sah e Nicoll, 1991), curiosamente, contrariando a definição clássica de PLD apresentada em 1.2, e a ativação intensa desta

estrutura pode inibir a consolidação da memória de esquiva inibitória (Santos-Anderson e Routtenberg, 1976), possivelmente por saturar ou desorganizar o sistema mediador da memória de trabalho.

Já o **córtex insular agranular** tem conhecida participação na aquisição, consolidação e evocação da memória da esquiva inibitória, embora não seja sítio de armazenamento (conforme abordado no Capítulo 6). Em nosso trabalho, mostramos que a excessiva ativação dos receptores 5-HT1_A no início da consolidação provoca amnésia quando a memória é medida 24 horas depois. É possível que tal efeito seja mediado por um mecanismo de retroalimentação negativa existente entre o **córtex insular agranular** e os núcleos da rafe, como abordado no Capítulo 6. Caso este seja o mecanismo, alguma estrutura do cérebro deve ter seus níveis de serotonina diminuídos ao ser infundido DPAT no **córtex insular agranular**, o que provocaria a amnésia. Esta estrutura não é, porém, o hipocampo, a amígdala, o córtex entorrinal ou o córtex parietal (Izquierdo e Medina, 1997). Uma outra possibilidade é de que a ativação dos receptores 5HT1_A esteja inibindo a ativação da cascata do AMPc/PKA no **córtex insular agranular**, como é sugerido que ocorra no hipocampo, no córtex entorrinal e no córtex parietal (Izquierdo e Medina, 1997). Um próximo estudo sobre os efeitos da infusão no córtex insular agranular de Rp-AMPS e do Sp-AMPS (um inibidor e um estimulador, respectivamente, da atividade da proteína quinase A) sobre a consolidação da memória da tarefa de esquiva inibitória, que por sinal já sendo implementado, poderá auxiliar no esclarecimento de tal questão. A participação de outros sistemas (*v.g.*, glutamatérgico) pode vir também a ser estudada.

7.1.3 Considerações Sobre a Medida da Memória através da Evocação

Avaliamos a memória, ao longo deste trabalho, através da mudança do comportamento animal na fase de teste. Esta é, de fato, a única forma, até o presente momento, que efetivamente possuímos para medir a memória, mesmo sendo esta uma forma indireta (Quillfeldt, 1994). Porém, alguns fatores podem dificultar a avaliação da memória segundo a perspectiva de sua evocação:

(1) Um traço pode ser reativado através de diferentes estratégias, ou seja, por diferentes combinações dos elementos adquiridos pelo sujeito durante a aquisição (Spear, 1978) e que foram modulados durante a consolidação (MgGaugh, 2000). Alguns destes

elementos poderiam não estar sob o controle (ou meramente a atenção) do experimentador (Spear, 1978);

(2) O estado neuro-humoral do sujeito experimental pode interferir na evocação (*v.g.*, dependência de estado) (Izquierdo, 1984);

(3) O grau de acessibilidade da memória à evocação, que de alguma forma é determinado durante sua consolidação e armazenamento (Izquierdo, 1984) (*v.g.*, tratamento pré-treino com cicloheximida, um inibidor da síntese protéica, é amnésico ao primeiro, mas não ao segundo dia de teste) (Quartermain e McEwen, 1970).

Nossa abordagem experimental, ao analisar o resultado a nível comportamental da interferência (direta) do funcionamento de apenas uma estrutura (ou apenas de parte desta), observa o efeito sobre a memória da exclusão funcional temporária ("lesões químicas reversíveis") de um componente de um ou mais sistemas operacionais atuantes durante a aquisição, consolidação ou evocação. Por sinal, esta tem sido a abordagem utilizada em trabalhos anteriores que verificaram a participação do hipocampo, da amígdala, do córtex entorrinal e do córtex parietal na memória da tarefa de esquiva inibitória (dentre estes, podemos citar Izquierdo *et al.*, 1997 e Zanatta *et al.*, 1997). Logo, a relevância desta interferência pode variar enormemente segundo vários fatores, tais como: (1) a riqueza desta memória, proporcionada pelo número de componentes/sistemas que ela possui/mobiliza; (2) a intensidade na qual ela foi gravada, que pode variar segundo a existência ou não de componentes emocionais (estes, por sinal, também podem variar em intensidade); (3) a natureza da interferência, se no núcleo ou na modulação do processo (Barros *et al.*, 1999); e (4) a importância intrínseca de cada componente e a relação entre eles. A natureza do engrama é, portanto, de difícil acessibilidade por esta via, provavelmente devido à sua complexidade. Assim sendo, é necessário um grande conjunto de evidências experimentais, através de diferentes abordagens, para que possamos definir conclusivamente se uma determinada estrutura participa, e de que forma o faz, em determinada memória.

Outras abordagens experimentais, como a observação do metabolismo das diversas regiões cerebrais (*v.g.* em Bontempi *et al.*, 1999 e Sif *et al.*, 1991) ou da atividade neuronal de diversas estruturas (*v.g.* em Nicolelis *et al.*, 1995), revelam a natureza do engrama sobre diferente perspectiva, ao revelarem algumas das propriedades globais dos sistemas intactos.

Porém, apesar de todo o esforço até então empreendido em diversos laboratórios ao redor do mundo, muitas questões, até de caráter mais básico, ainda permanecem em aberto. Esperamos ter dado uma pequena contribuição rumo às respostas das diversas questões aqui levantadas, através de um estudo de Neurofarmacologia Comportamental com enfoque anatômico, o qual verificou o envolvimento de algumas estruturas — **córtex cingulado posterior**, **córtex cingulado anterior**, **área pré-central medial (Fr2)** e **córtex insular agranular** — na memória da esquiva inibitória, que, agora, somam-se ao já conhecido envolvimento do hipocampo, da amígdala, do córtex entorrinal e do córtex parietal. Esperamos, mais ainda, termos suscitado novas questões.

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9. Lista de Publicações no Doutorado

9.1 Da Tese

1. **Mello e Souza T**, Roesler R, Madruga M, de Paris F, Quevedo J, Rodrigues C, Sant'Anna MK, Medina JH e Izquierdo I (1999). Differential effects of post-training muscimol and AP5 infusions into different regions of the cingulate cortex on retention for inhibitory avoidance in rats. *Neurobiology of Learning and Memory* **72:**118-127.
2. **Mello e Souza T**, Vianna MRM, Rodrigues C, Quevedo J, Madruga M, Medina JH e Izquierdo I (2000). Involvement of the medial precentral prefrontal cortex in memory consolidation for inhibitory avoidance in rats. *Pharmacology, Biochemistry and Behavior* **66**(3):615-622.
3. Barros DM, Izquierdo LA, **Mello e Souza T,** Ardenghi PG, Pereira P, Medina JH e Izquierdo I (2000). Molecular signalling pathways in the cerebral cortex are required for retrieval of one-trial avoidance learning in rats. *Behavioural Brain Research* **114**(1-2):183-92.
4. **Mello e Souza T**, Rodrigues C, Souza MM, Vinadé E, Choi H e Izquierdo I. Involvement of serotonergic type 1A (5-HT1A) receptor in the Agranular Insular Cortex in the Consolidation of Memory for Inhibitory Avoidance in Rats. *Behavioural Pharmacology* (submetido).

9.2 Sobre Cingulado ou Pré-Frontal (Fora da Tese)

1. Izquierdo I, Izquierdo LA, Barros DM, **Mello e Souza T**, de Souza MM, Quevedo J, Rodrigues C, Sant'Anna M Kauer, Madruga M e Medina JH (1998). Differential Involvement of Cortical Receptor Mechanisms in Working, Short- and Long-term Memory. *Behavioural Pharmacology* **9**(5-6):421-7.
2. Souza MM, **Mello e Souza T**, Vinadé ER, Rodrigues C, Choi HK, Dedavid e Silva TL e Medina JH e Izquierdo I. Effects of Post-training Treatments in the Posterior

Cingulate Cortex on Short- and Long-Term Memory for Inhibitory Avoidance in Rats. *Neurobiology of Learning and Memory* (submetido).

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9.3 Outras

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Hic jacet lepus.