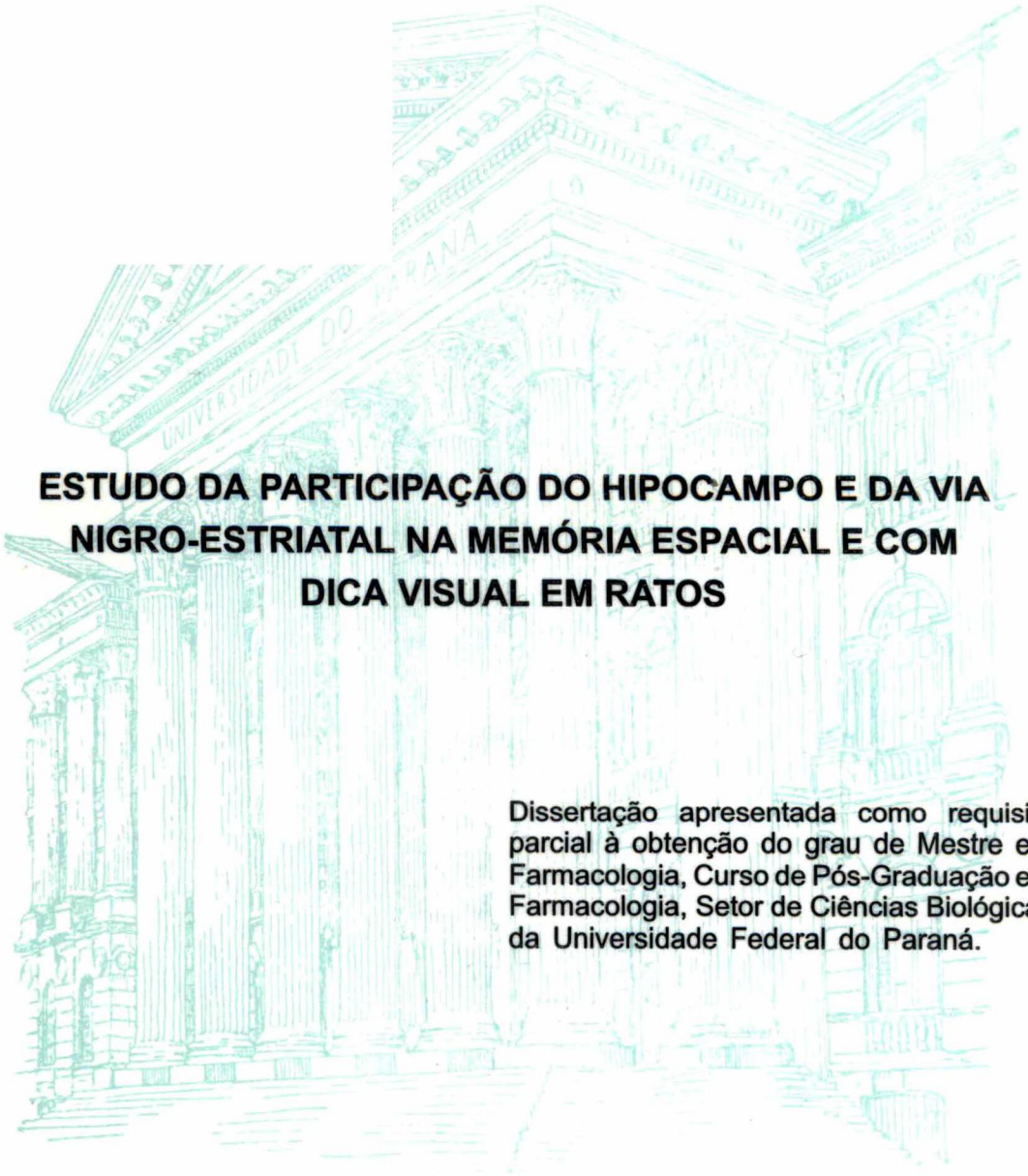
SAMANTHA WIETZIKOSKI



ESTUDO DA PARTICIPAÇÃO DO HIPOCAMPO E DA VIA NIGRO-ESTRIATAL NA MEMÓRIA ESPACIAL E COM DICA VISUAL EM RATOS

Dissertação apresentada como requisito parcial à obtenção do grau de Mestre em Farmacologia, Curso de Pós-Graduação em Farmacologia, Setor de Ciências Biológicas da Universidade Federal do Paraná.

CURITIBA

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Ministério da Educação
UNIVERSIDADE FEDERAL DO PARANÁ
Setor de Ciências Biológicas
Programa de Pós-Graduação em Farmacologia

PARECER

A Comissão Examinadora da Dissertação de Mestrado "**ESTUDO DA PARTICIPAÇÃO DO HIPOCAMPO E DA VIA NIGRO-ESTRIATAL NA MEMÓRIA ESPACIAL E COM DICA VISUAL EM RATOS**", de autoria da Pós-Graduanda **SAMANTHA WIETZIKOSKI**, e composta pelos Professores Dr. Cláudio da Cunha (Presidente); Dr. Newton Sabino Canteras (USP), Drª Roseli Boerngen de Lacerda (UFPR). De acordo com o Regimento Interno do Programa de Pós-Graduação em Farmacologia, a Pós-Graduanda foi APROVADA. Para a devida publicação o trabalho deve sofrer as modificações sugeridas, que serão conferidas pela coordenadora. Em Curitiba, 12 de dezembro de 2003.

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Esta dissertação foi realizada no Departamento de Ciências Biológicas Farmacologia – Universidade Federal do Paraná, com o apoio financeiro da CAPES.

Aos meus pais, Luiz Wietzikoski e Idalina Menossi Wietzikoski, de quem recebi o dom mais precioso: a Vida, e como se já não bastasse, revestiram minha existência de amor, carinho e dedicação.

Ao meu namorado, Humberto Itiro Sato, pela compreensão nos momentos de dificuldade e ausência. Obrigada pelo teu amor, estímulo e carinho.

A minha irmã, Evellyn Cláudia Wietzikoski, pelo apoio, incentivo e colaboração neste trabalho, obrigada por sempre torcer e confiar em mim. Espero que você concretize todos os seus sonhos. Estou torcendo por você.

"Há homens que lutam um dia e são bons.

Há outros que lutam um ano e são melhores.

Há aqueles que lutam muitos anos e são muito bons.

Porém, existem aqueles que lutam toda uma vida. Esses

são imprescindíveis".

(Bertold Brecht).

AGRADECIMENTOS

- A Deus, pois quando o menor apoio me pareceu distante e os objetivos inatingíveis, com fé, roguei pela única força de que realmente precisava, a força de Deus.

- Ao professor Dr. Cláudio da Cunha, pela orientação na elaboração e execução deste trabalho.

- Ao professor Dr. Newton Sabino Canteras, pela preparação do material histológico, fotográfico e colaboração científica.

- Ao professor Dr. Brás H. Oliveira, pela disponibilidade do laboratório e do HPLC.

- A mestre Irinéia Paulina Baretta, por sua valiosa colaboração, mostrando-se apaixonada pela ciência e ensino, ajudando-me a confirmar o sentimento de desejo pelo exercício da docência.

- A UNIPAR e todos os profissionais desta instituição, que marcaram e continuam marcando esta trajetória e que me deu a oportunidade de também fazer parte desta grande família.

- Aos queridos amigos: Marcelo, Daniel, Francine, Gianna, Ivana, Maria Inês, Andréia, Edmar, Kênia, Lilian, Márcio, Tadeu, Inajara, quanta alegria já me proporcionaram!

- Aos professores: Roberto, Hidevaldo, Míriam, Roseli, Maria, pelo apoio, críticas e sugestões sempre pertinentes.

- Aos professores Paulo e Herbet, pelo bom humor, trocadilhos, e piadinhas... o que faz o ambiente de trabalho ser mais alegre.

- À todos os pós-graduandos do departamento, pela amizade e companheirismo.

- À todos os funcionários do departamento, em especial à equipe de secretaria, limpeza e do biotério.

- À Lindacir e Nair, pela dedicação e cuidados com os animais.

- Ao Departamento de Pós-Graduação em Farmacologia da UFPR.

- À CAPES, pela bolsa de estudos.

Obrigada.

SUMÁRIO

LISTA DE ABREVIATURAS

SNc	- Substância negra, parte compacta
MPTP	- 1-methil-4-fenil-1,2,3,6-tetrahidropiridina
6-OHDA	- 6-hidroxidopamina
DA	- Dopamina
SHAM	- O mesmo que simulado, falsamente lesado
DP	- Doença de Parkinson

INTRODUÇÃO

A Doença de Parkinson é caracterizada por uma degeneração de neurônios dopaminérgicos na substância negra parte compacta (SNc) e pela presença de pequenas inclusões esféricas citoplasmáticas denominadas corpos de Lewy, que consistem de uma densa camada granular cercado por um halo que se propaga em filamentos (FLINT, 2001). A Doença de Parkinson é uma doença crônica e progressiva, e está citada como a segunda doença neurodegenerativa mais comum, afetando 1% da população acima de 50 anos de idade (FLINT, 2001). A progressão dos sintomas é usualmente lenta mas a velocidade com que esta progressão se desenvolve é bastante variável. As manifestações motoras incluem: acinesia, bradicinesia, tremor em repouso, rigidez muscular, distúrbios do equilíbrio e da marcha, porém evidências clínicas e experimentais indicam que esta desordem neurodegenerativa também causa déficits cognitivos (HIRSCH et al., 1988; MYOSHI et al., 2002), depressão, alterações do sono e distúrbios do sistema nervoso autônomo (FLINT, 2001).

O termo parkinsonismo é mais amplo e refere-se a um grupo de doenças que apresentam em comum os sintomas acima em combinações variáveis, associados ou não a outras manifestações neurológicas, sem que sua causa esteja necessariamente relacionada à presença de corpos de Lewy na SNc. A Doença de Parkinson é também chamada de parkinsonismo primário ou idiopático porque a causa etiológica específica da doença é desconhecida, isto dificulta desenvolver métodos ideais para o estudo da doença. (FLINT, 2001).

Os pacientes com Doença de Parkinson apresentam deficiências em tipos específicos de memória. Existem vários tipos de memória. A utilização de modelos animais e estudos com pacientes com lesões cerebrais vêm permitindo identificar estruturas e conexões cerebrais que quando seletivamente danificadas, produzem alterações no funcionamento normal dos diferentes tipos de memória. A memória pode ser classificada, quanto à sua natureza, em: memória operacional, memória explícita e memória implícita.

A memória operacional serve para o arquivamento de informações durante o desempenho de operações cognitivas por um período muito curto de tempo. Embora ela seja usualmente identificada como (e mesmo tratada como sinônimo de) memória de curta duração, esta última mostrou-se por demais simples para lidar com os tipos de retenção de informação por curtos períodos de tempo, evidenciados experimentalmente. Assim, desenvolveu-se o conceito de memória operacional como um sistema de capacidade limitada e com múltiplos componentes (BADDELEY, 1992). De acordo com BADDELEY e HITCH (1974), memória operacional em humanos compreende um sistema de controle de atenção, a central executiva, auxiliado por dois sistemas de suporte responsáveis pelo arquivamento temporário e manipulação de informações, um de natureza vísuo-espacial e outro de natureza fonológica. A central executiva, com capacidade limitada, proporcionaria a conexão entre os sistemas de suporte e a memória de longa duração e seria o responsável pela seleção de estratégias e planos (BADDELEY, 1992); sua atividade estaria relacionada ao funcionamento do lobo frontal que teria a função de supervisionar informações a serem codificadas,

armazenadas e evocadas, concomitantemente ao seu ingresso no sistema. Acredita-se que existem também outros tipos de memória de curta duração que podem armazenar informações por intervalos de até algumas horas.

Estudos de dissociação levaram COHEN (1984) a classificar as memórias de longa duração em memória declarativa (ou explícita) e memória não-declarativa (ou procedural ou ainda implícita). A memória declarativa episódica refere-se à retenção de experiências sobre fatos e eventos do passado. Por exemplo, o indivíduo tem acesso consciente ao conteúdo da informação, sendo adequada para o arquivamento de associações arbitrárias após uma única experiência. A memória implícita, de acordo com SCHACTER (1987), "é revelada quando a experiência prévia facilita o desempenho numa tarefa que não requer a evocação consciente ou intencional daquela experiência". De acordo com COHEN (1984), a aquisição de informações pelo sistema não-declarativo depende de mudanças cumulativas que ocorrem a cada ocasião em que o sistema é acionado. Isso implica que o sistema não-declarativo requer treinamento repetitivo para a aquisição do comportamento e que a aquisição ocorre de forma gradual. Pacientes com Doença de Parkinson apresentam deficiências de memória procedural e também de memória operacional e oferecem evidências do envolvimento dos gânglios basais nos processos de memória não-declarativa (DAMÁSIO E TRANEL, 1991; KNOPMAN E NISSEN, 1991). Sabe-se que os circuitos dos núcleos da base estão principalmente envolvidos com o controle de movimentos, porém evidências anatômicas, fisiológicas, clínicas e patológicas (MIDDLETON e STRICK, 2000) demonstram que os núcleos da base também

estão envolvidos nos processos cognitivos. Alguns estudos clínicos (DIVAC et al., 1967; MIYACHI et al., 1997) sugerem que os núcleos da base contêm circuitos motores e cognitivos separados. Em uma revisão de resultados de estudos anatômicos, ALEXANDER, DELONG E STRIK (1986) propuseram que os núcleos da base estariam participando de cinco circuitos paralelos "loops" com o córtex cerebral, que por sua vez explicaria, como que os núcleos da base poderiam estar modulando os processos comportamentais, assim como os processos envolvidos com os controles motores. Segundo esta hipótese, as áreas corticais seriam alvos das vias eferentes dos núcleos da base, incluindo não apenas a área motora primária (M1) responsável pelo controle de movimentos, mas também a área 9 responsável pelo planejamento de ações e memória de trabalho, incluindo subdivisões do córtex pré-motor, oculomotor, pré-frontal e inferotemporal. Partindo deste princípio, uma das hipóteses mais aceitas é que os núcleos da base estariam participando nos processos de aquisição de aprendizagem do tipo estímulo-resposta (S-R), que quando aprendidos de forma gradual e inconsciente, constituem um hábito. Existem muitos estudos (PACKARD et al., 1996) que foram realizados para demonstrar a dissociação dos núcleos da base de outro sistema de memória mediado pelo lobo medial temporal, na qual incluiria o hipocampo como um componente primário para a formação da memória declarativa. Evidências sugerem que durante o processo de aquisição da memória, o sistema dos núcleos da base e o sistema hipocampal seriam ativados simultaneamente e em paralelo (MCDONALD E WHITE 1994; PACKARD E McGAUGH, 1996), e que poderia ocorrer competição em algumas situações de aprendizagem causando uma interferência entre estes dois sistemas. Deste modo, o sistema hipocampal

estaria mediando uma rápida forma de aprendizagem que inicialmente controlaria o comportamento e o sistema dos núcleos da base mediariam uma aprendizagem mais duradoura e automática envolvendo a formação de aprendizado do tipo S-R.

Para se estudar a Doença de Parkinson, foram desenvolvidos diversos modelos animais. A 6-hidroxidopamina (6-OHDA) é uma das neurotoxinas mais utilizadas experimentalmente em modelos de degeneração da SNc, tanto "in vitro" como "in vivo" (BLUM, 2001). Em modelos animais de Doença de Parkinson, 6-OHDA é administrada diretamente na SNc ou no estriado, induzindo a degeneração de neurônios dopaminérgicos, produzindo características fisiopatológicas responsáveis pelos prejuízos motores na Doença de Parkinson (BLUM,2001). Animais com lesão da SNc pela neurotóxico 1-methil-4-fenil-1,2,3,6-tetrahidropiridina, ou MPTP (LANGSTON et al., 1983), também são utilizados como um modelo da Doença de Parkinson. A administração intra-nigral de MPTP na SNc dos ratos ocasiona perdas específicas de dopamina no estriado e córtex pré-frontal (GERLARCH e RIEDERER, 1996; BLUM, 2001). Estudos realizados por MIYOSHI (2002), demonstraram que esta lesão não altera o desempenho motor dos animais ou seu aprendizado no teste do labirinto aquático versão espacial, porém causa um prejuízo na memória de hábito (um tipo de memória não-declarativa em humanos) quando os animais são submetidos ao teste do labirinto aquático, versão com dica visual. Outros estudos demonstraram que animais com lesão no hipocampo desempenham normalmente a tarefa do labirinto aquático com dica visual, enquanto que esta lesão causa um prejuízo no desempenho da tarefa do labirinto aquático, versão espacial (MORRIS, GARRUD, RAWILINS & O´KEEFE, 1982). Estes resultados sugerem que existem estruturas

cerebrais diferentes que participam da aquisição e retenção de informações envolvendo memórias explicitas ou implícitas (PALLER, 1990).

OBJETIVOS

O presente estudo pretende:

- Avaliar a importância da SNc e do hipocampo no aprendizado na memória da tarefa do labirinto aquático nas versões com dica visual, um modelo de memória de estímulo – resposta (S-R), e espacial (um modelo de memória espacial dependente do hipocampo).

- Testar se o aprendizado de uma versão da tarefa do labirinto aquático reverte o déficit de aprendizado observado em ratos com lesão da (SNc).

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EXPERIMENTO 1

A SUBSTÂNCIA NEGRA, PARTE COMPACTA, COMO PARTE DE UM SISTEMA DE MEMÓRIA INDEPENDENTE DO HIPOCAMPO

Trabalho publicado na revista Neurobiology of Learning and Memory, 79, 236-242, 2003.

ACADEMIC PRESS

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Neurobiology of Learning and Memory 79 (2003) 236–242

Neurobiology of Learning and Memory

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Evidence for the substantia nigra pars compacta as an essential component of a memory system independent of the hippocampal memory system

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Received 2 July 2002; revised 13 November 2002; accepted 6 January 2003

Abstract

The aim of the present study was to test if the nigrostriatal pathway is an essential component for a water maze cued task learning and if it works independently of the hippocampal memory system. This hypothesis was tested using an animal model of Parkinson's disease in which male Wistar rats were lesioned in the substantia nigra pars compacta (SNc) by the intranigral infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), thus causing a partial depletion of striatal dopamine. SNc-lesioned and sham-operated animals were implanted bilaterally with guide cannulae above the dorsal hippocampus in order to be tested after the administration of 0.4 μl 2% lidocaine or saline into this structure. The animals were tested in a spatial or in a cued version of the water maze, memory tasks previously reported to model hippocampal-dependent spatial/relational and striatal-dependent S–R learning, respectively. Hippocampal inactivation, but not SNc lesion, impaired learning and memory in the spatial version of the water maze. An opposite situation was observed with the cued version. No significant interaction was observed between the SNc lesion and hippocampal inactivation conditions affecting scores in the spatial or in the cued version of the water maze. These results suggest that the nigrostriatal pathway is an essential part of the memory system that processes S–R learning and that it works independently of the hippocampal memory system that processes spatial/relational memories.

Keywords: Substantia nigra pars compacta; Hippocampus; Dorsal striatum; Memory; Spatial learning; Cued task learning; Parkinson's disease; Water maze

1. Introduction

Previous studies have suggested that spatial/relational and cued task learning are independently processed by different brain structures (Packard, Hirsh, & White, 1989; Packard & McGaugh, 1992; White & McDonald, 2002). Thus it has been shown that spatial learning in rats depends critically on the integrity of the hippocampus but not of the dorsal striatum, whereas cued task learning depends critically on the integrity of the dorsal striatum. Evidence supporting this double dissociation of the hippocampal and striatum memory systems stems from studies using lesions or drug administration in rats submitted to the spatial or to the cued version of water or radial maze memory tasks (Packard et al., 1989; Packard & McGaugh, 1996). Clinical studies also support the idea that hippocampal damage impairs the formation of some kinds of memory (e.g., episodic) without affecting habit formation, a kind of S–R learning (Milner, Squire, & Kandel, 1998). Conversely, early phase Parkinson's disease patients, in whom the nigrostriatal pathway is damaged, present impaired learning of habit tasks but

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1074-7427/03/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S1074-7427(03)00008-X

present preserved ability to form new episodic memories (Dubois & Pillon, 1997; Knowlton, Mangels, & Squire, 1996).

Previous studies from our laboratory have shown that bilateral MPTP-induced lesion of the rat nigrostriatal pathway impairs learning of the cued version of the water maze and that the partial depletion of striatal dopamine (DA) is sufficient to produce this effect (Miyoshi et al., 2002). The aim of the present investigation is to obtain further information about the nigrostriatal pathway as an essential component of the striatal memory system and to test if it works independently of the hippocampal memory system. This hypothesis was tested by analyzing the effect of lesion of the rat substantia nigra pars compacta (SNc) and/or inactivation of the dorsal hippocampus with lidocaine on learning of cued or spatial version of the water maze.

2. Materials and methods

2.1. Subjects

Fifty adult male Wistar rats from our own breeding stock weighing 280–320 g at the beginning of the experiments were used. The animals were maintained in a temperature-controlled room (22 ± 2 °C) on a 12/12-h dark/light cycle (lights on 07:00 a.m.) with food and water available ad libitum. All the behavioral experiments were conducted between 07:00 and 13:00 h. The animals were individually housed in plexiglas home cages (60 × 25 × 25 cm).

2.2. Surgery

The animals were divided into a control sham-operated group and an SNc-lesioned group of 20 and 30 animals, respectively. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was chosen to induce SNc lesion based on previous studies showing that its infusion into the rat SNc is effective to lesion dopaminergic cells to an extent that does not cause sensorimotor disabilities (Da Cunha et al., 2001; Gevaerd et al., 2001) and is more selective in depleting DA (Da Cunha et al., 2001; Gevaerd et al., 2001; Harik et al., 1987) compared to 1-methyl-4-phenylpyridinium ion (MPP^+) or 6-hydroxydopamine (6-OHDA) (Costall, Marsden, Naylor, & Pycock, 1977; Harik et al., 1987). Twenty-one days before the initiation of the behavioral experiments animals of the lesioned group received atropine sulfate (0.4 mg/kg, i.p.) to suppress salivation, penicillin G-procaine (20,000 U in 0.1 ml, i.m.), 3 injections of 120 mg/kg, acetaldehyde (Sigma Chemical St. Louis, MO, USA, i.p., 10 min before and 30 and 60 min after the beginning of surgery), and were anesthetized with 40 mg/kg sodium thiopental (i.p.). MPTP HCl (Sigma Chemical, 0.5 μmol, 1 μl in saline, 0.33 μl/min) was bilaterally infused through a 30-gauge needle according to the following coordinates: anteroposterior (AP), −5.0 mm from bregma; mediolateral (ML), ±2.1 mm from midline; dorsoventral (DV), −7.7 mm from the skull (see Fig. 1). Sham-operated animals were submitted to the same procedure but 1 μl saline was infused into the SNc instead of MPTP. After surgery the animals were allowed to recover from anesthesia in a temperature-controlled

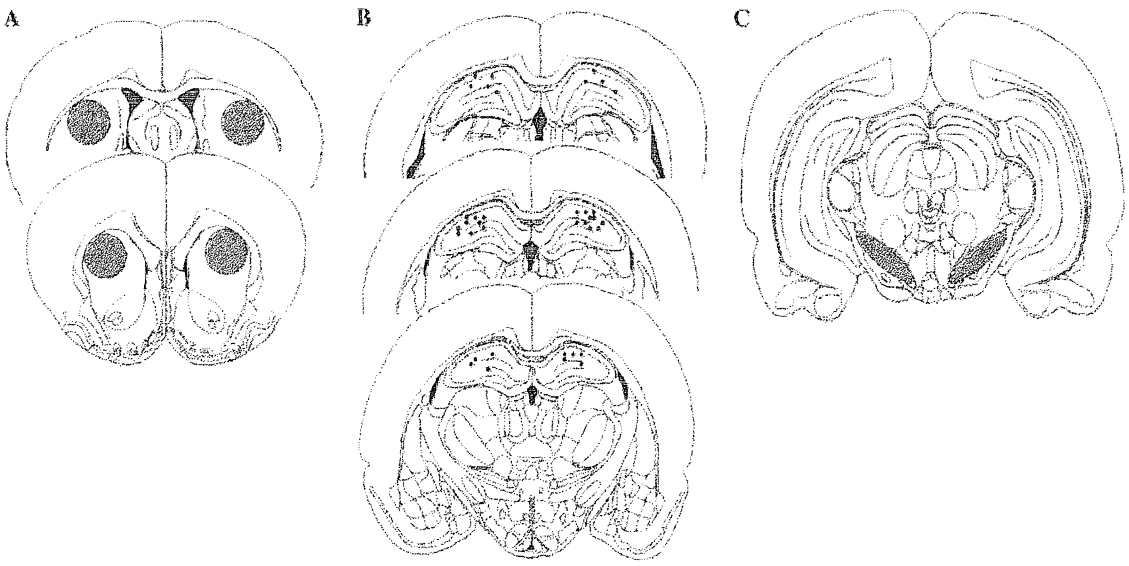

Fig. 1. Illustration of coronal sections of the rat brain areas manipulated in the present study. (A) Striatal tissue area punched for dopamine determination (striped), from +1.6 to −0.4 mm of bregma. (B) Sites where 2% lidocaine was microinfused into the hippocampus (dots) shown from −3.1 to −3.6 of bregma. (C) Mesencephalic area showing maximal cell loss after microinfusion of 1 μmol MPTP (striped), shown at −5 mm from bregma. Adapted from The Rat Brain in Stereotaxic Coodinates by Paxinos and Watson (1986), San Diego, Ca; Academic Press.

chamber and then returned to their home cage. During the first 5 postoperative days, the solid diet of both groups was replaced with a liquid diet to which the animals had free access. The liquid diet consisted of 16.6% Sustacal (Bristol-Myers Squibb, NY, USA) and 10.7% sucrose. Pilot experiments showed that this procedure prevented body weight loss in MPTP-injected rats, reducing mortality. Previous studies (Da Cunha et al., 2001) demonstrated no motor, behavioral, or neurochemical differences between sham-operated and non-operated animals. Five days before the behavioral test, the same animals were anesthetized with sodium thiopental as described above and implanted bilaterally with guide cannulae (7-mm long, 23 gauge) aimed 2.0 mm above the dorsal hippocampus according to the following coordinates: AP, −3.1 mm from bregma; ML ± 1.5 mm from midline; DV, −2.0 mm from the skull (see Fig. 1). Hippocampus and SNc coordinates were adapted from Paxinos and Watson (1986).

After the behavioral tests, the animals were sacrificed by decapitation and their dorsal striata were removed for the determination of DA levels as described below. The posterior part of the rat brain was preserved and left in formalin for subsequent histological analysis. Thus, for each brain, series of 30 μm sections were cut with a sliding microtome and stained with thionin to verify the placement of the hippocampal cannulae and the extent of MPTP-induced lesion in the SNc (see Fig. 1). Three sham-operated and 6 MPTP-injected animals died. The behavioral data of 4 other MPTP-injected animals were excluded from analysis. These were animals that presented lesions smaller than 40% of the SNc and animals with less than 30% depletion of striatal DA.

2.3. Behavioral procedures

Three weeks after surgery, the rats were submitted to one of two versions of the water maze task,both conducted in a round tank, 170 cm in diameter and 40 cm deep, filled with water. The water temperature was maintained at 22 °C. Several distal visual cues were placed on the walls of the water maze room. During the experiments, the tank was videotaped and the latency to reach the escape platform was measured.

Nine sham-operated rats and 9 MPTP-lesioned rats were submitted to a spatial version of the water maze. This consisted of 5 training days, 4 consecutive trials per day, during which the animals were left in the tank facing the wall and allowed to swim freely to a transparent acrylic escape platform (11 × 14 cm) submerged 2 cm under the water surface, placed on the center of one of the quadrants of the tank. The platform position was maintained constant throughout the 5 training days. The initial position in which the animal was left in the tank varied among trials in a pseudo-random way. If the animal did not find the platform during a period of 60 s it was gently guided to it. Then, it was allowed to remain on the platform for 20 s and removed from the tank for 30 s before being placed in the next initial starting position in the tank. On the 4th and 5th training days, the animals were submitted to a probe test before the routine training with the escape platform. The probe test consisted of allowing the animals to freely swim for one minute in the tank without the escape platform. The amount of time spent in the quadrant that contained the platform on the previous day (target) and in the opposite quadrant and the swimming paths were recorded using an image analyzer (CEFET; Curitiba, Brazil). This protocol is quite similar to that of Morris, Garrud, Rawlins, and O' Keefe (1982). On the 4th and 5th training day, 10 min before the probe test, 30-gauge needles were inserted into the cannulae, extending 2 mm beyond their tips, and 0.4 μl of saline (0.9% NaCl) or 2% lidocaine, respectively, was infused bilaterally into the dorsal hippocampus.

Two other groups of 8 sham-operated and 11 SNc-lesioned animals were submitted to a cued version of the water maze similar to the previous experimental procedure, except that the position of the escape platform was cued by a 7-cm diameter white ball attached to the top of the platform, protruding above the water. Furthermore, the position of the platform was always changed in each trial of the day. This protocol was adapted from Packard and McGaugh (1996). The effect of hippocampal inactivation with lidocaine on the escape latency was tested on the 5th training day, similarly to the spatial task, but without the probe test.

2.4. Determination of monoamine levels

The endogenous levels of DA were assayed by reverse-phase HPLC with electrochemical detection as described by Hallman and Jonsson (1984). Briefly, a C18 reverse phase column (Shim-pack, CLC-ODS 150 × 4.6 mm, Shimadzu), an amperometric detector (Amtec, Decade), and a liquid chromatography workstation CLASS-VP 5032 (Shimadzu) were used. The animals were sacrificed by decapitation 30 days after surgery, their brains were removed from the skull, frozen, and sliced and a 2-mm diameter (beginning 1.6 mm anterior to the bregma) circular punch from the dorsolateral striatum was sampled and stored at −70 °C (see Fig. 1). Frozen tissue was weighed and homogenized in 100 μl of 0.1 N HCl with a microultrasonic cell disrupter. After centrifugation (12,000*g*, 10 min), 20 μl of the supernatant was injected into the chromatograph. The mobile phase, used at a flow rate of 0.9 ml/min, was of the following composition: 292 mg NaCl, 15.7 g citric acid, 465 ml twice-distilled water, sufficient 10 N NaOH to bring the pH value to 3.0, 70 mg octyl sodium sulphate, 20 ml acetonitrile, and 10 ml tetrahydrofluran. The oxidation potential was fixed at 0.85 V using a glass

carbon working electrode versus an Ag/AgCl reference electrode. The peak areas of the external standards were used to quantify the sample peaks. The values obtained were expressed as ng/g tissue wet weight.

2.5. Statistical analysis

Differences in monoamine levels between groups were analyzed by one-way ANOVA. Escape latencies for the individual trials were averaged by day and analyzed by two-way ANOVA with repeated measures (session day). Time spent in the quadrants during the probe test was analyzed by three-way ANOVA considering SNc-lesion and intra-hippocampal administration of lidocaine as independent factors, and scores on the 4th and 5th days as repeated measures. Differences between groups and training days were analyzed by the post-hoc Duncan test and were considered to be statistically significant when $p \leqslant .05$.

3. Results

Histological analysis of brains from MPTP-treated animals revealed that the lesions were by and large confined to the SNc as illustrated in Fig. 1. Only occasionally was a minimum cells loss in the ventral tegmental area also observed. MPTP-induced lesion of the SNc caused a significant decrease in DA levels in the dorsal striatum of the rats: sham-operated group = 3726 ± 310; SNc-lesioned group = 2290 ± 169 (means ± SEM expressed as ng/g tissue wet weight; $F(1,35) = 17.89$, $p \leqslant .001$).

As observed in previous experiments (Da Cunha et al., 2001; Gevaerd et al., 2001), the animals swam normally and did not present any gross sensorimotor disturbances when submitted to the behavioral tests three weeks after surgery. The absence of motor impairments can also be ruled out by the observed lack of significant differences between control sham-operated and SNc-lesioned groups in the spatial version of the water maze (see Figs. 2 and 3).

As can be seen in Fig. 2, SNc lesion did not affect learning or memory in the spatial version of the water maze task since both the sham-operated and SNc-lesioned groups improved their mean escape latency throughout the first 4 training days ($F(4,56) = 7.36$, $p \leqslant .001$) and no significant difference was observed between them (lesion effect: $F(1,14) = 0.03$, $p \geqslant .2$); lesion vs. training day interaction: ($F(4,56) = 0.43$, $p \geqslant .2$). On the other hand, on the 5th training day, hippocampal

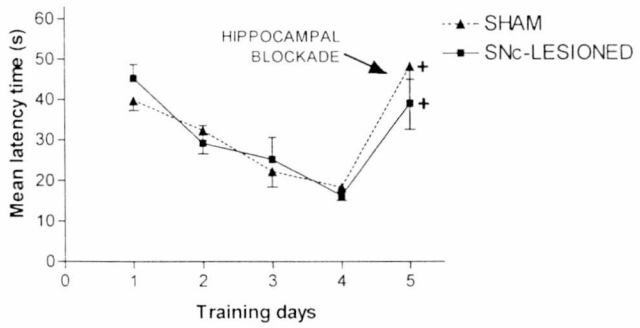

Fig. 2. Effect of MPTP-induced lesion of the SNc and lidocaine-induced hippocampal inactivation on the spatial version of the water maze task. The graph represents means ± SEM latencies to escape to a submerged platform during 5 training days, with 4 consecutive trials per day. Escape latencies for the individual trials were averaged by day. The arrow indicates the day when animals were trained after receiving an intra-hippocampal administration of 2% lidocaine. *$p \leqslant .05$ compared to the group that received intra-hippocampal administration of saline; +$p \leqslant .05$ compared to the 4th training day, Duncan test after ANOVA.

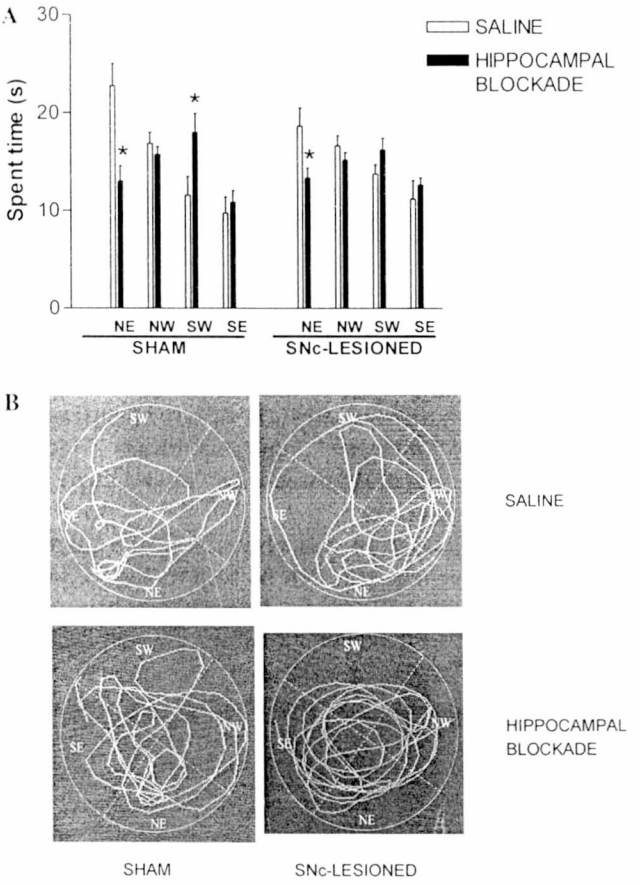

Fig. 3. Effect of MPTP-induced lesion of the SNc and lidocaine-induced hippocampal inactivation on the spatial version of the water maze task during a probe test in which animals were allowed to swim in the water maze without the escape platform. On the previous days the platform was present in the NE quadrant. (A) Bars represent the means ± SEM time spent in the quadrants of the water maze. (B) Swimming paths of the animals during the probe test. *$p \leqslant .05$ compared to the group that received intra-hippocampal administration of saline; +$p \leqslant .05$ compared to the 4th training day, Duncan test after ANOVA.

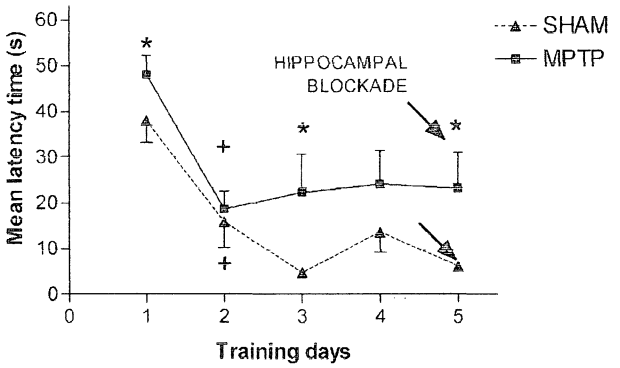

Fig. 4. Effect of MPTP-induced lesion of the SNc and lidocaine-induced hippocampal inactivation on the cued version of the water maze task. Values are expressed as means ± SEM. Data express the latency to escape to a cued platform during 5 training days, with 4 consecutive trials per day. Escape latencies for the individual trials were averaged by day. Arrows represent the day when animals were training after receiving an intra-hippocampal administration of 2% lidocaine. *$p \leq 0.05$ compared to the group that received intra-hippocampal administration of saline; +$p \leq .05$ compared to the previous training day, Duncan test after ANOVA.

inactivation prevented the animals from finding the platform (4th vs 5th day: $p \leq .05$, post-hoc Duncan test). This effect was also observed in the analysis of probe test scores as shown in Fig. 3. Three-way ANOVA showed that the time spent by the animals in different quadrants of the water maze was not affected by the SNc lesion (interaction lesion factor and quadrant ($F(3,58) = 1.35$, $p = .26$), three-way ANOVA). On the other hand, the rats spent less time in the target quadrant (NE) after intra-hippocampal administration of lidocaine (interaction repeated measure and quadrant factors, $F(3,58) = 10.49$, $p \leq .001$, three-way ANOVA; $p \leq .001$ post-hoc Duncan test).

An opposite situation was observed in the cued version of the water maze, as can be seen in Fig. 4. In this version of the task both groups learned to reduce escape latency ($F(4,56) = 24.44$, $p \leq .001$), but the SNc-lesioned animals spent longer times finding the cued platform compared to the sham-operated animals ($F(1,14) = 5.37$, $p \leq 0.05$, see Fig. 4 for individual differences during the training days). No significant interaction was observed between the lesion factor and the training day ($F(1,14) = 5.37$, $p \geq .2$). On the other hand, the administration of lidocaine 10 min before testing the animals on the 5th training day did not affect their latencies to find the cued platform compared to the latencies of the 4th day ($p \geq .2$, Duncan test).

4. Discussion

The intranigral administration of MPTP caused a partial depletion of striatal DA as observed in previous work on rats (Da Cunha et al., 2001; Gevaerd et al., 2001; Harik et al., 1987; Miyoshi et al., 2002). According to a previous study from our laboratory, this depletion is specific for DA and metabolites while the levels of other monoaminergic neurotransmitters are preserved (Gevaerd et al., 2001). The fact that the DA depletion was only partial can explain why motor disabilities were not observed in the rats of this and previous studies of MPTP-induced SNc lesions (Da Cunha et al., 2001; Heikkila, Sonsalla, & Duvoisin, 1989; Miyoshi et al., 2002). In the present study, consistent evidence that the MPTP-induced SNc lesion did not affect the swimming performance of the rats was provided by the observation that the mean latencies to find the platform in the spatial version of the water maze task did not differ significantly between control and SNc-lesioned animals (see Fig. 2). Further evidence that the MPTP-induced SNc lesion did not affect rat swimming was presented in a previous study (Miyoshi et al., 2002).

The fact that the SNc-lesioned rats performed normally in the spatial memory version of the water maze (see Figs. 2 and 3) suggests that this task is not critically dependent on the modulatory influences of dopaminergic neurons of the SNc on the dorsal striatum. Previous studies have shown that this protocol of SNc lesion is selective in depleting DA in the dorsal striatum, sparing dopaminergic projections to the ventral striatum (nucleus accumbens), hippocampus, and other limbic brain regions (Gevaerd et al., 2001; Miyoshi et al., 2002). Conversely, the inactivation of the dorsal hippocampus with lidocaine caused an expressive and significant increase in the time the rats spent to find the submerged platform in the spatial task, independent of the integrity of the SNc. This result agrees with previous studies showing that place navigation learning in a water maze depends on hippocampal (DiMattia & Kesner, 1988; Morris et al., 1982; Sutherland, Whishaw, & Kolb, 1983), but not on striatal integrity (Packard & McGaugh, 1996). Moreover, disruption of learning and memory for the spatial version of the water maze by damaging the hippocampus (DiMattia & Kesner, 1988; Morris et al., 1982; Sutherland et al., 1983) or the fimbria-fornix (McDonald & White, 1994; Nilsson, Shapiro, Gage, Olton, & Bjorklund, 1987; Sutherland & Rodriguez, 1989) was further demonstrated in many other studies, all coherent with the present results. The critical role of the hippocampus in spatial learning in rats was also demonstrated in many other memory tasks that require discrimination between sets of cues that contain common elements, like the extra-maze cues visible from different points of the water maze. These tasks include the win-shift 8-arm radial maze task (Jarrard, 1993; McDonald & White, 1993; Olton & Papas, 1979; Packard & White, 1990; Packard et al., 1989) and the T-maze forced alternation task (Aggleton, Hunt, & Rawlins, 1986). Interestingly, rats with lesions

in the hippocampus can perform normally all of these tasks when the right response can be associated with a single and not ambiguous cue (see White & McDonald, 2002).

The results of the present study also showed that SNc lesion impaired learning of the cued task while the hippocampal inactivation with lidocaine did not affect it (see Fig. 4). Morris et al. (1982) first demonstrated that lesions in the hippocampus specifically prevent rats from learning to escape to a submerged fixed platform in a water maze in the absence of intra-maze cues (spatial task) but that they learn to escape like control animals when the platform protrudes above the water (cued task).

Later, Packard and McGaugh (1996) showed that dorsal striatum-lesioned rats learn the spatial but not the cued version of the water maze and proposed that the cued version is a kind of S–R task since the animals learn to associate a single stimulus (cue) with a reinforced response to approach it. Within this context, it is noteworthy that nigrostriatal dopaminergic cells increase their firing rate when unpredictable reward stimuli are presented to monkeys (Shultz, 2001). This so-called "learning signal" of these dopaminergic cells represents their key role in S–R learning.

The present results suggest that the dorsal striatum memory system is under the modulatory control of the dopaminergic neurons projecting from the SNc. The data also show that the nigrostriatal pathway is an essential part of the dorsal striatum memory system since SNc disruption impairs its proper function. This idea agrees with studies showing the critical effect of dopamine on striatal synaptic plasticity (Calabresi, Centonze, & Bernardi, 2000). In agreement with the present results, a previous report by Packard and McGaugh (1994) showed that a dopaminergic D2 agonist can improve learning in the cued version of the water maze. In the same direction, Packard and White (1991) showed that the administration of D1 (SKF 38393) or D2 (LY 171555) receptor agonists into the dorsal striatum but not into the hippocampus of rats improves learning of an 8-arm win-stay task. This is another S–R task in which the places of 4 food-baited arms are randomly cued with a light. Notably, opposite effects were observed with the win-shift version of the 8-arm radial maze in which the animals had to remember the location of previously visited arms using multiple environmental spatial cues. More recently, Setlow and McGaugh (2000) showed that the systemic administration of sulpiride, a D2 receptor antagonist, to rats impairs both the spatial and cued versions of the water maze. Indeed, it has been shown in rats that DA depletion in the hippocampal formation by local application of 6-OHDA produces a deficit in the spatial version but not in the cued version of the water maze (Gasbarri, Sulli, Innocenzi, Pacitti, & Brioni, 1996). Previous studies from our laboratory indicated that the protocol presently used for MPTP-induced lesions yields selective DA depletion in the dorsal striatum, sparing the hippocampus and other limbic brain regions (Gevaerd et al., 2001; Miyoshi et al., 2002). Moreover, according to the present histological analysis, the ventral tegmental area and the interfascicular nucleus, thought to represent the main sources of dopaminergic inputs to the hippocampus (Swanson, Kohler, & Bjorklund, 1987), were fairly preserved in the MPTP-treated animals.

Our findings are also consistent with human studies reporting that episodic hippocampal-dependent mnemonic functions are spared in early phase PD patients while these patients fail to perform other learning and memory tasks sharing common elements with habit learning (Fama et al., 2000; Pillon, Deweer, Agid, & Dubois, 1993; Rieger & Markowitsch, 1996; Sullivan & Sagar, 1991). In addition, Knowlton et al. (1996) showed that non-demented PD patients are impaired in a probabilistic classification task also proposed as a habit task, although they present intact memory for the test episode.

In summary, the present results strongly suggest that the dopaminergic nigrostriatal pathway is an essential component of the memory system that processes cued task learning in a way independent of the hippocampal memory system. The present study also supports the theory that the hippocampal memory system in not necessary for some kinds of S–R learning and that it processes spatial memory in a way independent of and not affected by the integrity of the nigrostriatal pathway.

Acknowledgments

This research was partly supported by CNPq and CAPES. The skillful technical assistance of Adriana Freitas in the neurochemical experiments and the comments of Dr. Tadeu Mello e Souza are acknowledged.

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EXPERIMENTO 2

INTERAÇÕES ENTRE OS SISTEMAS DE MEMÓRIA DEPENDENTE DA SUBSTÂNCIA NEGRA, PARTE COMPACTA, E DO HIPOCAMPO, RESPECTIVAMENTE

Trabalho submetido para publicação na revista Trends in Neurosciences, 2003.

Crosstalks, secret talks, and forbidden talks between basal ganglia- and hippocampal-based memory systems

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This research was partly supported by CNPq, CAPES.

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Running Title: Reversing a memory deficit in a rat model of Parkinson's disease

ABSTRACT

The bilateral intranigral infusion of 1 μmol 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in adult male Wistar rats caused a specific and partial loss of substantia nigra pars compacta (SNpc) dopaminergic neurons, a partial depletion of striatal dopamine (DA), and a deficit in learning the cued version of the water maze. This deficit was reversed by pretraining the SNpc-lesioned rats in the spatial version of the water maze. Lesion of the SNpc did not affect learning of the spatial version of the water maze. Pretraining non-lesioned rats in the cued version also improved their performance in the spatial version. These results suggest that hippocampal and nigrostriatal memory systems can work independently but can share information and mutually compensate learning if one system is damaged. This interpretation encourages cognitive training to compensate for memory deficits in Parkinson's disease patients.

Key Words: substantia nigra pars compacta; dorsal striatum; memory; spatial learning; habit learning; Parkinson's disease.

The hippocampus and the basal ganglia seem to be critical components of memory systems that process memories of different nature. The hippocampus is proposed to process spatial/relational memories in which a large cluster of temporally related stimuli (or goals) are processed at the same time (1-4), while the basal ganglia are related to learning of associations of a single set of stimuli to a response (4-7). In humans these memory systems are related to declarative (explicit) and non-declarative (implicit) memories, respectively (1,3). There is consistent documentation of failure of declarative memory after hippocampal and related cortex damage due to traumatic lesions or Alzheimer's disease (3,8). More recently, some kinds of implicit memories, like habits (9) and other kinds of procedural memories (10,11) have been reported to fail in Parkinson's disease due to the progressive loss of nigrostriatal neurons that modulate the function of information processing by basal ganglia (12).

Many studies using rat hippocampal and basal ganglia-dependent memory tasks support the idea that these memory systems can operate independently. The spatial versions of the water maze (6,13) and 8 arm radial maze tasks (14-18) are examples of memory tasks affected by lesion or pharmacological manipulation of the hippocampus, but not of the caudoputamen or of the substantia nigra pars compacta (SNpc). Conversely, lesion or pharmacological inactivation of the dorsolateral striatum or of the SNpc, but not of the hippocampus, affects learning of the cued versions of the water maze (6,7) and the 8 arm radial maze (15).

In previous studies we showed that SNpc-lesioned rats show impaired learning of a version of the water maze task in which rats need to associate a hidden platform with a visual cue, i.e. a ball attached to it (6,19). On the other hand, this task was not affected by

lesion of the hippocampus (6,7,13). Conversely, hippocampus-lesioned rats show impaired learning of the spatial version of the water maze in which they need to associate multiple spatial distal cues to navigate and find a spatially fixed hidden platform (6,7,13). However, the lesion or blockade of the rat dorsolateral caudoputamen (20) or of the SNpc (6) did not affect learning of this task.

Are there crosstalks between the hippocampal and basal ganglia- based memory systems? Can the latter use the information stored independently of the former? The answer to this question can be found in the results presented in Fig. 1A: pretraining SNpc-lesioned animals in the spatial version reversed their deficit in learning the cued version of the of the water maze task. This means that, although learning the cued version is affected by damage of the SNpc, but not of the hippocampus, and learning the spatial version is affected by lesion of the hippocampus, but not of the SNpc (6), once the spatial version is learned, a crosstalk between these systems can share the stored information to compensate for the learning deficit due to damage in the SNpc.

What is the precise nature of the information shared by these systems? Can the SNpc-lesioned rats learn the spatial nature of the environment and use this information to compensate for a deficit in learning the cued version? The answer is no, since, as shown in Fig. 1B, pre-exposure of the animals to the water maze tank without the escape platform, i.e., simply letting them swim freely in the water maze pool for 8 days, was not sufficient to reverse the impairing effect of the SNpc-lesion on the learning of the cued version. Therefore, the stored information about the spatial nature of the environment is a forbidden talk between the hippocampal and the basal ganglia memory systems if the SNpc is damaged. However, as shown in Fig. 1C, animals with an intact SNpc obtain a smaller, but

significant, benefit after a simple exposure to the water maze pool. In their first trials they remained motionless for a time, perhaps just looking for the spatial distal cues of the water maze room, and then swan more directly to the cued platform. As expected, this behavior improved more the traveled distance than the latency to escape to the platform. Therefore, a kind of secret talk between these two learning and memory systems can occur but requires an intact SNpc.

Although the spatial version can be learned by SNpc-lesioned but not by hippocampus-lesioned animals (6,13), another crosstalk between these systems can be deduced from the results presented in Fig. 2A, which show that pretraining the animals in the cued version improved learning of the spatial version. However, SNpc-lesioned rats obtained a smaller benefit from the pretraining improving effect. Again, this benefit is more evident in traveled distance than in latency to escape to the platform, particularly in the first trial. The pretrained animals waste some time exploring the environment in the water maze room before going more directly to the platform placed in a spatially fixed position, indicating that some important information is learned during the pretraining session in the cued version with the participation of basal ganglia and that this information was transferred to the hippocampal system and helped the animal to learn the spatial version faster. This is another kind of secret talk between these two memory systems. Again, the information shared between these two systems is not the spatial nature of the environment since the simple exposure of non-lesioned rats to the pool (swimming sessions without the platform) did not improve their performance in the spatial version, as can be seen in Fig. 2B.

Besides learning the spatial nature of the water maze room, what other relevant aspect of the pretraing sessions would improve cued learning? It is possible that, when the animals are pretrained in the spatial task, they learn that there is an escape platform to be looked for in the maze and that it can be found in the center of one of the quadrants of the pool. This kind of secrete talk between these systems can share information useful to find the cued platform easily later on. Indeed, if the animal knows that there is a platform to be looked for, the probabilistic nature of the task decreases considerably. In naive rats, the first association of the platform position with the cue occurs by chance, while pre-trained animals know that the platform can be found in the center of one of the four quadrants, one of those positions matching the cue position. This interpretation is also consistent with the path followed by naive compared to pre-trained animals (see Fig. 1D). In the first trial of the cued version, pre-trained animals typically went directly to the position where they used to find the submersed platform while performing the spatial version. After that, they swam in a circle in the middle of the pool that matched the position of the center of the other quadrants. It was in one of these positions that they found the cue and the submersed escape platform. This navigation pattern resulted in a reduction of the thigmotaxis behavior observed in the pre-trained rats. Similar swimming paths are observed for rats pretrained in the cued version and later tested in the spatial version, as shown in Fig. 2C. However, as pointed out above, an intact SNpc is required for this benefit. So, if the nigrostriatal system is necessary for association of a single stimulus (cue) with a specific response, pretraining the animals in the spatial task simplifies this task enormously by reducing a large number of possible positions, virtually the number of dots of the size of the platform that can be contained in the maze field, to four possible positions. This simplification can

permit even animals with partial lesion of the system used to do this kind of computation to solve this problem. In this respect, learning a stimulus-response rule in a probabilistic way (i.e. habit learning) would be replaced by a cognitive learning by steps, each of them consisting in memorizing parts of a general and complex rule. In the specific case of the cued task, instead of learning by chance the rule – by approaching the cue you can escape to the platform - the animal sequentially learns: 1) an escape platform can be found in the pool; 2) it is placed in the center of one quadrant of the pool; 3) there is a white ball placed in the center of one quadrant of the pool; 4) go to the ball to find the escape platform. Note that just learning about the spatial nature of the water maze (without a platform, as shown in Fig. 2B) does not reduce the probability of solving the maze problem in this way.

Basal ganglia functioning is highly modulated by firing of nigral dopaminergic neurons (21). The dramatic effect of the dopaminergic nigral cell loss on motor and some cognitive functions can be observed in PD patients. It is exactly the loss of this kind of modulation that is produced by the intranigral administration of MPTP into the rat substantia nigra, as shown in Fig. 3 and 4. Some studies propose that mesencephalic dopaminergic neurons fire to introduce an error signal when a behavior is not rewarded as expected (22). Perhaps the function of the nigrostriatal pathway is to signal situations in which the animal needs to adjust its behavior to be better rewarded in a trial and error way. If so, during Pavlovian or instrumental (stimulus-response) learning, the mesencephalic dopaminergic neurons are activated by the conditioned stimuli (23).

The extrapolation of the present results to the therapy of Parkinson's disease patients suggest that habit learning, affected by the disease, would be improved by replacing the way patients normally learn these habit tasks. At least for early phase

Parkinson's disease patients whose hippocampal cortex is preserved, automatic learning of many motor tasks such as posture maintenance, walking, rising from bed, etc, would be relearned based on memorizing the rules of how to execute the movements, step-by-step, in a conscious way with active participation of the hippocampus. An example of a successful attempt to test this practice was reported by Piemonte and Xavier (24) who were able to help akinetic Parkinson's disease patients improve their daily life activities after memorizing a sequence of sub-components of movements that constituted the task. Similarly, our results suggest that demented patients with a compromised hippocampal memory function would benefit from learning some tasks, like going from one room to the other in their house, by habit learning that would consist of automating this learning by a repetitive association of single cues with the target place.

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FIGURE LEGENDS

Fig. 1: Effect of pretraining rats in the spatial version of the Morris water maze task on learning of the cued version of the water maze. Values are expressed as mean ± SEM latency and distance traveled to escape to a cued platform during 3 training days, with 4 consecutive trials per day. The values for the individual trials were averaged by day. (A) Rats were pretrained to find a hidden platform kept in the same position (spatial version) for 8 days, 4 trials per day, and then were tested in a cued version in which they have to find a platform cued by a white ball. The platform position was changed in each new trial. Three-way ANOVA showed significant effects ($P < 0.05$) for lesion, pretraining, and training day factors, both for latency and traveled distance dependent variables. A significant increase in swimming speed was observed only for training day factor, but not for lesion or pretraining factors. (B) Rats were pre-exposed to the pool without the escape platform (free swimming) for 8 days, 4 x 1-min session per day, and then were tested in the cued version. Two-way ANOVA showed significant effects for lesion and training day factors, both for latency and traveled distance dependent variables. (C) Non-operated rats were pre-exposed to the pool as in "B" and compared to naive (non-exposed) animals in a subsequent test in the cued version. Two-way ANOVA showed significant effects for pre-exposure to the pool and training day factors, both for latency and traveled distance dependent variables. (D) Typical swimming path of the first trial of animals of "A" in the cued test. (E) Typical swimming path of the last trial of animals of "A" on the last training day in the cued test. SNpc = rats with a lesion in the substantia nigra pars compacta induced

by the 1 μmol MPTP; Sham = sham-operated rats (n = 7-10 each). * $P < 0.05$ compared to the sham, not pre-trained group (post hoc Duncan test).

Fig. 2: Effect of pretraining rats in the cued version of the water maze on learning of the spatial version of the water maze task. Values are expressed as mean ± SEM latency to escape to a cued platform during 3 training days, with 4 consecutive trials per day. Escape latencies for the individual trials were averaged by day. (A) Rats were pretrained to find a platform cued by a white ball that changed position every new trial (cued version) for 8 days, 4 trials per day, and then were tested in the spatial version in which they had to find a hidden platform kept in the same position. (B) Non-operated rats were pre-exposed to the pool without the escape platform (free swimming) for 8 days, 4 x 1-min session per day, and compared to naive (non-exposed) animals in a subsequent test in the spatial version conducted as described in (A). SNpc-lesioned = rats with a lesion in the substantia nigra pars compact induced by 1 μmol MPTP; Sham = sham-operated rats. Naive = non-operated rats not pre-exposed to the maze. * $P < 0.05$ compared to the sham, not pre-trained group (Duncan test after two-way ANOVA).

Fig. 3: Brightfield photomicrographs of a tyrosine hydroxylase- immunostained section illustrating the appearance of a control (A) and an MPTP SNpc-lesioned rat (B). SNCd =

Substantia Nigra, compact, dorsal part; SNCv =Substantia Nigra, compact, ventral part; cp - cerebral peduncle.

Fig. 4: Effect of the administration of 1 μmol MPTP into the rat SNpc on cerebral levels of monoamines assayed by reverse-phase high performance liquid chromatography with electrochemical detection. The bars represent the mean ± SEM (n = 8 each). DA = dopamine; DOPAC = 3,4-dihydroxyphenylacetic acid; HVA = homovallinic acid; 5-HT = serotonin; 5-HIAA = 5-hydroxy-indoleacetic acid; NE = norepinephrine. * $P < 0.05$, compared to the sham-operated group, Student t-test.

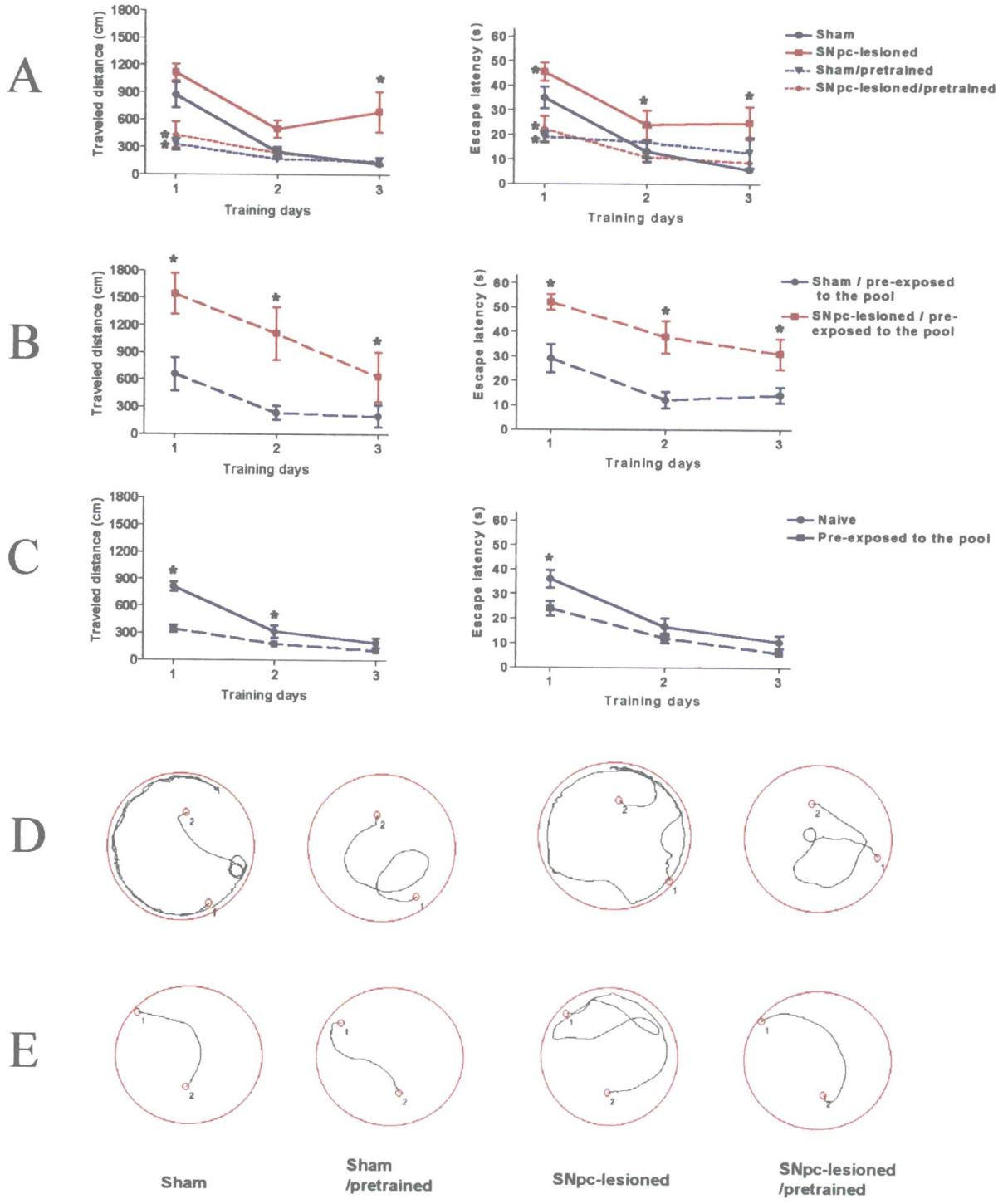

A
Traveled distance (cm)
Training days
Escape latency (s)
Training days
Sham
SNpc-lesioned
Sham/pretrained
SNpc-lesioned/pretrained
B
Traveled distance (cm)
Training days
Escape latency (s)
Training days
Sham / pre-exposed to the pool
SNpc-lesioned / pre-exposed to the pool
C
Traveled distance (cm)
Training days
Escape latency (s)
Training days
Naive
Pre-exposed to the pool
D
E
Sham
Sham /pretrained
SNpc-lesioned
SNpc-lesioned /pretrained

Figure 1

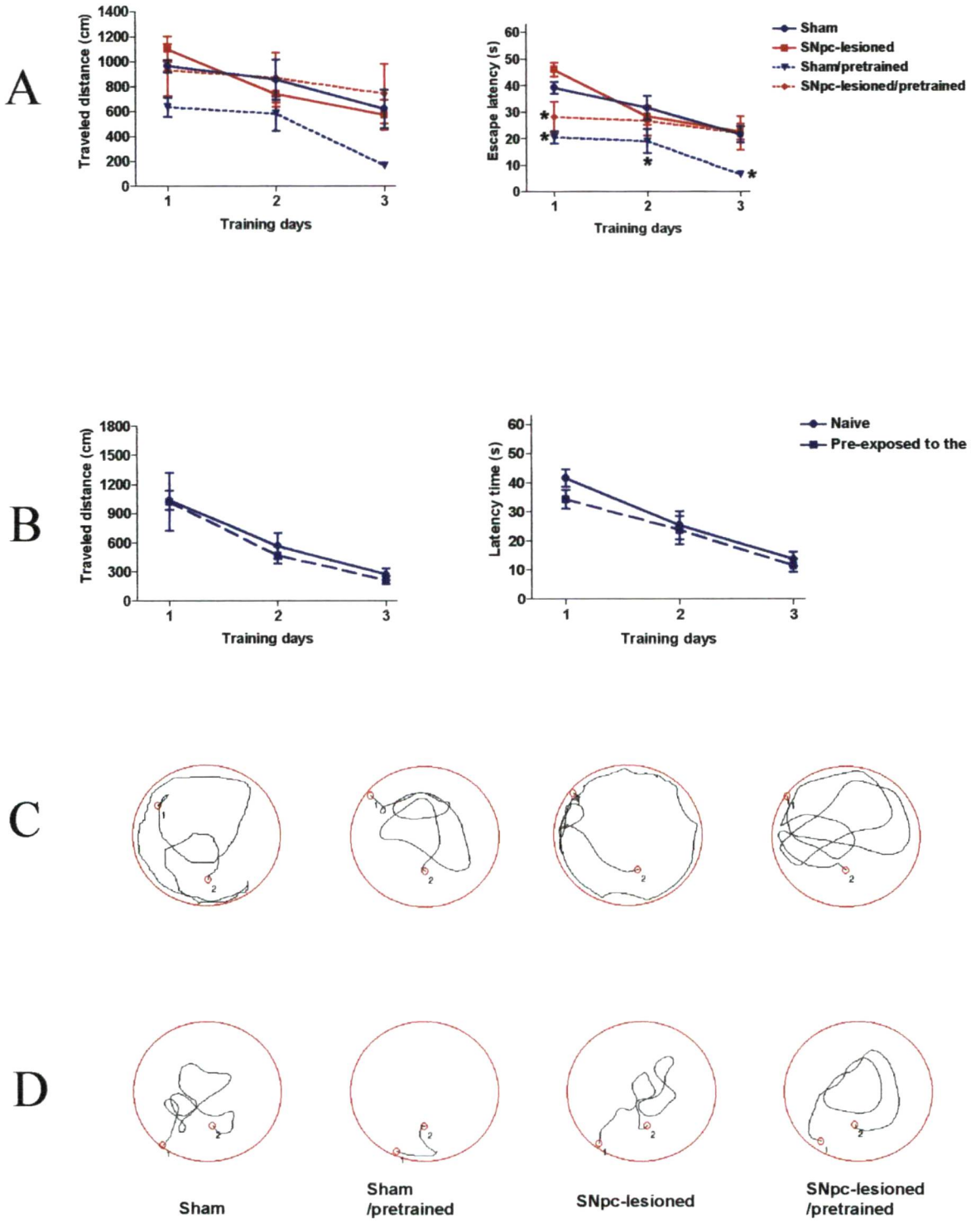
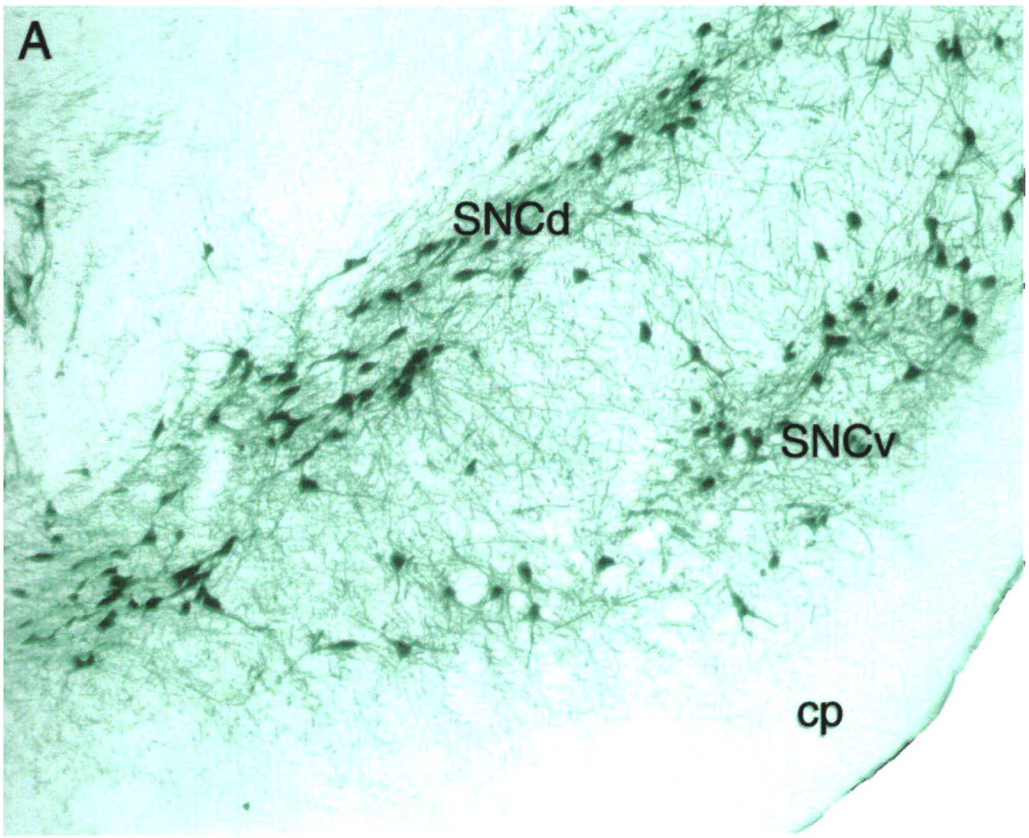
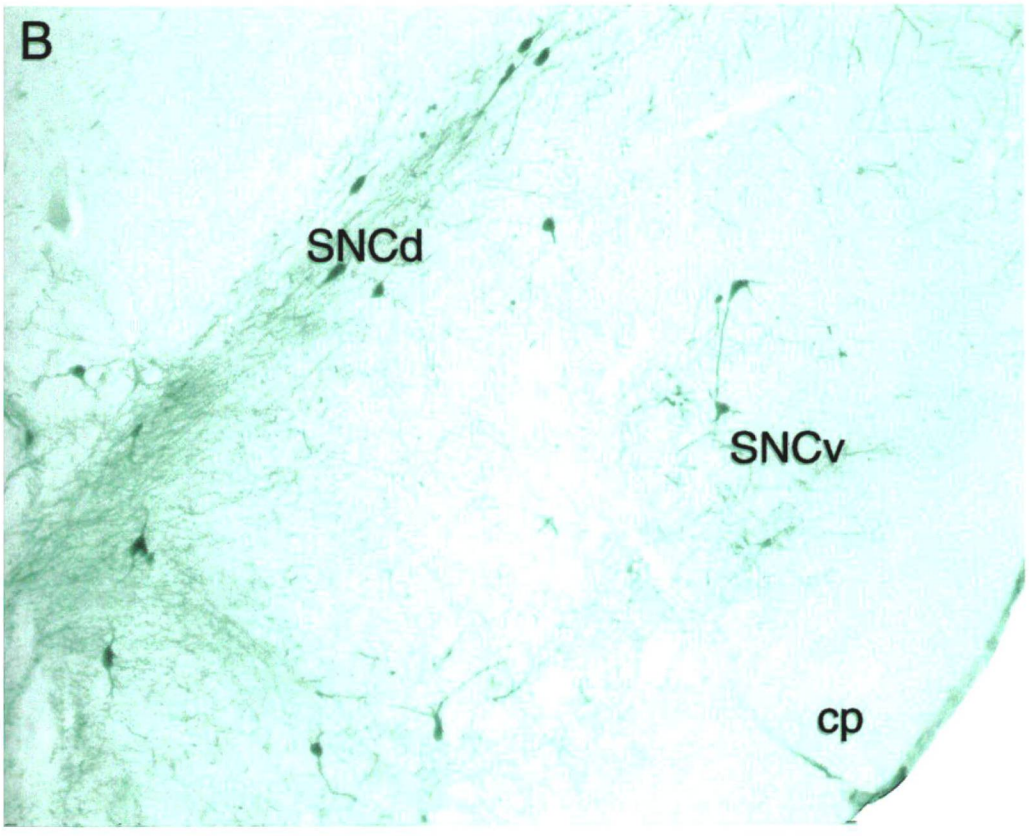

A
Traveled distance (cm)
1400
1200
1000
800
600
400
200
0
1
2
3
Training days
Escape latency (s)
60
50
40
30
20
10
0
*
Sham
SNpc-lesioned
Sham/pretrained
SNpc-lesioned/pretrained
B
Traveled distance (cm)
1800
1500
1200
900
600
300
Latency time (s)
Naive
Pre-exposed to the pool
C
D
Sham
Sham /pretrained
SNpc-lesioned
SNpc-lesioned /pretrained

Figure 2


A
SNCd
SNCv
cp


B
SNCd
SNCv
cp

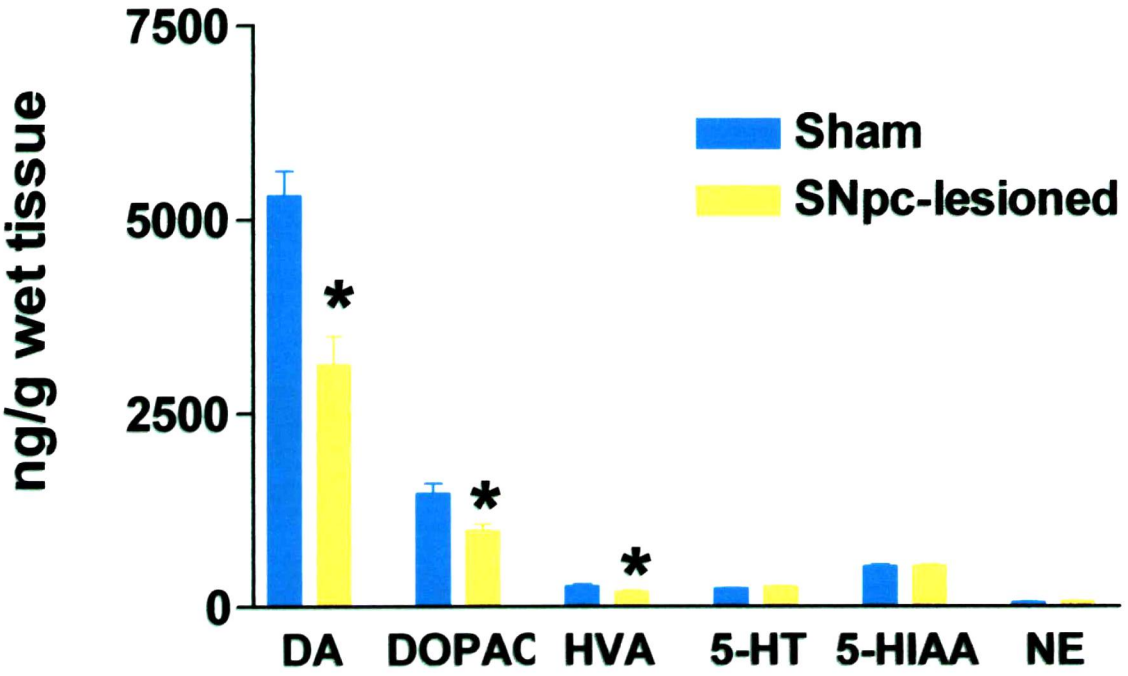

7500
5000
2500
0
ng/g wet tissue
Sham
SNpc-lesioned
*
*
*
DA
DOPAC
HVA
5-HT
5-HIAA
NE

Figure 4

Supplementary Material

Materials and Methods

Animals

Male Wistar rats from our own breeding stock weighing 280-320 g at the beginning of the experiments were used. The animals were maintained in a temperature-controlled room (22 ± 2 °C) on a 12/12-h dark/light cycle (lights on 07:00 a.m.) with food and water available *ad libitum*.

Lesion of the substantia nigra pars compacta

Twenty-one days before the beginning of the behavioral experiments, animals of the lesioned group received atropine sulfate (0.4 mg/kg, i.p.) to suppress salivation, penicillin G-procaine (20,000 U in 0.1 ml, i.m.), 3 injections of 120 mg/kg acetaldehyde (i.p., 10 min before and 30 and 60 min after the beginning of surgery), and were anesthetized with 40 mg/kg sodium thiopental (i.p.). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine HCl (MPTP, Sigma, 0.5 µmol, 1 µl in saline, 0.33 µl/min) was bilaterally infused through a 30-gauge needle according to the following coordinates of the Paxinos and Watson Atlas (1986) : anteroposterior (AP), -5.0 mm from bregma; mediolateral (ML), ± 2.1 mm from midline; dorsoventral (DV), -7.7 mm from the skull. Sham-operated animals were submitted to the same procedure but 1 µl saline was infused into the SNpc instead of MPTP.

Behavioral Procedures

All the behavioral experiments were conducted between 07:00 and 13:00 h. The animals were submitted to two different versions of the water maze task or to a simple exposure to the maze without the platform (a swimming session), all conducted in a round tank, 170 cm in diameter and 40 cm deep, filled with water. The water temperature was maintained at 22°C. Several distal visual cues were placed on the walls of the water maze room. During the experiments, the tank was videotaped and the latency to reach the escape platform was measured.

The spatial version consisted of training the animals for various consecutive days, 4 consecutive trials per day, during which the animals were left in the tank facing the wall and allowed to swim freely to a transparent acrylic escape platform (11 x 14 cm) submersed 2 cm under the water surface, placed in the center of one of the quadrants of the tank. The platform position was kept constant throughout the training days. The initial position in which the animal was left in the tank varied among trials in a pseudorandom way. If the animal did not find the platform during a period of 60 s it was gently guided to it. Then, it was allowed to remain on the platform for 20 s and removed from the tank for 30 s before being placed in the next initial starting position in the tank.

The cued version of the water maze task was similar to the previous experimental procedure, except that the position of the escape platform was cued by a 7-cm diameter white ball attached to the top of the platform and protruding above the water. Furthermore, the position of the platform was always changed in each trial of the day.

The session of *pre-exposure* of the animals to the water maze pool consisted simply of letting the them swim freely in the water maze tank without the escape platform for 4

consecutive trials of 60 s each. After each trial the animals were removed from the tank for 30 s before being placed in the next initial starting position.

Lesion evaluation by histology and determination of striatal dopamine concentration

After the behavioral tests, the operated animals were sacrificed by decapitation and their dorsal striata were removed for the determination of DA concentration. The posterior part of the rat brain was preserved in formalin for 1 week and placed in 20% sucrose formalin 48 h before sectioning. Four series of 30 μm thick sections were cut on a sliding microtome in the frontal plane and collected from the caudal diencephalon to the caudal midbrain. One series was immunostained for tyrosine hydroxylase (TH) with a monoclonal antibody to TH raised in mice (1:5000 dilution; Incstar). The antigen-antibody complex was localized using a variation of the ABC system with a commercially available kit (ABC Elite kit, Vector Laboratories). Slides were then dehydrated and coverslipped with DPX. An adjacent series was stained with thionin to serve as a reference series for cytoarchitectural purposes.

The endogenous levels of DA were assayed by reverse-phase HPLC with electrochemical detection. The system consisted of a C18 reverse phase column (150 x 4.6 mm i.d.; 5 μm particle size, Varian), a 9080 electrochemical detector (Varian), a 9012Q pump (Varian), and an AI 200 autosampler (Rainin). The column was maintained inside a temperature-controlled oven (29°C, Varian) and the system was controlled with the Star software, version 5.3 (Varian). The oxidation potential was fixed at +0.85 V using a glass carbon work electrode. The tissue samples were homogenized in 0.1 M perchloric acid, 147

ng/ml 3,4-dihydroxybenzoic acid (DHBA, Sigma, used as internal standard) with a microultrasonic cell disrupter. After centrifugation at 10,000 g for 40 min, 20 µl of the supernatant was injected into the chromatograph. The mobile phase, used at a flow rate of 1 ml/min, was of the following composition: 7.16 g Na2HPO4.12H2O, 4.2 g citric acid, 0.04 g EDTA, 0.55 g octyl sodium sulfate, 100 ml methanol, and 800 ml water. The water was twice distilled and processed with a water purification system (Millipore). The pH was adjusted to 4.7. The peak heights of the internal standards were used to quantify the sample peaks.

Test schedules

Table 1 shows the groups and the order of the behavioral tests in each experiment. Experiment 1 was planned to test if pretraining the animals in the spatial version of the water maze would reverse the deficit in learning the cued version of the water maze observed in the SNpc-lesioned animals. In this experiment the animals were submitted to the cued version of the water maze for 3 consecutive days. Other sham-operated and SNpc-lesioned animals were pre-trained in the spatial version of the water maze for the 8 previous days and than submitted to the cued version for the 3 subsequent days (see results in Fig. 1A). Experiment 2 was planned to test if this impairment of the SNpc-lesioned rats in learning the cued version of the water maze would be reversed just by pre-exposing the animals to the maze without the escape platform. In this experiment other sham-operated and SNpc-lesioned animals were pre-exposed to the maze (pool) for the 8 previous days and than submitted to the cued version for the 3 subsequent days (see results in Fig. 1B).

Experiment 3 was planned to test if pre-exposure of non-operated animals to the pool would affect their learning of the cued task. In this experiment other rats were pre-exposed to the maze for the 8 previous days and their learning scores in the cued task on the 3 subsequent days were compared to the scores of naive animals (see results in Fig. 1C). Experiment 4 was planned to test if learning the cued task would affect learning of the spatial task and if the integrity of the SNpc would be necessary for it. In this experiment the scores for other sham-operated and SNpc-lesioned animals submitted to the spatial version of the water maze for 3 consecutive days were compared to the scores of sham-operated or SNpc-lesioned animals that were pre-trained in the cued version of the water maze for the 8 previous days (see results in Fig. 2A). Experiment 5 was planned to test if the simple pre-exposure of non-operated animals to the pool would affect their learning of the spatial task. In this experiment other non-operated animals were pre-exposed to the pool for 5 days and their learning scores in the spatial task on the 3 subsequent days were compared to the scores of naive animals (see results in Fig. 2B).

Statistical analysis

Differences between groups in dopamine levels were analyzed by the Student t-test. Escape latencies and traveled distances for the individual trials were averaged by day and analyzed separately by three-way ANOVA with repeated measures (session day) followed by the Duncan test and were considered to be statistically significant when $P < 0.05$.

References and Notes

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CONCLUSÕES

Os resultados deste estudo sugerem que os sistemas de memória que integram o hipocampo e a via nigroestriatal são responsáveis por mediar diferentes tipos de memória (espacial ou estímulo resposta, respectivamente). Embora dissociados, estes dois sistemas podem compartilhar informações para tentar compensar uma lesão da via nigroestriatal durante o processo de aprendizagem.

Uma extrapolação dos resultados obtidos neste estudo, sugere que muitas das habilidades aprendidas na vida cotidiana são prejudicadas pela Doença de Parkinson. Este déficit poderia ser compensado pelo aprendizado consciente dessas tarefas, uma vez que em estágios iniciais da doença, o córtex e o hipocampo estão preservados. Deste modo, se o sistema nigroestriatal é importante na associação de um único estímulo a uma resposta específica, a evocação consciente destas tarefas pelo hipocampo poderia ajudar a simplificar este aprendizado.