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MEDIAÇÃO DAS VIAS DOPAMINÉRGICAS MESO-ESTRIATAIS NO COMPORTAMENTO DE ESCOLHA DE AÇÕES MOTORAS E NA FORMAÇÃO DE MEMÓRIAS DE PROCEDIMENTO

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

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CURITIBA

Julho de 2009

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

Ao meu marido, Ricardo, pelo incentivo, pela dedicação, por ser meu companheiro, por estar sempre presente. Te amo!!!

Aos meus pais, Luiz e Idalina, pela excelente formação, por toda dedicação, por terem me ensinado todos os valores e principalmente por sempre me apoiarem em minhas decisões. Amo vocês demais!!!

À minha única irmã, Samantha, você é maravilhosa, tenho muito orgulho por ser sua irmã, eu admiro demais o seu trabalho e você como mãe. Ao meu cunhado, Humberto, pelo apoio e conselhos.

Ao meu lindo sobrinho, Henri Gabriel. A tia ama você demais!!!

MAIS UMA VEZ, ESTA VITÓRIA É NOSSA!!!

AGRADECIMENTOS

A Deus e a Nossa Senhora, a quem eu sempre busquei conforto nos momentos difíceis.

Ao Ricardo, o meu amorzinho, nós finalmente estamos casados e iniciando uma vida juntos, que seja repleta de amor, felicidade e compreensão como foi toda a nossa longa trajetória de namoro. Eu agradeço pela paciência, por você estar sempre do meu lado, por ser meu amigo, meu amor, meu marido...

Aos meus pais, irmã, cunhado, sobrinho, eu sei que vocês sempre estarão presentes. Eu sou muito grata por todo o apoio recebido.

Ao meu orientador, Prof. Dr. Cláudio da Cunha, agradeço todo o incentivo e dedicação. Muito obrigada pela confiança depositada em mim e por todas as oportunidades que o senhor tornou real durante todo o período em que trabalhei em seu laboratório. Muito obrigada!

A Universidade Paranaense pelo apoio nesta etapa final de realização desta tese. Muito obrigada!!!

Á Prof. Dr. Maria de Fátima da Costa Almeida, muito obrigada pela sua amizade, por torcer por mim e estar presente em mais esta jornada.

Ao Prof. Dr. Newton Sabino Canteras pela preparação do material imunoistoquímico, orientação sobre a análise histológica e colaboração científica.

Ao Prof. Dr. Marcos Lira Brandão por ter colaborado neste trabalho.

Aos professores Dr. Phillip Win e Dr. Michael Frank por terem concedido parte das drogas utilizadas neste projeto.

Aos amigos de laboratório que vou lembrar por toda minha vida, Marcelinho, Léa, Edmar, Lucélia, Mariza, Patrícia, Suelen, Fernanda, Adriana, Jamile, Bruno, Flávia. Obrigada pelo apoio e amizade!!!

Á todos os alunos da pós-graduação por proporcionarem um ambiente de trabalho agradável, com cooperatividade, respeito e companheirismo.

Á farmacêutica do Laboratório Central, Silvia, minha amiga, você nunca negou uma ajuda por mais simples que fosse, e ainda, sempre me ouviu e me aconselhou. Muito obrigada!

Á professora de inglês, minha amiga e minha madrinha, Suzana, pelo bom humor, palavras de ajuda sempre na hora certa, apoio, incentivo, enfim, por torcer por mim.

Aos funcionários do departamento, Lindacir, Nair e Sr. Jorge.

Ao secretário do Departamento de Farmacologia, Erich, por todo trabalho e dedicação aos alunos da pós-graduação.

Á CAPES pelo apoio financeiro.

Agradeço todos vocês.
Muito obrigada!!!

Não se pode ensinar tudo a alguém.

Pode-se apenas ajuda-lo a encontrar por si mesmo".

(Galileu Galilei)

SUMÁRIO

LISTA DE ABREVIATURAS

6-OHDA	-	6-Hidroxidopamina
CPF	-	Córtex pré-frontal
CS	-	Estímulo condicionado (do inglês, *conditioned stimulus)*
DA	-	Dopamina
DAT	-	Transportador de dopamina (do inglês, *dopamine transporter)*
DP	-	Doença de Parkinson
GPe	-	Globo pálido externo
GPi	-	Globo pálido interno
LTD	-	Depressão de longa duração (do inglês, *long-term depression*)
LTP	-	Potenciação de longa duração (do inglês, *long-term potention*)
MPTP	-	1-metil-4-fenil-1,2,3,6-tetrahidropiridina
NAc	-	Núcleo Accumbens
O	-	Conseqüência (do inglês, *outcome)*
SEA	-	Sistema encefálico aversivo
SHAM	-	O mesmo que simulado, falsamente lesado
SNC	-	Sistema Nervoso Central
SNc	-	Substância negra parte compacta
SNr	-	Substância negra parte reticulata
S-R	-	Memória estímulo-resposta (do inglês, *stimulus-response)*
US	-	Estímulo incondicionado (do inglês, *unconditioned stimulus)*
VTA	-	Área tegmental ventral (do inglês, *ventral tegmental area)*

RESUMO

Nesta tese propomos explicações de como drogas dopaminérgicas modulam processsos de tomada de decisão, aprendizagem e memória de acordo com o modelo do mosaico dos espelhos quebrados. Este modelo atribui um papel crítico à dopamina na plasticidade sináptica córtico-estriatal, necessária para a aprendizagem instrumental e para a tomada de decisões sobre ações motoras. Mais especificamente mostramos que antagonistas dos receptores D1 prejudicam o aprendizado e a memória da tarefa de esquiva ativa de duas vias. De acordo com nosso modelo a dopamina atua ativando a via direta (do estriado ao globo pálido interno e substância negra reticulata) para liberar a resposta de cruzamento. Ainda segundo este modelo, a dopamina atua de tal forma porque durante a aprendizagem ela propiciou o fortalecimento das sinapses entre neurônios corticais que representam o estímulo (luz) e a resposta motora, neurônios estes que convergem para os mesmos neurônios estriatais. Nossos resultados mostraram também que diferentes partes do estriado exercem funções diferentes na aprendizagem: o núcleo accumbens participa de um aprendizado rápido e o estriado dorsolateral de um aprendizado lento da associação estímulo-resposta. Mostramos também que animais com lesão unilateral por MPTP apresentaram rotações ipsiversivas quando desafiados com agonistas dopaminérgicos diretos e indiretas e também várias drogas usadas no tratamento da doença de Parkinson. De acordo com nosso modelo estes animais apresentam o comportamento rotatório porque perdem a capacidade de iniciar ações para o lado contrário à lesão. Estes experimentos validaram a proposta de ratos com lesão unilateral por MPTP como um modelo animal útil para testar drogas da fase inicial da doença de Parkinson.

ABSTRACT

This thesis explains how dopaminergic drugs modulate processes of decision-making, learning, and memory, according to the Model of the Mosaic of Broken Mirrors. According to this model, the dopamine plays a critic role in the corticostriatal synaptic plasticity that supports instrumental learning and decision-making processes. More specifically, we showed that a D1-like dopamine receptor antagonist impaired the learning of the two-way active avoidance task. According to our Model, the dopamine activates the direct pathway (from the striatum to the substantia nigra pars reticulata/intrapeduncular nucleus) that releases the crossing response. In addition, it proposes that the dopamine causes the strengthening of the synapses between the cortical neurons that represent the stimulus (light) and the motor response and that converge to the same striatal neurons. Our results also showed that different parts of the striatum play different roles in learning, the nucleus accumbens eliciting a fast learning and the dorsolateral striatum eliciting a slow learning of the stimulus-response association. We also showed that rats with a unilateral lesion of the substantia nigra pars compacta (SNc) induced by MPTP responded with ipsiversive turns to a challenge with both direct and undirect dopamine receptor agonists and other drugs used to treat the early phase of Parkinson's disease (PD). This result is explained by our Model as a loss of ability to inicitate actions directed to the side contralateral to the lesion. This experiment validates the rats with unilateral lesion of the SNc induced by MPTP as a model for the screening of drugs used to treat the early phase of PD.

1 INTRODUÇÃO

Diz um velho ditado que “a vida é feita de escolhas”. Viver em um mundo que está em constante mudança é um desafio que impõe aos animais a escolha de ações baseada na expectativa de suas conseqüências. Para tanto se fez necessário o desenvolvimento de sistemas neurais especializados na tomada de decisões e no aprendizado que leve à formação de memórias das ações tomadas no passado e de suas conseqüências. Memórias sobre como fazer a coisa certa na hora certa.

É baseado nestas **memórias de procedimentos** que os animais tomam decisões que resultam em conseqüências reforçadoras e evitam ações com conseqüências aversivas. Este tipo de aprendizagem é chamado de **instrumental** ou **operante** (Domjan e Burkhard, 1982; Eichenbaum, 2008). Ele permite que o indivíduo escolha uma ação motora que lhe permita atuar sobre seu ambiente de forma a produzir uma conseqüência. A escolha destas ações está baseada na presença de deteminados **estímulos** ambientais que sinaliza qual é a **resposta motora** ou ação apropriada. Por esta razão, as memórias resultantes do aprendizado instrumental são chamadas de memórias estímulo-resposta (S-R, do inglês *stimulus-response*) (White e Mcdonald, 2002). Em situações onde as conseqüências (O, do inglês *outcome*) de uma resposta a um estímulo não mudam, a repetição deste pareamento S-R-O leva a uma automação da resposta, de forma que o indivíduo a escolhe e executa de forma automática. Este tipo de memória é chamado de **hábito S-R**. Os aspectos chave do comportamento habitual incluem: (a) aprendizado lento, (b) relativamente estável no tempo, exceto sob condições de extinção, (c) pequena transferência entre os sistemas efetores e o contexto comportamental, e (d) indisponível aos mecanismos da consciência (Wise, 1996). Em função da forma automática com que estas respostas são escolhidas e executadas, as memórias para a escolha destes procedimentos e da sua execução são também chamadas de **memória implícita** ou **não-declarativas** (Squire, 2004).

Muitos psicólogos contemporâneos discriminam os comportamentos resultantes do aprendizado instrumental em ações direcionadas a um objetivo ou **R-O** e **hábitos S-R** (Yin e Knowlton, 2006; Horvitz, 2009). O comportamento R-O é controdado por sua conseqüência. Os estímulos que, quando apresentados de forma contingente a uma resposta, aumentam sua freqüência são chamados de reforçadores positivos. Aqueles que diminuem a freqüência da responta são chamados de punidores. Nas situações de reforço negativo, a remoção de um estímulo punidor ou aversivo na contingência de uma resposta resulta no aumento de sua freqüência (Domjan e Burkhard, 1982; Eichenbaum, 2008). Já nos hábitos S-R o comportamento é controlado pelo estímulo neutro que o precede, sendo mais resistente à extinção por desvalorização do reforço, tal como em situações de saciedade e reforço alimentar (Yin e Knowlton, 2006). Porém o reforço e punição têm um papel determinante na aprendizagem tanto do comportamento R-O como dos hábitos S-R. Por esta razão autores como Norman White e Mark Packard consideram todas as memórias resultantes do comportamento instrumental de hábitos S-R, na mesma concepção dos primeiros teóricos do aprendizado instrumental, tais como Clark Hull (Packard e Mcgaugh, 1992; Salmon e Butters, 1995; White e Mcdonald, 2002).

Em muitas situações os animais podem antecipar a iminência da apresentação de um estímulo reforçador ou aversivo (US, do inglês *unconditioned stimulus*) associando-o a outro estímulo neutro que o precede (CS, do inglês *conditined stimulus*). Este tipo de aprendizagem é chamado de **condicionamento clássico** ou **Pavloviano**, em homenagem ao pesquisador russo que o descobriu (Pavlov, 1927; Schultz, 2006). A memória resultante do condicionamento clássico também é considerada como implícita ou não-declarativa, tendo em vista que seu aprendizado não é necessariamente um processo consciente (Squire, 2004). Os US apresentados tanto no condicionamente clássico como no instrumental geram também uma resposta emocional em função da recompensa ou

punição. Por esta razão, seu aprendizado e a evocação de suas memórias são também estudados dentro do contexto de **comportamentos motivados**.

Entre os comportamentos motivados altamente adaptativos estão as reações de defesa. O condicionamento clássico e os hábitos S-R constituem recursos preciosos com os quais os animais conseguem prever situações de risco. Dessa forma, o animal pode antecipar reações de defesa, tais como a **imobilidade**, a **luta**, **fuga** e a **esquiva**.

Muitas vezes, em situações em que o perigo está distante, tal como na presença de um predador, a melhor resposta é a imobilidade. Com a sua aproximação, a melhor resposta pode ser a fuga. Além da presença do predador, outros estímulos aversivos que põem em risco a integridade física, tais como os que causam dor (choque elétrico, objetos cortantes, altas temperaturas, etc.), também desencadeiam reações de medo (Brandão e Graeff, 2006).

Um modelo animal que é muito usado para estudar comportamentos motivados que envolvem os condicionamentos clássico e instrumental é a esquiva ativa de duas vias. Nele o animal aprende a emitir uma resposta de esquiva (resposta condicionada) ou a antecipar a resposta de fuga (resposta incondicionada) sob a apresentação de um CS, o qual é tipicamente um sinal auditivo ou visual. Este estímulo é sempre pareado a um US, usualmente um choque nas patas. Quando a resposta ao CS não é emitida no tempo estipulado, o US é então aplicado. Quando a resposta ao CS é emitida, tanto o CS quanto o US são finalizados. O animal aprende a antecipar a fuga do choque por condicionamento clássico e a se esquivar dele por condicionamento instrumental motivado por reforço negativo (Wadenberg e Hicks, 1999).

A utilização de técnicas de lesões cerebrais e de infusão intra-cerebral de drogas que atuam sobre determinados sistemas de neurotransmissores vem permitindo identificar estruturas e conexões cerebrais envolvidas no comportamento motivado, tomadas de decisão e na formação das memórias de procedimento. O trabalho de muitos pesquisadores contemporâneos,

entre os quais nos incluímos, sugere fortemente que os gânglios da base são a solução apresentada pela evolução para a aprendizagem e a escolha de ações motoras apropriadas para as diversas demandas do ambiente (O'doherty, 2004; Balleine, Delgado *et al.*, 2007; Nicola, 2007; Redgrave, Gurney *et al.*, 2008; Da Cunha, C., Wietzikoski, E.C. *et al.*, 2009).

Os gânglios da base são formados por vários núcleos localizados abaixo na base do cérebro. Entre eles encontram-se o núcleo estriado, formado pelo caudado-putâmen (neoestriado ou estriado dorsal), núcleo accumbens (NAc) e tubérculo olfatório (estriado ventral), o globo pálido (externo (GPE) e interno (GPI)), substância negra (reticulada (SNr e compacta (SNc)) e o núcleo subtalâmico.

O estriado é a principal porta de entrada dos gânglios da base, recebendo aferências de todo o neocórtex e também de estruturas subcorticais muitas das quais envolvidas também em comportamentos motivados e emoções, tais como a amígdala e os colículos superior e inferior (Silveira, Sandner *et al.*, 1993; Zanoveli, Ferreira-Netto *et al.*, 2007; Redgrave, Gurney *et al.*, 2008). O processamento dessas informações se inicia no estriado é comunicado pelos gânglios da base a estruturas efetoras, de forma a desinibir as respostas adequadas. Entre os efetores estão o córtex frontal e as estruturas do sistema encefálico aversivo (SEA), tais como a substância cinzenta periaquedutal dorsal, o hipotálamo medial e também regiões autonômicas do tronco encefálico (Nicola, 2007). A desinibição desses efetores determina as características motoras, endócrinas e vegetativas das reações de defesa e também da expressão de memórias de procedimento.

Do estriado partem duas vias de saída para o tálamo: a via direta e a via indireta. Na via direta, os neurônios que saem do estriado, liberam o neurotransmissor inibitório ácido gama-aminobutírico e o neuropeptídeo substância P que vão inibir os neurônios GABAérgicos no GPi e na SNr, liberando desta forma a ação motora escolhida. Na via indireta, o estriado projeta neurônios contendo GABA e encefalinas que inibirão o GPe. O GPe

ao ser inibido, faz com que o núcleo subtalâmico libere glutamato no GPi e SNr excitando os neurônios GABAérgicos que vão inibir os neurônios do tálamo, inibindo a iniciação de determinas ações (Alexander, Delong *et al.*, 1986; Delong e Wichmann, 2007).

As sinapses córtico-estriatais são moduladas por neurônios dopaminérgicos da SNc. A alça frontocorticoestriatal é importante para a escolha de respostas motoras frente a um estímulo. A integridade da via nigroestriatal é crítica tanto para a escolha de ações motoras como para o seu aprendizado, pois a dopamina (DA) estimula a via direta (através de receptores da família D1, veja abaixo) e inibe a via indireta (através de receptores da família D2). Desta forma a DA exerce um papel permissivo na escolha, iniciação e aprendizagem de ações motoras (Da Cunha, Wietzikoski et al., 2003; Da Cunha, Silva et al., 2006; Da Cunha, Wietzikoski et al., 2007).

Um desequilíbrio nesse sistema é a causa de algumas patologias neurológicas, entre elas a doença de Parkinson (DP) caracterizada pela perda progressiva dos neurônios dopaminérgicos da SNc.

A DA, o neurotransmissor dos neurônios nigroestriatais, foi descoberta há mais de 50 anos atrás por Arvid Carlsson e seu papel no SNC tem sido estudado intensamente (Iversen e Iversen, 2007; Da Cunha, 2009). Assim como a noradrenalina e a adrenalina, a DA é um neurotransmissor que pertence à família das catecolaminas. Ela é sintetizada a partir do aminoácido tirosina pelas enzimas tirosina hidroxilase e DOPA descarboxilase (Siegel, Albers *et al.*, 2006).

Os receptores dopaminérgicos são classificados em duas famílias: tipo-D1 e tipo-D2. A família D1 inclui os receptores D1 e D5, e a família D2 é composta pelos receptores D2 (incluindo as isoformas curta e longa, D2S e D2L), D3 e D4 (Missale, Nash *et al.*, 1998; Neve, Seamans *et al.*, 2004). Desses, os receptores D1, D2 e D4 são expressos no estriado. Seus efeitos celulares são mediados por proteínas G que controlam a produção de AMPc, sendo esta estimulada pelos receptores da famiília D1 e inibida pelos receptores da família D2 (Lichter, Barr *et al.*, 1993; Jaber, Robinson *et al.*,

1996; Grady, Mcintosh *et al.*, 2003; Siegel, Albers *et al.*, 2006). A DA exibe tanto ações excitatórias, mediadas pelos receptores tipo-D1 (embora haja exceções), como inibitórias, mediadas por receptores D2.

Os neurônios dopaminérgicos mesencefálicos liberam DA no estriado de forma tônica ou fásica (Goto, Otani *et al.*, 2007). Uma pequena quantidade de DA é liberada de forma tônica espontânea e continuamente por estes neurônios, estabelecendo um conteúdo basal de DA extrasináptica necessário para a escolha dos programas motores que já estão programados para ser deflagrados frente a determinado estímulos. A falta deste nível basal de DA é a causa da dificuldade de iniciar ações em pacientes portadores da DP (Olanow e Tatton, 1999).

Na DP e em outras patologias com disfunções dopaminérgicas, tais como no transtorno de hiperatividade com déficit de atenção e esquizofrenia ocorrem também déficis cognitivos (Dougherty, Bonab *et al.*, 1999; Abi-Dargham, Rodenhiser *et al.*, 2000; Ilgin, Senol *et al.*, 2001). Notavelmente, estes déficits cognitivos são similares àqueles observados em pacientes com lesões no córtex pré-frontal (CPF) (Willcutt, Brodsky *et al.*, 2005). Conseqüentemente, existe uma tendência na literatura em considerar que os déficits cognitivos em pacientes ocorrem devido a disfunções dopaminérgicas somente no CPF. Entretanto, existem evidências tanto em animais como em humanos de que disfunções dopaminérgicas nos gânglios da base podem ocasionar déficits cognitivos semelhantes com os observados no CPF (Rinne, Portin *et al.*, 2000; Da Cunha, Wietzikoski *et al.*, 2003; Frank, 2005; Da Cunha, Silva *et al.*, 2006).

O papel da DA nas disfunções motoras e cognitivas da DP tem sido extensivamente estudado em modelos animais. Entre eles, trabalhamos com os modelos da 6-hidroxidopamina (6-OHDA) e do 1-metil-4-fenil-1,2,3,6-tetrahidropiridina (MPTP) em ratos. Animais lesados bilateralmente com 6-OHDA demonstram os sinais motores da DP, entretanto, as lesões bilaterais não constituem um modelo comum (Cenci, Whishaw *et al.*, 2002). A 6-OHDA é usualmente injetada unilateralmente, constituindo um modelo de

hemiparkinsonismo, que é caracterizado por um comportamento motor assimétrico (rotatório) após a administração de drogas dopaminérgicas, devido a um desequilíbrio funcional entre o lado lesado e o não-lesionado (Ungerstedt e Arbuthnott, 1970; Ungerstedt, 1971; Betarbet, Sherer *et al.*, 2002). No contexto do comportamento de escolha, o comportamento rotatório pode ser visto como a escolha de virar para a direita ou para a esquerda. Na literatura dos modelos de DP, o comportamento rotatório de ratos é denominado de contraversivo (direcionado para o lado contrário à lesão) e ipsiversivo (para o lado da lesão). Os animais 6-OHDA perdem a capacidade de escolher virar para o lado lesado quando estimulados por agonistas dopaminérgicos (Schwarting e Huston, 1997).

O modelo de ratos com lesão da SNc pela toxina MPTP foi proposto pelo nosso grupo e é usado para o estudo de alterações cognitivas da DP (Da Cunha, Angelucci *et al.*, 2002; Da Cunha, Wietzikoski *et al.*, 2008). A validação farmacológica deste modelo para o estudo dos sinais motores da DP (dificuldade de iniciar ações para o lado contrário à lesão) é parte do trabalho desta tese (veja também em Da Cunha, Wietzikoski *et al.*, 2008). Para a validação do modelo do MPTP, testamos o efeito de agonistas dopaminérgicos diretos e indiretos (e também várias drogas usadas no tratamento desta doença) sobre o comportamento rotatório e comparamos os resultados com os obtidos em ratos com lesão unilateral por 6-OHDA. A 6-OHDA é citado na literatura como o “modelo-ouro” no teste de rotação.

O papel dos gânglios da base no aprendizado e escolha de ações motoras permanece enigmático, apesar de décadas de intensa investigação. Mas sua participação na formação de memórias de hábito S-R é uma hipótese antiga (Wise, 1996). Entre os trabalhos incluídos nesta tese está um onde propomos um modelo para explicar como os gânglios da base formam memórias de procedimentos que determinam o comportamento de escolha. Este modelo foi denominado de “Mosaico dos Espelhos Quebrados” (Da Cunha, C., Wietzikoski, E.C. et al., 2009). De acordo com esse modelo, projeções convergentes do córtex para neurônios estriatais formam unidades

sensoriomotoras no estriado, representado partes do corpo, objetos e locais próximos ao sujeito. Este modelo explica o aprendizado de associações S-R e R-O (memórias de procedimento) pelo fortalecimento das sinapses córtico-estriatais representando ações de partes do corpo em direção outra parte do corpo ou em direção a objetos ou do sujeito em direção a um lugar (Da Cunha, C., Wietzikoski, E.C. et al., 2009).

A liberação fásica de DA no estriado é crítica para o aprendizado dessas associações, tal como na tarefa da esquiva ativa de duas vias (Gevaerd, Miyoshi *et al.*, 2001; Gevaerd, Takahashi *et al.*, 2001; Da Cunha, Angelucci *et al.*, 2002) e na versão S-R do labirinto aquático de Morris (Miyoshi, Wietzikoski *et al.*, 2002; Da Cunha, Wietzikoski *et al.*, 2003; Da Cunha, Silva *et al.*, 2006; Da Cunha, Wietzikoski *et al.*, 2007). Pacientes com DP também apresentam déficits equivalentes na formação destas memórias de procedimento (Knowlton, Mangels *et al.*, 1996). Diversos trabalhos produziram modelos que simulam as oscilações nos níveis de DA durante a apresentação de estímulos e seus diferentes efeitos sobre a via direta e indireta dos gânglios da base (Frank, 2005; O'Reilly e Frank, 2006). A DA pode aumentar a freqüência do sinal via receptor D1 na via direta (Frank, 2005), ou seja, o efeito da estimulação do receptor D1 no estriado depende da excitabilidade do potencial de membrana do neurônio. A DA tem como função excitar neurônios com alto potencial de membrana (próximo do limiar para despolarização) enquanto é capaz de inibir aqueles neurônios com baixo potencial de membrana (hiperpolarizado) (Hernandez-Lopez, Tkatch *et al.*, 2000).

A liberação fásica de DA no estriado é uma condição necessária para que ocorram os fenômenos de plasticiadade sináptica necessária para fortalecer as sinapses córtico-estriatais ativas (Beninger, 1983; Di Filippo, Picconi *et al.*, 2009). Neurônios dopaminérgicos mesencefálicos da SNc e da área tegmentar ventral (VTA, do inglês *ventral tegmental area)* disparam de forma fásica na presença de estímulos desconhecidos e salientes, tais como estímulos que funcionam como reforçadores ou punidores de primeira (US)

ou segunda ordem (CS). (Beninger, 1983; Berridge, 2007; Di Filippo, Picconi *et al.*, 2009). Após a liberação fásica de DA, as sinapses córtico-estriatais nas unidades representando ao mesmo tempo a parte do corpo e os objetos envolvidos na ação (p.ex. patas (correr), grades do piso (choque), campainha (estímulo neutro) são reforçadas. Estas sinapses podem ser mais fortalecidas ainda quando a conseqüência da ação é reforçadora e prolonga a atividade fásica dos neurônios dopaminérgicos. Isto ocorre devido a projeções do CPF, dos córtices límbicos e da amígdala para a VTA e SNc (Oades e Halliday, 1987; Bacon e Totterdell, 2000; Georges e Aston-Jones, 2002; Paxinos, 2004).

Existem evidências de que a ativação fásica dos neurônios dopaminérgicos seja mediada pelo colículo superior (Comoli, Coizet et al., 2003). Fibras glutamatérgicas de outras regiões subcorticais, tais como o tegmento pontino, pode também contribuir para a resposta fásica dos neurônios da VTA e SNc (Omelchenko e Sesack, 2007). A SNc e VTA também recebem projeções do CPF, amígdala extendida e núcleos da rafe, mas é improvável que estas estruturas possam desencadear a resposta fásica dos neurônios dopaminérgicos, uma vez que elas respondem a estímulos salientes com uma latência maior (Oades e Halliday, 1987; Bacon e Totterdell, 2000; Georges e Aston-Jones, 2002; Paxinos, 2004). É mais provável que estas estruturas contribuam para sustentar o padrão de disparo dos neurônios dopaminérgicos quando o estímulo desencadeador é reforçador. A resposta fásica dos neurônios dopaminérgicos não apresenta esta latência curta quando o estímulo desencadeador é aversivo ou sinaliza um estímulo aversivo. Estímulos aversivos são processados pela habênula lateral que inibe a resposta fásica dos neurônios dopaminérgicos (Gao, Jeaugey *et al.*, 1990; Ji e Shepard, 2007; Matsumoto e Hikosaka, 2009). Os níveis baixos de DA favorecem a indução de depressão de longa duração (LTD, do inglês long-term depression) e de potenciação de longa duração (LTP, do inglês long-term potention) nas sinapses córtico-estriatais dos neurônios ativos (Di Filippo, Picconi et al., 2009). Esta LTD pode diminuir a

probabilidade de que esta ação seja apresentada no futuro, quando o sujeito se defrontar com estes mesmos estímulos (Schultz, 2007a).

Portanto, a resposta fásica dos neurônios dopaminérgicos pode ser considerada como um sinal de que as sinapses córtico-estriatais dos neurônios que representam o estímulo e a ação devem ser reforçadas. Esta resposta é deflagrada pelo estímulo saliente que indica que existe algo relevante e novo para ser aprendido. Se a resposta fásica dos neurônios dopaminérgico for prolongada por uma conseqüência reforçadora, a informação sobre a conseqüência também será associada à ação escolhida. Quando o estímulo sinalizar uma conseqüência já conhecida, a resposta fásica dos neurônios dopaminérgicos não ocorre e a memória da associação S-R não é alterada. Quando a conseqüência for aversiva, os neurônios dopaminérgicos serão inibidos e esta memória será enfraquecida.

Nos dias atuais, o estudo do papel dos gânglios da base nesses processos de aprendizagem e da seleção da melhor ação para cada situação ambiental (tomada de decisão) é uma das áreas mais estudadas da neurociência. Embora existam pontos de consenso, a questão do significado e função da liberação de DA no estriado é um dos pontos de maior controvérsia (Harper, 2006; Horvitz, 2006; Lekne e Tracey, 2006; Scott, Heitzeg *et al.*, 2006; Barbano e Cador, 2007; Schultz, 2007b; Redgrave, Gurney *et al.*, 2008). A sua liberação frente a um estímulo apetitivo é explicada como a representação do reforço para uns (Lekne e Tracey, 2006); como uma medida da discrepância entre a expectativa de reforço e sua contingência para outros (Schultz, 2007b); como uma sinalização da saliência de incentivo de estímulos ambientais (Berridge, 2007); ou simplesmente como uma sinalização de novidade (Redgrave, Gurney *et al.*, 2008).

As alterações na liberação de DA frente a estímulos aversivos são ainda mais controversas. Sabe-se que estímulos aversivos também desencadeiam uma resposta fásica da DA, mas estudos de microdiálise in vivo e de registro de célula unitária em animais acordados divergem sobre a

natureza inibitória ou excitatória dessa resposta (Nicola, 2007; Horvitz, 2009). Há até pouco tempo, acreditava-se que as alterações na liberação fásica de DA sinalizassem apenas reforço. Porém, estudos mais recentes mostram que a aplicação de um estímulo aversivo (como o choque nas patas) resulta em uma inibição da liberação de DA, seguida de sua liberação de forma exacerbada após a interrupção do estímulo (Redgrave, Gurney *et al.*, 2008). São também objeto de debate as diferenças funcionais entre o estriado dorsal e o NAc (Nicola, 2007). Embora o NAc seja parte do estriado ventral, ele era tradicionalmente relacionado ao aprendizado e aos mecanismos de abuso de drogas (Everitt e Robbins, 2005), enquanto o estriado dorsal era relacionado às funções motoras (Delong e Wichmann, 2007). Nos modelos atuais do envolvimento dos gânglios da base no aprendizado e na escolha de ações motoras, tal como no "mosaico dos espelhos quebrados", tanto o estriado dorsal como o NAc fazem o mesmo tipo de computação, diferindo mais no padrão de conexões de seus inputs corticais e subcorticais. Uma outra diferença, apontada recentemente por Wickens (2007), é que o estriado dorsal tem uma maior densidade da proteína que faz a recaptação de DA (DAT, do inglês *dopamine transporter*). Com isso, após a sua liberação fásica, o clearance da DA das fendas sinápticas seria mais acelerado no estriado dorsal que no NAc. Como a avaliação da valência do estímulo (como tendo propriedades de reforço ou punição) demora mais tempo que a latência da resposta fásica de DA (Redgrave, Gurney *et al.*, 2008), no NAc, haveria mais DA e, portanto, facilitaria a plasticidade sináptica para associar uma ação sinalizada por um estímulo com sua contingência de reforço ou punição. Isso resultaria em um aprendizado mais rápido e mais susceptível à extinção, quando mediado pelo NAc. De forma concomitante, o estriado dorsal mediaria um aprendizado mais lento, porém mais resistente à extinção. Esse tipo de aprendizado mediado pelo estriado dorsal, onde o estímulo que sinaliza reforço passa a ter maior controle sobre o comportamento que a própria contingência de reforço, resulta na formação de hábitos S-R.

2 OBJETIVOS

- Apresentar o modelo do mosaico dos espelhos quebrados.
- Estudar o papel da estimulação da via direta por agonistas e antagonistas do receptor D1 na aprendizagem da escolha de uma ação motora mediada por reforço negativo.
- Validar o comportamento rotatório de ratos com lesão unilateral da SNc por MPTP como um modelo de acinesia (dificuldade de escolher ações direcionadas ao lado contralateral à lesão), útil para o estudo de drogas antiparkinsonianas usadas no estágio inicial da DP.

3 PARTE 1

Nesta publicação apresentamos o “modelo do mosaico dos espelhos quebrados”. Tal como descrito na Introdução desta tese, o modelo do mosaico dos espelhos quebrados explica como os gânglios da base e os neurônios dopaminérgicos da SNc e VTA participam dos processos de aprendizagem instrumental e da seleção de ações (Da Cunha, C., Wietzikoski, E. C. *et al.*, 2009). Os experimentos apresentados nas Partes 2 e 3 desta tese podem ser explicados dentro do contexto deste modelo.

LEARNING PROCESSING IN THE BASAL GANGLIA: A MOSAIC OF BROKEN MIRRORS

Trabalho publicado na revista Behavioural Brain Research, 2009.

Behavioural Brain Research 199 (2009) 157–170

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Review

Learning processing in the basal ganglia: A mosaic of broken mirrors

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ARTICLE INFO

Article history:
Received 16 May 2008
Received in revised form 1 October 2008
Accepted 2 October 2008
Available online 11 October 2008

Keywords:
Basal ganglia
Striatum
Substantia nigra
Associative learning
Memory
Cognition
Dopamine
Reinforcement
Reward

ABSTRACT

In the present review we propose a model to explain the role of the basal ganglia in sensorimotor and cognitive functions based on a growing body of behavioural, anatomical, physiological, and neurochemical evidence accumulated over the last decades. This model proposes that the body and its surrounding environment are represented in the striatum in a fragmented and repeated way, like a mosaic consisting of the fragmented images of broken mirrors. Each fragment forms a functional unit representing articulated parts of the body with motion properties, objects of the environment which the subject can approach or manipulate, and locations the subject can move to. These units integrate the sensory properties and movements related to them. The repeated and widespread distribution of such units amplifies the combinatorial power of the associations among them. These associations depend on the phasic release of dopamine in the striatum triggered by the saliency of stimuli and will be reinforced by the rewarding consequences of the actions related to them. Dopamine permits synaptic plasticity in the corticostriatal synapses. The striatal units encoding the same stimulus/action send convergent projections to the internal segment of the globus pallidus (GPi) and to the substantia nigra pars reticulata (SNr) that stimulate or hold the action through a thalamus-frontal cortex pathway. According to this model, this is how the basal ganglia select actions based on environmental stimuli and store adaptive associations as nondeclarative memories such as motor skills, habits, and memories formed by Pavlovian and instrumental conditioning.

Contents

Abbreviations: CAR, conditioned avoidance response; CS, conditioned stimulus; GP, globus pallidus; GPe, external segment of the globus pallidus; GPi, internal segment of the globus pallidus; LTD, long-term depression; LTP, long-term potentiation; MSNs, medium spiny neurons; NAc, nucleus accumbens; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; S-R, stimulus-response; STN, subthalamic nucleus; TANs, called tonically active neurons; US, unconditioned stimulus.

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doi:10.1016/j.bbr.2008.10.001

1. Introduction

At the first half of the last century, Parkinson's and Huntington's diseases were known by their motor disabilities. The discovery that these diseases are caused by the degeneration of components of the basal ganglia led to the theory that this system is exclusively involved in motor functions [13,55,164]. Over the last decades a growing body of evidence has shown that Parkinson's and Huntington's disease patients also present marked cognitive disabilities [78,112,127,142,155]. It also became evident that the malfunctioning of components of the basal ganglia contributes to cognitive disabilities in mental diseases such as schizophrenia [93], attention-deficit/hyperactivity disorder [24], and addiction [11,58].

The involvement of the basal ganglia in cognitive processes also became evident from studies on learning and memory carried out after the second half of the last century. Studies involving patients who became amnesic after lesion to the medial temporal lobe (such as patient H.M.) have shown that these patients conserved some learning and memory abilities later named nondeclarative or procedural memories [190,196]. These clinical studies, complemented by investigations on animals with experimental brain lesions (i.e., the hippocampal formation and the dorsal striatum), supported the theory of multiple memory systems in the brain ([136,137,157,159–162], see also Refs. [196,214] for a review). In this context, the hippocampus and the adjacent cortex of the medial temporal lobe were considered to be components of the declarative memory system and the striatum was considered to be a critical component of the nondeclarative or procedural memory system.

Nowadays there are many theories to explain the role of the basal ganglia in cognitive and motor functions. One view accepted by many researchers is that the basal ganglia form a system selecting actions appropriate under specific circumstances [6,30,64,83,102,108,114,135,174,191]. In this context, procedural memories are products of basal ganglia processing. Motor skills [51,52,95,189], Pavlovian conditioning [10,187], action-outcome instrumental conditioning [7,143,173,217,222], and habits [7,136,214,222] are examples of procedural memories processed by the basal ganglia.

What kind of computation do the basal ganglia do that result in these types of procedural memory? The term procedural memory means knowing "how to do something" rather than "what to do", which is a kind of knowledge encoded as a declarative memory. As suggested by some authors, the expression of procedural memories is the product of an action selection process [6,83,135,149,174] based on associations, i.e., sequential associations of a chain of movements in skill learning; association of an action-eliciting stimulus with a neutral stimulus in Pavlovian conditioning; association of a discrete stimulus with the outcome of a specific action in instrumental conditioning. In all of these cases, the choice of the most adaptive association in a given situation is learned in a reinforcement-driven gradual process [53,158,214].

The present paper proposes a unified model to explain how the basal ganglia process learning and memories. This model, here named the 'mosaic of broken mirrors', is based on the known circuit and properties of the basal ganglia, most of them reviewed in this special issue of *Behavioural Brain Research*. It explains how the associative process occurs in the basal ganglia and how the choice of the most adaptive associations increases as a function of the novelty and salience of a stimulus and the outcome of the action associated with it.

2. The basal ganglia circuitry

A detailed review of the anatomy, physiology, and biochemistry of the basal ganglia is beyond the scope of this article and can be found elsewhere [15,48,163]. The description that follows is a concise view of the basal ganglia components and properties sufficient for readers to understand the model proposed in the article to explain the basal ganglia processing of learning and memory.

The core components of the basal ganglia are the dorsal and ventral striatum and the globus pallidus (GP). The dorsal striatum is formed by the caudate nucleus and the putamen. Many authors refer to the ventral striatum as the nucleus accumbens (NAc), its main part. The GP consists of an internal (GPi) and an external (GPe) segment and of the ventral pallidum. Due to their reciprocal connections with these core structures, the substantia nigra, ventral tegmental area, and subthalamic nucleus (STN) are considered to be associated basal ganglia structures. The substantia nigra comprises two parts: the substantia nigra pars compacta (SNc), and the substantia nigra pars reticulata (SNr) parts [163].

The basal ganglia nuclei form partially closed loops with the neocortex and thalamus (Fig. 1). Neurons from most parts of the neocortex project to the striatum [48]. Sensorimotor subthalamic structures also project directly to the striatum or by innervating other thalamic regions that project to the striatum [131]. Striatal neurons project to the GP or to the SNr which projects to specific thalamic nuclei that, in turn, project back to the frontal cortex. Projection neurons of the neocortex, STN, and thalamus are excitatory (glutamatergic), whereas projection neurons of the striatum, GP, and SNr are inhibitory (GABAergic). Therefore, the activity of different regions of the neocortex affects the activity of the basal ganglia that, in turn, modulate motor and cognitive parts of the frontal cortex. The positive modulation exerted by thalamic neurons in the frontal cortex is under inhibitory control of the GPi and SNr. This inhibition can be either blocked by a direct pathway or can be increased by an indirect pathway of neurons that arise in the striatum. The direct pathway is a projection of the striatum to the GPi/SNr. The indirect pathway is formed by striatal neurons that project to the STN which, in turn, projects to the GPe. The latter then sends projections to the GPi/SNr. Both the GPe and the STN present reciprocal projections to many nuclei of this circuit, thus working as relay stations. Midbrain dopaminergic neurons project mainly to the striatum. Dopamine released by these neurons activates the direct pathway and inhibits the indirect pathway by acting on 'D1-like' (D1 and D5) or on 'D2-like' (D2, D3, and D4) dopamine receptors, respectively. Both actions result in a positive modulation of the motor and cognitive functions of the frontal cortex [2,30,48,163]. The segregation of the direct and indirect pathways seems to be

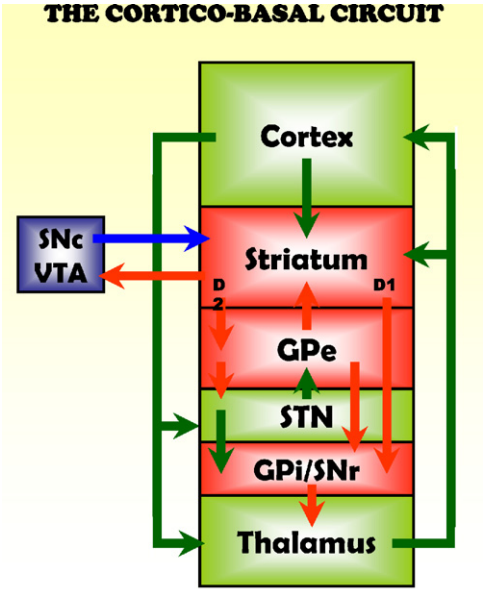

Fig. 1. An updated and simplified diagram of the Alexander et al. [2] cortico-basal ganglia network. Glutamatergic synapses are indicated by green arrows, GABAergic synapses by red arrows and dopaminergic synapses by blue arrows. Abbreviations: D, dopamine receptors; GPe, external globus pallidus; GPi, internal globus pallidus; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; VTA, ventral tegmental area.

incomplete, with many projection neurons of the striatum expressing both D1 and D2 receptors [199]. In these cases, one family of dopamine receptors may predominate in each subpopulation of neurons.

Almost 95% of the neurons of the striatum consist of GABAergic projection neurons called medium spiny neurons (MSNs). The other striatal neurons are interneurons that interact and modulate the activity of MSNs, including parvalbumin-containing, GABA-releasing interneurons; NADPH diaphorase- and somatostatin-positive interneurons, and giant cholinergic aspiny interneurons, also called tonically active neurons (TANs) [107,166,201].

The homogeneity of the cytoarchitecture of the striatum is only apparent. The MSNs of the direct and indirect pathways are homogenously mixed [71,72]. However, the MSNs form patches of acetylcholinesterase-poor but μ opioid receptor-rich regions, named striosomes. Striosomes are surrounded by a dense acetylcholinesterase-rich matrix [81].

The striatum is the input unit of the basal ganglia. Practically all modalities of cortical regions project to the striatum. Elegant studies conducted by [62,63] regarding the projections of the primary somatosensory and motor cortices of monkeys to the striatum have revealed that units of different modalities of somatosensory and motor information, encoded in different areas of the cortex, project to the same area of the striatal matrix. The authors called each region of the matrix representing a part of the body a matrisome. The cortical regions encoding, for example, the motor and sensory (pain, temperature, and pressure sensitivity) properties of a finger of a monkey overlap in the same matrisome. More intriguing, the authors found several matrisomes in the striatum encoding for the same functional part of the body. This indicates that a regions in the cortex that represent a body part project to several matrisomes in the striatum. In this respect, the distribution of matrisomes in the striatum is a mosaic of multiple sensorimotor units that are repeatedly represented.

The concept of corticostriatal convergence and disperse repetition of matrisomes in the striatum is in contrast to the concept of segregated and parallel corticostriatal circuits. There is a current debate about which of these concepts better explains corticostriatal functioning [22,72]. Many studies have shown convergent and overlapping corticostriatal projections, including regions beyond the somatosensorimotor areas such as the prefrontal [22,87,192], posterior parietal [28,175], secondary visual [28,175], and cingulate cortex [224], among others [123,150,179,221].

Zheng and Wilson [224] showed that the axonal arborizations of corticostriatal neurons form a pattern of multiple focal and dense innervations dispersed within a vast area of the striatum, similar to the matrisomes. The same pattern of multiple focal cortical projections with widespread terminal fields in the striatum have also been reported by other investigators [22,72]. In addition to these patchy corticostriatal projections, these authors also found diffuse projections that would "broadcast" the cortical activity to different areas of the striatum, thus increasing the probability of corticostriatal convergence.

However, corticostriatal convergence may not be complete and is certainly not homogeneous throughout the striatum. Areas of predominantly (but not absolutely segregated) sensorimotor, associative or limbic cortical projections in the striatum exist, as proposed by the parallel segregated loops model [2] and in agreement with experimental evidence [105,177].

3. The 'mosaic of broken mirrors' model

The model is inspired by the properties of the cortico-basal circuitry described above. It proposes that the striatum processes cortical information in an operation similar to the generation of images of a person and his environment in a mirror house. The images are repeatedly represented in the many mirrors. The mirrors are broken into many pieces that conserve fragments of the image. The repetition of the multiple pieces facilitates their combination into a mosaic. The mosaic is the product of a particular combination.

3.1. Breaking the mirrors: functional convergence and widespread repetition

The first postulate of this model is based on the generalization of the finding that corticostriatal projections from the somatosensory and motor cortex form matrisomes in the striatum [62,63]. According to this postulate, all cortical projections to the striatum are functionally convergent and form 'matrisome-like' units widely dispersed within the striatum (see Figs. 3 and 4). The term matrisome was proposed by Flaherty and Graybiel because they found out that all corticostriatal projections from the somatosensory and motor cortices made synapses with MSNs of the matrix and not of the striosomes [62,63]. However, more recent studies have reported focal projections from other cortical regions forming 'matrisome-like' terminals in both the matrix and the striosomal compartments of the striatum [224]. Thus, these "matrisome-like" units will be named here 'functional units' of the striatum.

3.2. Building functional units

3.2.1. Body parts

The first question is what do these 'functional units' represent? Let us go back to the 'functional units' called matrisomes by Flaherty and Graybiel [62,63]. The matrisomes integrate different sensory and motor properties of articulated parts of the animal's body, i.e., a functional part with motion properties. The model proposes that functional units allow the striatum to program actions based on the

movement of articulated parts of the body in relation to each other and to the environment.

3.2.2. Objects

What about the representation of sensory information of the surrounding world in the striatum? We propose that they are also encoded in the pieces of the 'broken mirrors'. Each piece individualizes an object with which a part, or the whole body, can interact. Each object is repeatedly encoded in many striatal units. These units are the same that also represent each body part in a repeated and random way. Therefore, when an object appears in the receptive field of a unit representing a body part, the firing of its neurons will increase. In other words, the firing of the neurons of a unit representing a body part increases when an object is close enough to that body part (see Fig. 2). Touching the left eye with the right index finger, kicking a ball, eating an apple, sitting on a chair, are examples of such actions. Therefore, we propose that, due to the repetition of the units representing the same objects and body parts, the increased excitation of a unit representing an object can move though the units representing different body parts as illustrated in Fig. 2. We also propose that objects are encoded in the striatum in a multisensory way. That means that the units encoding the body part that is approaching an object will respond to the view, touch, smell, or sound of that object.

Many known characteristics of the cortical projections to the striatum are coherent with our model. The ventral stream of visual information concerning object cognition is directed into the area TE, located in the inferior temporal cortex [212]. In primates, TE projects to the tail of the caudate nucleus and caudal/ventral

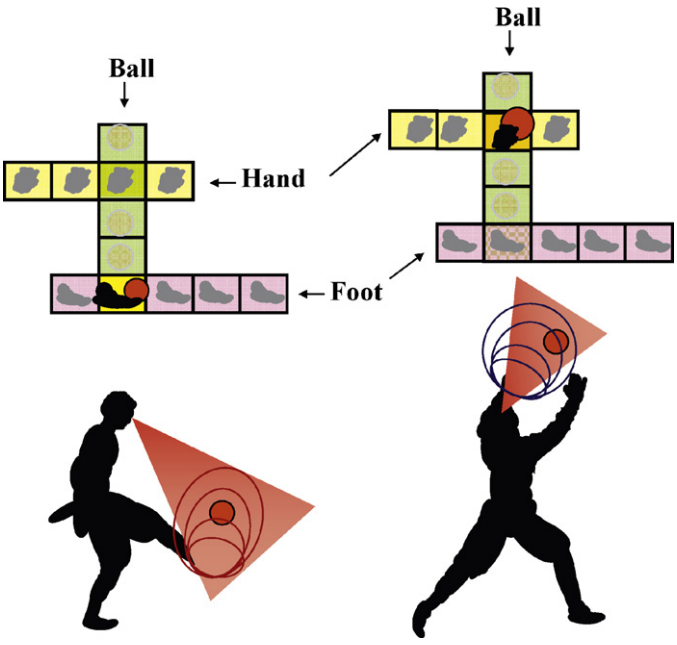

Fig. 2. These diagrams illustrate how the striatum encodes actions of a body part towards an object, according to the 'mosaic of the broken mirrors' model. Functional units of the striatum are represented by interlinked squares. They encode body parts that can interact with objects of the nearby environment. These objects are also represented by these units in a repeated way. The representation of an object and a body part can overlap in the same unit. Overlapping representation of a specific body part with an object seen, heard or smelled occurs by chance, due to the widespread distribution of these units. Each unit encodes an object in body part-coordinates, i.e., in coordinates centered in the body part that it also represents. Polymodal neurons of these units, like a hand-vision neuron, respond to an object only when it is seen near the hand. In the left sketch, a striatal foot-unit is activated to release a movement of the foot towards a ball seen close to it. In the right sketch, a striatal hand-unit is activated to release a movement towards a ball that approaches that hand.

portions of the putamen in a patchy manner [88,212]. The striatum, in turn, projects back to TE via SNr/thalamus [134]. This remarkable exception of the rule that basal ganglia output is exclusively directed at the frontal cortex, stress how important representing objects in the striatum is. The striatal neurons receiving these patchy projections from TE are intermixed by striatal neurons with receptive fields of one or more sensory modalities: visual [18,31,33,60,82,88,89,96,104,130,146,148,150,167,176], somatosensory [62,96,148], auditory [29,148,184], gustatory [67], and olfactory [193]. Inputs from sensory neurons of other higher visual cortical areas, extra-geniculate sensory thalamus, and the superior colliculus are also likely to contribute to the sensory and movement properties of the objects represented in the striatum [148]. In agreement with the view that the striatum encodes body parts and objects, visual and somatosensory modalities predominate among striatal neurons [82,148] and many of them are selective to approaching stimuli [82,150,194]. Except for the patchy projections from TE [88], these neurons present large size receptive fields and no signs of retinotopic or continuous somatotopic organization ([147], but see Refs. [36,82]). Their receptive fields cover the whole visual field, auditory perimeter, and body surface [148].

The striatum is widely regarded as being involved in sensorimotor integration [9,48,163,121,214,222]. According to our model this integration can be achieved if the locations of an object are encoded in the striatum, not in the retinotopic-, but in body part-coordinates. In other words, we propose that the striatal neurons located in the unit representing a hand will respond to the vision of an object only when it is near to that hand (see Fig. 2). This model predicts that the closer the hand is to the object, the higher the firing rate of the visual neurons of that unit will be. It is exactly the picture found by Graziano and Gross [82] while recording from the ventral putamen of anesthetized monkeys. They reported that some neurons presented a tactile receptive field covering the whole body and visual fields restricted to a visual angle. Others, responsive to the touch of a cotton swab in the monkey's face while its eyes were covered, increased their firing after the animal had its eyes uncovered so that it could see this object approaching its face. The same neuron did not respond before the object was 10 cm or less from the animal's face. They defined the visual receptive field of this neuron as "corresponding to the solid angle centered at the tactile receptive field and extending out approximately 10 cm" [82]. They reported receptive fields centered in other body parts extending from some centimeters (e.g., a hand) to more than a meter away out to the wall of the room (e.g., an arm). Coherent with the hypothesis that these striatal neurons encode objects that can be manipulated by a body part, when the arm of the animal was moved out of its vision, a typical "arm + vision neuron" no longer responded to the presence of the object to its field of view. Based in these findings they propose that the striatum encodes objects located in the visual space surrounding the subject in body part, rather than in retinotopic coordinates. Our model not only incorporates this theory, but also proposes a mechanism by which this body part-centered coordinates may arise in the striatum (see Fig. 2).

Such model also explains why the dysfunctions of the basal ganglia (and their loop with TE) lead to alterations in visual perception, like visual hallucinations [134], impaired reaction times in visual search [116], and impaired pattern/object location associative learning [60,116,134].

3.2.3. Locations

While the actions towards objects located in the space immediately surrounding the subject demand body part-centered coordinates, actions toward distal targets demand spatial coordinates. No consensus exists that the spatial context is represented in the striatum [49,128,139,141,214,222]. Behavioural studies report-

ing a double dissociation between the dorsolateral striatum and the hippocampus for spatial and stimulus-response (S-R) learning tasks have initially led to the view that the striatum is not important for spatial tasks. These studies included spatial and cued versions of the Morris water maze [156,159], radial maze [159], and plus-maze tasks [160]. Other studies from our group have also shown this dissociation between the SNc and the hippocampus [38,40,42,43,61,138].

However, even the cued tasks mentioned above require some degree of spatial information to be solved. In those studies, the cue (i.e., a ball, a salient platform, a light) can be conceived as an object which the animal needs to approach in order to be rewarded. Since this object is located in a specific place of the maze, the behaviour of the rat can be conceived as "to go to that object located in that place". In some instances, such as in a plus-maze or T-maze, the reference is not an object but a hemi-side of the animal's body (egocentric orientation) which permits encoding behaviours such as making a right or left turn to be rewarded [16,106]. Even in these cases, the task involves performing an action (turn) in a specific place.

Evidence that the striatum encodes spatial information about the environment came from studies reporting that, like the hippocampus [153], the striatum also contains place-related cells, neurons that discharge when the animal is in a particular place of the environment [57,139–141,172,213]. Compared to the hippocampal place cells, those found in the striatum are more influenced by other parameters of the task [111]: they also encode egocentric movements and are more sensitive to visual cues [141] and reward variables [111,126,140,141,194]. The striatum, as well as the hippocampus, also contains a subpopulation of neurons called head direction cells that fire preferentially when the animal's head is aligned with a particular orientation, irrespective of the animal's location [139,141]. These neurons are probably involved in egocentric movement.

The difference between the tasks depending on the dorsal striatum and those depending on the hippocampus is that, in the former, the location of the target does not need to be defined in terms of multiple relations between distal cues. In a recent study, we have shown that inactivation or lesion of the striatum or of the SNc does not impair the ability of rats to navigate in a water maze when they always depart from the same starting point to find a hidden platform kept in the same place in the maze ([40], see Ref. [159]). The animals learn this task probably by using a single object of the environment as a distal cue. Animals with intact striatum and a lesion in the hippocampus may orient themselves in an environment, but this orientation is not sufficient to disambiguate places equidistant to the same environmental object. This dissociation has been shown by McDonald and White [128] in rats searching for food in two adjacent arms of an 8-arm radial maze. Rats with a hippocampal disconnection, but with an intact striatum, were unable to solve this task. However, the same rats were not impaired to discriminate in which of the two arms, separated by other two or more arms, they would find the food. In the latter case the animals probably use different distal cues to discriminate between arms.

According to the 'mosaic of broken mirrors' model, the representation of space in the striatum may account for the characteristics of the tasks that can be learned with the participation of the striatum. This model postulates that cortical projections to the striatum are fragmented into pieces, with each piece representing a location. In other words, this model assumes that, while the hippocampus represents space as a continuum, the place fields in the striatum are repeated and intermixed. This configuration facilitates the association of objects (cues) with particular places, but breaks the orthogonal relationships among different locations. Therefore, the hippocampus is in a position to compare the current spatial context

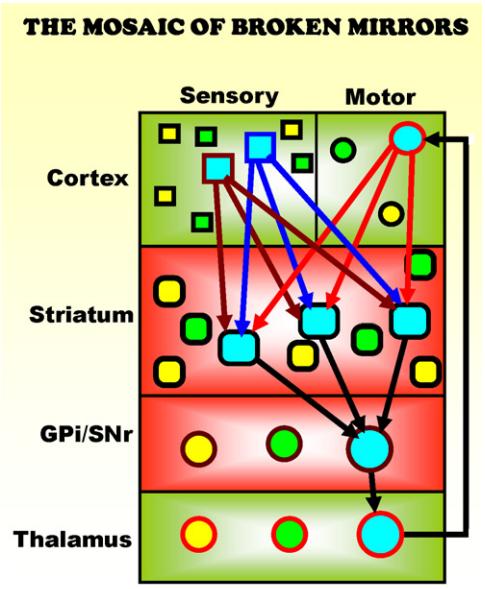

Fig. 3. This diagram illustrates the redundancy and functional convergence properties that the 'mosaic of broken mirrors' model proposes to the functional units of the striatum. The indirect pathway and the dopaminergic modulation are not represented in order to simplify the diagram. Abbreviations: GPi, internal globus pallidus; SNr, substantia nigra pars reticulata.

of the environment with the context found in the past. On the other hand, the striatum is in a position to choose an action that can move the "pieces of the mosaic (the subject's body, body's parts, objects) to a particular location. According to this view, the hippocampal representation of the environment is globally oriented, while the striatal actions depend on breaking the environment into pieces in order to move them. Hence, tasks such as the cued version of the water maze or the win-stay version of the radial maze can be easily solved by the striatum by associating the approaching action with the place in which an object (cue) is located.

The action of approaching a location cannot be encoded in the hippocampus since it does not have direct connections with motor areas of the neocortex. This location-approaching action association is probably done in the striatum that receives direct inputs from the hippocampal formation to the shell region of the NAc, and indirect inputs to the core of the NAc through the prefrontal cortex and to the ventromedial striatum through the medial entorhinal cortex [66,119,129,202].

We recently obtained some curious results in experiments of latent learning that can be explained by the assumption of the 'mosaic of broken mirrors' model that the striatum represents space in a fragmented way. We found that the impairment of SNc-lesioned rats to perform the cued version of the water maze disappeared when the animals were pre-trained in the spatial version of this task [42]. Curiously, SNc-lesioned rats were not impaired to perform the spatial version. A series of control experiments showed that the presence of the hidden platform and the view of the distal cues during the pre-training sessions were critical for that beneficial effect. More intriguing was the finding that this improvement was observed even when the locations of the distal cues (posters fixed on a curtain around the maze) were changed in relation to the pre-training session. Our model explains these data by assuming that the spatial map formed during the pre-training sessions was broken into pieces, each containing a distal cue. Hence, a particular cue could be associated with the action of approaching it, irrespective of its relationship with the other cues.

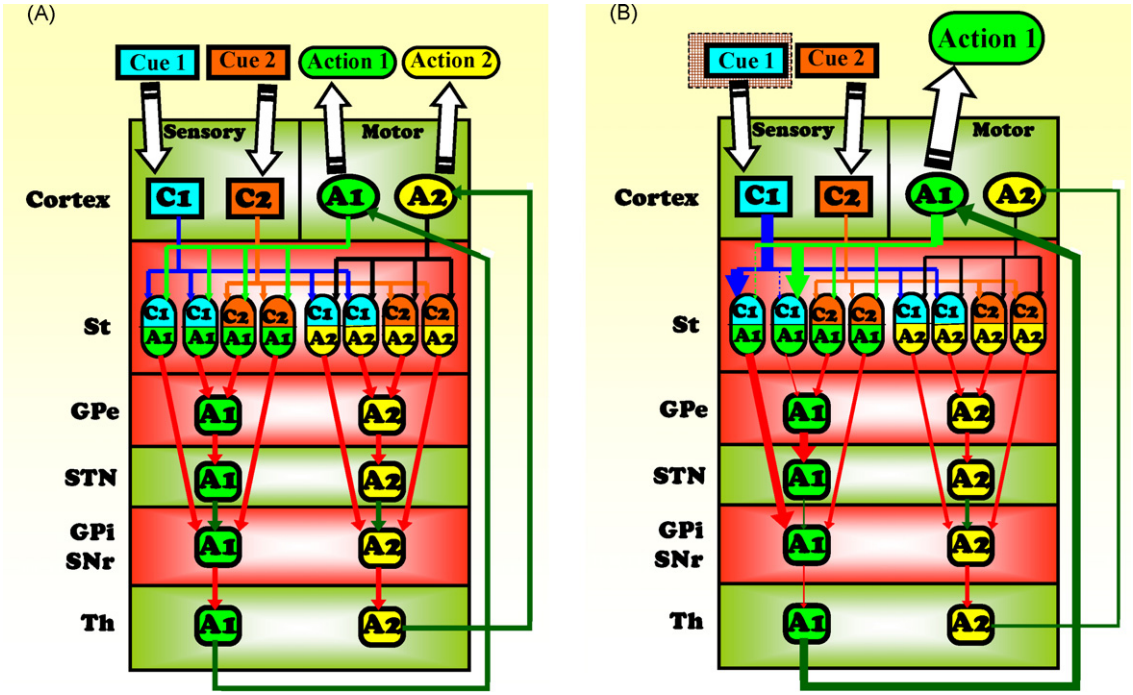

Fig. 4. This diagram demonstrates the combinatorial, associative, learning, and action selection properties of the mosaic of broken mirrors model. Neurons are represented by boxes and circles. The colours of the arrows linking glutamatergic cortical neurons to striatal neurons denote their origins. Arrows linking the other components of the basal ganglia circuit represent axons of GABAergic (red) or glutamatergic (green) neurons. (A) Before learning occurs, the circuit allows the association of any environmental cue with any action. (B) After pairings of the salient Cue 1 with Action 1, coincident with a phasic release of dopamine (not shown), the following alterations occur, restricted to the synapses between cortical neurons representing Cue 1 and those representing Action 1 that converge to the same striatal neurons: LTP in the direct pathway for Cue 1; LTD in the indirect pathway for Cue 1; LTP in the indirect pathway for Action 1; LTD in the direct pathway for Action 1. Alterations in the synapses of Cue 1 increase the probability that it will induce the choice of Action 1. The alterations in the synapses of Action 1 lead to the conclusion of the action. Abbreviations: GPe, external globus pallidus; GPi, internal globus pallidus; SNr, substantia nigra pars reticulata; St, striatum; STN, subthalamic nucleus; Th, thalamus.

In another study carried out in our laboratory, we found further evidence that units of the striatum encode actions directed at a goal (unpublished results). In that study, rats with complete hemilesion of the SNc induced by 6-hydroxydopamine were trained to enter the lighted arm of a radial maze in order to find a sucrose pellet. The lesion prevented the animals from running directly to the lighted arm when it was located on the side contralateral to the lesion. However, these animals made ipsiversive turns in order to adjust their pathway and enter the lighted arm. This result suggests that the action of approaching a goal, but not the goal *per se*, depends on the release of dopamine in the striatum contralateral to the goal location. Although SNc-hemilesioned rats have lost the basal ganglia modulation that helps them to choose making contraversive turns, they could still approach a goal located on their contralateral side by means of other actions (i.e., ipsiversive turns). When the dopaminergic receptors of the hemilesioned striatum were stimulated by the administration of a dopamine receptor agonist (i.e., apomorphine), these animals did not only recover their ability to perform contraversive turns, but also overdid this action due to supersensitization of D2 dopamine receptors [41]. These results are in agreement with the postulate of the "mosaic of broken mirrors" model that the activation of specific actions (such as turns) directed at a goal is encoded by the functional units of the striatum. Other actions involved in the practice of innate behaviours, such as grooming [34] and predatory hunting [183], have also been reported to depend on the striatum.

Therefore, the model proposes that not only the hippocampus, but also the striatum, is needed to solve spatial versions of water and radial mazes. The poor performance of striatum-lesioned rats in these tasks has been attributed to lesions more restricted to the dorsolateral striatum, sparing other regions that receive direct or indirect projections from the hippocampus, i.e., the dorsomedial striatum [49,141,222]. According to this view, spatial navigation depends on both the hippocampus and the striatum. The hippocampus provides the map and the striatum the pathway to navigate through it. Coherent with this postulate, neurons encoding for particular behaviours such as turns have been found in the striatum, but not in the hippocampus [141]. Mulder et al. [145] reported the existence in the striatum of "goal"-like neurons that fire continuously while a rat moves from one location to another in a plus-maze. These neurons may encode the paces of movements between landmarks of a route made up by pieces of the spatial map.

3.2.4. *Other functional units of the striatum*

The inputs to the striatum are not restricted to sensory, spatial or motor areas of the cortex. Prefrontal and limbic areas of the cortex also project to the striatum in a convergent and widespread manner. Convergence refers to afferents departing from different regions of the cortex to overlap in restricted areas of the cortex forming 'matrisome-like' functional units. These units are widely distributed in vast regions of the striatum. What is the functional nature of these units? They may refer to affective meaning and to abstract information such as symbols, words, digits, thoughts, and plans. The processing of these functional units by the basal ganglia would explain the involvement of the latter in working memory and executive and affective functions [26,132,154].

3.3. *Building associative units*

Once objects, locations, body parts, symbols, and associated actions or plans are individualized into functional units in the striatum, what is the function of their repeated representation?

The answer is associative learning. The body, the surrounding world and the mental world can be combined into more flexible associations if they are broken into pieces (see Fig. 3). Repetition increases the probability of association among pieces and explains the involvement of the basal ganglia in different kinds of associative learning: Pavlovian conditioning for the association between a conditioned stimulus (CS) (a neutral stimulus) and an unconditioned stimulus (US) (a rewarding or aversive outcome) [10,187]; instrumental or operant conditioning for the association of a predictive cue with an action outcome (reinforcement or punishment) [143,173,217]; addiction for the association between a drug with strong rewarding properties and its compulsive consumption [11,58]; skill learning for the association of a sequence of motor actions [1,51,52,95,189]. The associative property of basal ganglia proposed by this model also permits the striatum to play a role in action selection based on reinforcement of previous cue-action associations [6,7,30,64,114,191]. The ingredients for these associations are the synapses between the corticostriatal neurons and the MSNs encoding the functional units of the striatum.

3.3.1. Synaptic plasticity in the striatum

What are the mechanisms underlying the association of functional units of the striatum? The most likely candidates are the synaptic plasticity phenomena known to occur in the striatum. Both long-term potentiation (LTP) and long-term depression (LTD) have been reported to occur in synapses between the corticostriatal neurons and MSNs [20,50,218]. According to Hebb's rule, LTP occurs when presynaptic and postsynaptic neurons are depolarized at the same time. LTP can be induced in the striatum by repeated activation of cortical terminals [27]. Therefore, corticostriatal synapses are the binding elements associating information arriving from different regions of the cortex. This association may occur when LTP is induced in the synapses of the two corticostriatal neurons with the same MSN and requires a triple coincidence: the two cortical neurons and the MSN must be depolarized at the same time. Such coincidence fulfil the needs for the induction of heterosynaptic associative LTP [124]. The partially closed loops between the striatum–GPi–thalamus–striatum and the striatum–GPi–thalamus–cortex–striatum (Fig. 1) may result in reverberant activation of MSNs, a factor contributing to keep these neurons depolarized. Other loops involving the GPe and/or the STN may also play a role in such reverberation and/or in the modulation of this circuit. High-frequency firing of the corticostriatal neurons may also induce LTD in their synapses with MSNs ([19,120,211], see also Refs. [50,218] for a review). The concentration of dopamine and how dopamine receptors are distributed among MSNs are critical factors to determine the induction of LTD or LTP, as will be discussed in the next section. LTP and LTD of synapses associating different cortical inputs with the same MSNs may build the memory trace of associative learning mediated by the basal ganglia (see Figs. 3 and 4).

3.3.2. Dopamine-dependent synaptic plasticity

The synaptic plasticity necessary for the occurrence of associative learning in the striatum requires a learning signal, a message that signals when and how learning occurs. This message seems to be the release of dopamine ([99,187], but see Ref. [218]). The activation of dopamine receptors in MSNs is necessary for the induction of LTP or LTD. D2-like and (maybe) D1-like dopamine receptors are required for the induction of LTD, but the activation of D2 receptors favours the induction of LTD over LTP in some instances ([21], see also Ref. [218] for a different view). Activation of DB1 cannabinoid and adenosine A2A receptors also seems to be involved in the induction of the striatal LTD [50,70,218]. On the other hand, LTP requires the activation of D1 receptors [25] and is inhibited by the activation of D2 receptors [21].

D1 receptors occur mainly in MSNs of the direct pathway (those projecting to the GPi/SNr), whereas D2 receptors are mainly expressed in neurons of the indirect pathway (those projecting to the GPe) [71,72]. Therefore, in the presence of dopamine, LTP is more likely to occur in the direct pathway and LTD in the indirect pathway. The direct pathway positively modulates actions encoded by the frontal cortex, while the indirect pathway inhibits their occurrence (see Section 2 above). According to the 'mosaic of broken mirrors' model, in the presence of dopamine, the concomitant activation of corticostriatal neurons encoding, for example, an object and the action of approaching it, would induce LTP in their synapses with MSNs of the direct pathway and LTD in synapses with MSNs of the indirect pathway. This feature would increase the firing probability of MSNs encoding the association between the stimulus (object) and the action of approaching it [101].

The complete segregation of the direct and indirect pathways is currently a matter of debate [48,87,218]. Induction of LTD that requires the activation of D2 receptors occurs in most MSNs [19,50]. In addition, there is evidence for the co-expression of D1 and D2 receptors in a subpopulation of neurons [199]. In these neurons the induction of LTP or LTD depends on the level of dopamine and on the depolarization state of MSNs. D2 receptors present a higher affinity for dopamine than D1 receptors [103]. As a consequence, lower levels of dopamine favour the induction of LTD and higher levels favour the induction of LTP [25].

What happens when the act of approaching an object is reinforced? The corticostriatal neurons encoding the object and the action of approaching it are activated at the same time. As a consequence, LTP or LTD would occur in the connections of MSNs that receive overlapping projections from these active corticostriatal neurons, with the occurrence of LTP in MSNs of the direct pathway and LTD in those of the indirect pathway (see above). This feature would increase the firing probability of these MSNs and the consequent occurrence of the approaching action when the same object is seen by the subject in the future.

3.3.3. Novelty-driven reinforcement learning

Midbrain neurons release dopamine in the striatum in tonic or phasic patterns [68,75–77]. A small amount of dopamine is spontaneously and continuously released by these neurons in a tonic pattern, providing a baseline level of extrasynaptic dopamine required to run the motor programs already set up [75]. The phasic firing of dopaminergic neurons causes a transient and robust release of dopamine and serves as a learning signal, inducing neural plasticity in the striatum. Coherent with this theory, the phasic release of dopamine is critical for Pavlovian conditioning [10,187] instrumental learning [143], and other types of associative and reinforcement learning [114,185].

The influential studies by Schultz and other groups suggested that the phasic release of dopamine occurs in response to unpredicted rewarding stimuli [10,143,188], with the amount of dopamine released being proportional to the difference between expected and obtained reward [188]. This difference is called reward prediction error. More recently, this theory has been contested by the argument that the latency for a stimulus to induce the phasic release of dopamine is too short to permit the sensory processing necessary to evaluate the stimulus identity and reward value [173]. The fact that the unpredicted presentation of non-rewarding salient stimuli such as light flashes or tones elicits a phasic dopamine response also disagrees with the reward prediction error theory [99,100,118]. Habituation to a stimulus abolishes the phasic dopamine response [118,187]. The omission of an expected reward causes a brief cessation in the firing of midbrain

dopaminergic neurons at the time the stimulus was expected to occur [186]. Aversive or detrimental stimuli (usually those that cause pain) induce a pause in the firing of dopaminergic neurons for the duration of the event, followed by a rebound response [32,210]. Therefore, the phasic dopamine response seems to signal the presence of new biologically significant stimuli, with a positive response (increased release do dopamine) to non-harmful stimuli (neutral or rewarding) and a negative response to harmful stimuli [173].

As stressed above, striatal synaptic plasticity depends on the activation of dopamine receptors. Therefore, the phasic release of dopamine serves as a permissive signal for learning processes that occur in the striatum. The fragmentation of the sensory representation of the environmental world and functional parts of the body involved in actions permits the individualization of these elements and their repetition increases the combinatorial association among them. After repeated presentation of novel stimuli associated with actions, the continuous reinforcement of the associations between pairs of stimuli or stimulus-action units that always appear together causes them to be more strongly associated than the stimuli and actions that are associated only occasionally. According to the 'mosaic of broken mirrors' model, it is the principle of the associative learning that forms expectations based on current stimuli and actions (see also [114,149,207,219]). After learning, the occurrence of a salient stimulus can be predicted and it will no longer induce the phasic dopamine response. The memory for this association becomes stable.

According to this model, the association of an action with its outcome depends on their representation in the striatum at the same time as the concentration of dopamine in the synapses are high due to the phasic response. Otherwise, the synaptic plasticity to strengthen the synapses between overlapping corticostriatal neurons and MSNs would be lacking. The phasic dopamine response seems to appear too early and to be too short [65,84,99,188] to permit the association of a stimulus with an action and its rewarding outcome [173]. However, the clearance of dopamine released in the striatum, particularly in the NAc and medial regions of the striatum, takes longer compared to the dorsolateral striatum [151,198,216]. This fact would explain MSNs in the striatum responding to previous actions and their reward outcome [35,92,97,114,115]. The clearance of dopamine may range from a few hundreds of milliseconds in the dorsolateral striatum to several seconds in the NAc [151,198,216]. This difference can account for the higher involvement of the NAc in action-outcome reinforcement learning and of the dorsolateral striatum in S-R habits [149,222]. The fast clearance of dopamine in the dorsolateral striatum opens a time window too tight to include the reward outcome to the S-R association. This might be the reason for the slow learning rate of S-R habits and for the fact that these habits are relatively insensitive to reward devaluation. On the other hand, in the NAc the slow clearance of dopamine after a phasic response is probably long enough to associate the outcome (reward) with the action, a fast learning that fades more easily after reward withdrawal or devaluation.

This postulate is in line with imaging and electrophysiological studies showing increased activity in the striatum in response to a reward [47,91,113,114] and reward prediction errors [90,152]. It is also supported by studies reporting that the lesion or manipulation of the rat SNc or striatum disrupts associative reinforced learning in various tasks such as the cued version of the Morris water maze [42,43,61,138], two-way active avoidance task [39,73,74,110], inhibitory avoidance [23,46,133,165,170,171,181], Pavlovian conditioning [168], and cued instrumental tasks [12,59,168,169]. Similar associative reinforced and habit learning deficits have also been observed in mouse and monkey models of Parkinson's disease, as well as in Parkinson's disease patients [60,78,109,112,178,182,200].

3.3.4. Aversively motivated learning

Associative learning mediated by appetitive reinforcement can be easily explained by the postulates of the 'mosaic of the broken mirrors' model since a short latency phasic dopamine response follows the reward presentation [186], as mentioned above. However, aversively motivated associative learning demands further elaboration since, as also mentioned above, aversive stimuli may induce a pause in the firing of midbrain dopaminergic neurons for the duration of the event, followed by a rebound response [101,210]. How can a reduction in the extracellular dopamine levels in the striatum induce learning, a process that demands neuronal plasticity? Let us discuss two popular models of aversively motivated learning: the active and the inhibitory avoidance tasks.

Learning the two-way active avoidance task, a kind of conditioned avoidance response (CAR), demands from a rodent to actively run away from a footshock (unconditioned stimulus) signalled by a cue (usually a tone or the light of the chamber, i.e., the conditioned stimulus) [39]. Training is carried out by the pairing of the CS and US in a two-chamber shuttle box. The CS starts before and turns off together with the US. After many consecutive pairings, the animal learns to avoid the US by crossing to the opposite chamber just after the presentation of the CS. Electrophisiological studies reported that most, if not all [210], midbrain dopamine neurons respond to noxious stimuli with a short latency increase in the firing rate, followed by a rebound offset ([32,69,98,122,173,208,210], but see Ref. [125]). The temporal resolution of microdialysis studies is not enough to detect the decrease in dopamine release in the striatum after a footshock, but these studies consistently detect the increase that may result from the rebound response that follows the ending of the noxious stimulus [100,205,223].

Thus, the increase in the extracellular concentration of dopamine probably coincide with the presentation of the "crossing" action that turns the US and CS off. The higher level of dopamine favours the induction of LTP between the corticostriatal neurons encoding the CS that converge to MSNs to which the corticostriatal neurons encoding the "crossing" action also project (see Section 3.3.2). This "crossing response" of the animal may be seen as the action of running away from the CS. Note that running away from a painful stimulus (US) is an innate behaviour, independent of learning.

Inhibitory avoidance, also called passive avoidance, demands that the animal (usually a rodent) avoids entering a particular place. Inhibitory avoidance training may be performed in the same two-chamber box used for two-way active avoidance conditioning [3]. The animal is placed in a lit chamber and receives a brief footshock when it enters the dark chamber. Usually only one session is needed for the animal to learn to inhibit the innate tendency of entering the dark chamber. In other words, it learns not to go to that location. The novelty of exploring the lit chamber probably induces a phasic response of the midbrain dopaminergic neurons ([117,118], but see Ref. [44]). The footshock probably induces the cessation of their firing [32,173,187]. Therefore, the act of remaining in the lit chamber will coincide with higher levels of extracellular striatal dopamine and the act of entering the dark chamber with the lowering in the level of dopamine. The former situation favours the induction of LTP between the corticostriatal neurons encoding the location of the lit chamber and MSNs receiving projections of corticostriatal neurons encoding the action of remaining there (see Section 3.3.2).

Therefore, we propose that in aversively motivated learning, it is not the reduction of the firing of midbrain dopamine neurons that induces learning, but the increase in the release of dopamine in the striatum before and after the aversive stimulus. In both active and inhibitory avoidances, the action that coincides with higher levels of dopamine is associated with the concomitant cue or location.

This hypothesis is coherent with the findings that manipulations in the SNc [39,73,74] or in the striatum [23,46,110,133,165,170,171,181] impair learning of these tasks.

Note that inhibitory avoidance may be learned as the association of an action with a place. However, such association would impair learning of the two-way active avoidance task in which the animal must successively return to the place in which it was punished. In this situation, the hippocampus, that encodes an environment as a place [153], is expected to play a detrimental influence. This prediction is in agreement with studies reporting that the lesion of the septum [180,206] or fimbria-fornix [85] improves learning of the inhibitory avoidance task. This illustrates a case in which the striatum and the hippocampus play competitive roles on learning [214]. It is coherent with the present view that the striatum encodes discrete stimuli and locations (see Section 3.2.3). The representation of both discrete cues and locations in the striatum does not mean that they will be always associated with the current actions. Only the activation of the striatal units that coincide with an action performed under high levels of striatal dopamine will be associated to this action. During learning of the two-way active avoidance, the act of running to a specific location (chamber) will be coincident with the release of dopamine only in 50% of the trials. On the other hand, the action of running from the CS will be reinforced by the release of dopamine in all occasions. As a consequence, the competition between the associations of the CS-"running from it" and the location-"avoid running to it" will be won by the former as trials go on. Such learning may be faster if the influence of the hippocampus is inhibited.

3.4. Building action units

3.4.1. Driving MSNs to an 'up' or 'down state'

The membrane potential of MSNs oscillates between 'up' (subthreshold depolarized) and 'down' (hyperpolarized) states [220]. LTP is more likely to occur during the former and LTD during the latter state [20,50]. The higher activity of corticostriatal neurons representing actions and current features of the external or internal environment favours the 'up state' in MSNs to which they converge [197]. Since these functional units are represented in a repeated way [62,63,87], at least some of them probably overlap, thus presenting a higher probability to be in the 'up state' or depolarized. This probability is increased by the diffuse corticostriatal projections to a broader area of the striatum [22].

3.4.2. Go/NoGo units

The result of striatal processing flows to the GPi and SNr, the output doors of the basal ganglia through the direct or indirect pathway (see Figs. 1 and 4). They build the 'Go' and 'NoGo' products of the basal ganglia processing [64] (see Figs. 3 and 4). The direct pathway is a GABAergic (inhibitory) connection between the striatum and GPi/SNr. The indirect pathway connects the striatum to the GPi by a sequence of neurons that finally exert an excitatory effect. Therefore, the direct pathway (Go) relieves the thalamocortical neurons from the tonic inhibition of the GPi/SNr. The indirect pathway (NoGo) results in the opposite effect [2] (see Section 2 above).

Since the 'Go' and 'NoGo' units affect almost exclusively the frontal cortex (through thalamocortical projections) and subcortical motor areas, they result in the induction/repression of actions, action planning, and other executive functions.

3.5. Gathering action units

The smaller number of neurons in the striatum, compared to the neocortex, imposes a convergence of the information originating from the neocortex to transform it into functional units [8,144]. In rats, 17×10^6 corticostriatal neurons converge onto 1.7×10^6 MSNs in the striatum [224]. The corticostriatal convergence is probably higher due to the repetition of the functional units (see Figs. 3 and 4).

The lateral inhibition among MSNs is seen as evidence for parallel and independent processing in the striatum [215]. However, other studies reported that this lateral inhibition is unilateral and restricted to less than one-third of the tested pairs [37,209], a finding favouring the proposal that the functional units of the striatum are formed by patches of MSNs receiving convergent and overlapping cortical projections. In this case, lateral inhibition may help isolate neighbouring functional units from one another. Since the functional units are repeated and widespread throughout the striatum, they may be distant enough to avoid lateral inhibition from their peers.

This repeated and widespread distribution of the functional units imposes a binding problem to coordinate the firing and plasticity between equal units. Recent studies suggest that this problem might be solved by a class of interneurons, presumed to be cholinergic, called TANs (see Section 2). These interneurons present a broad distribution, lying mainly at the borders of the striosome-matrix [4], and a low spontaneous firing rate that results in inhibitory effects on the excitability of MSNs [225]. TANs respond to rewarding events with a phasic decrease in their firing rate, at the same time that dopaminergic neurons increase their firing rate [5,14,79,143,195]. However, while in some instances the response of dopaminergic neurons seems to be proportional to the reward prediction error (see Section 3.3.2), the response of TANs is indifferent to reward predictability [143]. The dopamine response is timed to novel salient stimuli (including rewarding stimuli), but the time necessary to remove dopamine from the synapse is longer compared to the rapid removal of acetylcholine by dense acetylcholinesterase [225]. The sharp response of TANs to rewarding stimuli may result in a temporal synchronization of the repeated functional units formed by the patches of MSNs spread throughout the striatum. In other words, TANs may signal to MSNs when to learn, midbrain dopaminergic neurons may signal how to learn, and corticostriatal neurons may signal what to learn [143]. Coherently, the number of TANs responding to the reward signal increases in parallel with learning of Pavlovian [4] and instrumental [143] learning tasks. Learning probably results in a gradual recruitment of the numerous functional units of the striatum as learning progresses.

The projection of the striatum to the GPi and SNr imposes a second convergence of the order of 10^2–10^3 [8] (see Figs. 3 and 4). This convergence probably accounts for the re-unification of the repeated functional units of the striatum [79], i.e., as learning progresses by recruiting a larger number of repeated units of the striatum, the activation of these convergent units of the GPi/SNr increases. Since the GPi/SNr projects almost exclusively to the frontal cortex (through the thalamus) and brainstem motor nuclei, they probably encode mainly actions and plans.

4. Emergent properties of the 'mosaic of broken mirrors' model

Most of the attributes of nondeclarative memories are emergent properties of the 'mosaic of broken mirrors' model. These memories are said to be implicit (unconscious) [196], rigid (inflexible) [56], procedural (expressing how to do something) [196], and suitable to guide cue-based and egocentric navigation [214]. The learning of most of these memories is a slow and gradual [54,158,222] associative process that depends on reinforcement [58,94,204], and sometimes forms habits after overtraining [136,222].

The implicit nature of memories that depend on basal ganglia processing is explained by the fragmentation of the information that occurs in the striatum, so that neither the subject's own body nor its environment are globally perceived during learning. Instead, few components of the environment are associated with discrete actions. This learning process is highly adaptive in order to adjust automatic responses (actions) to discrete changes in environmental elements. However, the meaning of this behaviour does not make sense in the global environment, simply because it is not globally oriented.

The rigid or inflexible aspects of these memories may be explained by this model for the same reasons. Since these memories are formed by associations of fragments of information about the environment and specific actions, their expression cannot be flexibly used in another context of the environment because of the lack of a global view of the environment. Even chains of actions performed in a skill are not oriented as an action of the subject in a complex environment, but as an automatic sequence of single actions.

Since the output of the basal ganglia is almost exclusively the frontal cortex and brainstem motor nuclei, the memories encoded by this system must be expressed as actions. This explains the procedural nature of these memories.

The fragmented representation of the environment in the striatum also explains the cue-based and egocentric navigation during basal ganglia-dependent learning. This type of navigation is not oriented towards a global view of the environment, but rather relies on discrete environmental cues or sequences of movements based on egocentric orientation [17,45,203]. The broken representation of the environment favours the association of units of information (cues) relevant as reward predictors with actions performed to approach the place in which the reward is delivered. However, this fragmentation does not allow multiple relations between environmental elements to form a spatial map. As a consequence, it stores information sufficient only to guide the navigation by steps based on sequential approaches to cues or sequences, for example, of right/left turns at specific locations.

One of the most evident properties of the 'mosaic of broken mirrors' model is that it is ideal to perform reinforcement associative learning. The repetition of the functional units formed in the striatum by convergent projections of the cortex amplifies the combinatorial power of the system. The dependence on dopamine to strengthen or weaken the associations among stimuli, actions and outcomes makes this associative process conditional. The release of dopamine only when the stimulus or the outcome are unpredictable (unlearned) becomes the driving force of learning mediated by this system.

The slow and gradual learning of procedural memories can be explained by two characteristics of this model. Reinforcement learning starts with trial and error associations, followed by evaluation of the outcome, and progresses by multiple comparisons between the reward prediction and/or the novelty of stimuli and the outcome during each trial. It is by definition a gradual process. The gradual recruitment of the functional units that are repeated in the striatum also contributes for learning to become slow and gradual.

Some types of instrumental learning result in a strong association between a stimulus and an action that becomes resistant to reward devaluation. This kind of associative memory, in which the stimulus becomes stronger than the outcome to trigger the response, is called habit [222]. The repetition of the functional units in the striatum mediating this association after extensive learning may partly account for this property. The more this associative memory becomes represented by a larger number of associative units, the more difficult it will be to erase them when the reward outcome or the novelty decreases. In addition, the spreading of these associative units throughout the striatum increases the probability of their occupying striatal regions less sensitive to the reward outcome. Recent findings suggesting a gradient from the ventral to the dorsal striatum in the clearance of dopamine and regional differences in dopamine-dependent synaptic plasticity may account for these differences [216]. The formation of association units less sensitive to a reward is slower and so is their dissociation after reward withdrawal. If this is the case, the ventral striatum (NAc) would account for a fast and transient learning observed during the first trials of an instrumental task, while the dorsal striatum would account for the slow and strong (more resistant to reward devaluation or withdrawal) learning (habit) achieved after overtraining.

5. Conclusion

In figurative words, we propose that the cortico-basal processing of procedural memories is similar to a mosaic consisting of pieces of images of several broken mirrors. According to this model, neurons of the sensory, motor and associative cortices send convergent projections to the striatum that result in functional units (see Figs. 3 and 4). These striatal units encode articulated parts of the body and portions of the surrounding world that can be moved or manipulated, such as surrounding objects (Fig. 2). These units also encode specific locations to which the subject can move. The association of these functional units results in programs to perform motor skills and movements of the arms, eyes, or other body parts to a specific target (object or location), or in the locomotion of the subject to specific targets. The combinatorial power of these associations is amplified by the repeated and widespread distribution of the functional units in the striatum.

According to this model, learning in this system depends on the alteration in the strength of the synapses between the corticostriatal neurons and MSNs that encode the functional units (Fig. 4). It occurs when an environmental stimulus becomes salient in an unpredictable way. At this time, the midbrain dopaminergic neurons release dopamine in the striatum in a phasic pattern. The activation of dopaminergic neurons is a condition for the occurrence of synaptic plasticity in the striatum. The synchronization of neurons of the repeated functional units encoding the same action in relation to the salient stimulus is performed by a pause in the release of acetylcholine by TANs. The striatal units encoding the same stimulus/action send convergent projections to the GPi and SNr that, in turn, drive the encoded action to the frontal cortex (passing by the thalamus) (Fig. 3). The partially closed loops involving the GPe, STN, thalamus, and striatum may result in reverberation that facilitates the induction of LTP or LTD in the striatum. These loops may also have other modulatory functions in this system.

Still according to this model, the stronger association between the functional units of the striatum encoding an action triggered by a stimulus makes the occurrence of this association no longer unpredictable. As the novelty is reduced, the salience of the stimulus decreases and no further learning occurs. In this respect, this learning system is driven by novelty.

After a phasic dopamine response, the high concentration of dopamine takes longer to be cleared in the synapses of the NAc compared to the dorsal striatum [151,198,216]. In other words, the learning signal that allows synaptic plasticity lasts longer in the NAc than in the dorsal striatum. Accordingly, this learning signal is long enough to incorporate the evaluation of the reward value of the action outcome in the NAc, but not in the dorsal striatum. It explains why learning mediated by the NAc is driven by the reward outcome of the action, while learning mediated by the dorsal striatum forms S-R habits that are less sensitive to reward. This

model explains the gradual learning and many known properties of different types of procedural memories, such as allowing cue and egocentric navigation and their implicit, inflexible and associative nature.

Several postulates of the 'mosaic of broken mirrors' model need to be tested in future studies, particularly those that are the core of this model and differentiate it from other models of basal ganglia functioning: the postulation of the existence of repeated functional units in the striatum and their associative combination to form procedural memories. Nevertheless, these postulates are coherent with current findings, such as the "matrisomes" discovered by Flaherty and Graybiel [62,63], evidence for convergence and widespread projections from different regions of the cortex to the striatum [22,28,72,123,150,175,179,192,221,224], cue and egocentric navigation mediated by the basal ganglia [38,40,42,43,61,138,159], and place-related cells in the striatum that also encode movements [141], among other findings reported in this review. The remaining postulates of this model were mainly incorporated from existing models [2,8,64,80,86,144,158,173,214,216], except for the mechanism proposed to explain how the NAc and dorsal striatum encodes action-outcome expectancies and S-R habits, respectively.

A model can be considered as equivalent to a map of a new land based on the landmarks discovered by explorers that made blind navigations through it. This map results from the recreation of the cartographer that tries to accommodate the landmarks to his logic and imagination. This map is not an infallible orientation to new explorers, but it can provide routes to the exploration of this land. The explorers may confirm or not the locations in this land according to the map. Such is the case for the striatum according to the 'mosaic of broken mirrors' model; the map can be improved based on the outcome of these intents. We hope that the 'mosaic of broken mirrors' model may be of some help to guide the work of researchers interested in understanding how the basal ganglia mediate procedural learning.

Acknowledgements

The editorial comments of Rainer K.W. Schwarting, Philip Winn, Jorge Medina, and Ivan Izquierdo are acknowledged. This work was supported by grants of Institutos do Milenio (CNPq/MCT), Pronex Paraná, and Fundação Araucária, and FAPESP.

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4 PARTE 2

Em muitas situações de perigo, tal como no confronto com um predador, os animais apresentam uma série de comportamentos defensivos que vão da fuga à luta e que incluem comportamentos relacionados ao medo. Além disto, outros estímulos aversivos, tais como os que causam dor (choque elétrico, objetos cortantes, altas temperaturas, etc.), também desencadeiam reações de medo. Apesar dos mecanismos não serem totalmente esclarecidos, sabe-se que a DA participa da formação de memórias aversivas, através da modulação da plasticidade córtico-estriatal tanto no estriado dorsal como no NAc. Em especial, existem evidências de que os receptores D1 participam de respostas de esquiva ativa, pois a ação da DA ativando a via direta é necessária para o aprendizado da associação entre um estímulo preditivo de uma conseqüência (CS) e a escolha de uma resposta motora para evitar ou interromper sua apresentação. Dessa forma, o objetivo deste estudo foi avaliar o efeito de drogas dopaminégicas seletivas para os receptores D1, os receptores que são expressos na via direta, no teste da esquiva ativa de duas vias. O efeito destas drogas foi avaliado após a sua administração intraperitoneal, intra estriado dorsolateral e intra-NAc.

FAST AND SLOW LEARNING OF A CONDITIONED AVOIDANCE RESPONSE DEPENDS ON THE ACTIVATION OF D1 RECEPTORS IN THE NUCLEUS ACCUMBENS AND DORSOLATERAL STRIATUM, RESPECTIVELY

Trabalho submetido para publicação na revista European Neuropsychopharmacology, 2009.

Fast and slow learning of a conditioned avoidance response depends on the activation of D1 receptors in the nucleus accumbens and dorsolateral striatum, respectively.

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Running title: Roles of the NAc and DLS D1 receptors on conditioned avoidance learning

Abstract

We investigated the effect of the i.p., intra-NAc, and intra-DLS administration of the D1 dopamine receptor agonist SKF 81297 and of the D1 receptor antagonist SCH 23390 on learning of the two-way active avoidance, an aversively motivated conditioned avoidance response (CAR) task. Following administration into the NAc, SCH 23390 induced a learning impairment. In contrast, administration into the DLS resulted in impairment only in a second session, carried out 24 h after drug administration. The i.p. administration of this drug impaired learning scores in both sessions. No effect was observed after the administration of SKF 81297. These results were taken as evidence that, during CAR learning, dopamine is phasically released at an optimal level to activate D1 receptors in the NAc and DLS so that they can mediate, respectively, fast and slow learning.

Keywords: dorsolateral striatum; nucleus accumbens; D1 dopamine receptor; two-way active avoidance; learning; Parkinson's disease

Abbreviations:

CAR, conditioned avoidance response; CS, conditioned stimulus; DLS, dorsolateral striatum; DMS, dorsomedial striatum; DS, dorsal striatum; ITC , inter-trial crossings; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NAc, nucleus accumbens; SNr, substantia nigra pars reticulata; S-R, stimulus-response; S-R-O, stimulus-response-outcome; US, unconditioned stimulus.

1. Introduction

Unpleasant events can be avoided by the performance of a particular action in response to a warning stimulus. This kind of behavior, called conditioned avoidance response (CAR), can be assessed with the two-way active avoidance task in which a rat learns to avoid a footshock (unconditioned stimulus, US) by crossing to the opposite side of a two-chamber shuttle box in response to the presentation of a light or sound cue (conditioned stimulus, CS). Learning this task involves classical and instrumental conditioning (Bolles, 1970). The instrumental conditioning, or stimulus-response (S-R) learning, consists in turning off the CS or the US by crossing to the opposite chamber, behaviors called active avoidance and escape, respectively (Carvalho et al., 2009).

The learning of the two-way active avoidance task and other CAR tasks is impaired by systemic (Iorio et al., 1991; Ogren and Archer, 1994; Wadenberg et al., 2001; Reis et al., 2004) or intra-striatal administration of dopamine receptor antagonists, as well as by lesion of the substantia nigra pars compacta (Timar et al., 1974; Da Cunha et al., 2001). These findings support the mosaic of broken mirror model that proposes that S-R learning depends on the strengthening of the synapses between the cortical neurons representing the stimulus and the response and the striatal neurons to which they converge (Da Cunha et al., 2009; see also Frank et al., 2005; Wickens et al., 2007). Such synaptic plasticity occurs only when the contingency between the stimulus and the avoidance or escape response is new and triggers a phasic release of dopamine in the striatum. According to this view, the reinforced synapses constitute the memory trace that will guide the selection of the crossing action triggered by the cue. In this sense, learning to avoid an aversive outcome leads into similar mechanisms that support learning with appetitive reward, in that outcomes that are better than expected trigger phasic dopamine release and reinforce actions that preceded them (e.g., Seymour et al., 2005).

Even after learning, a basal level of striatal dopamine release is needed for the activation of the striatal neurons encoding the avoidance actions in response to the warning cue. The failure of Parkinson's disease patients to initiate habitual actions triggered by environmental stimuli probably results from the requirement for striatal dopamine to exceed a critical level (Lang and Lozano, 1998). Learning and action-selection abnormalities observed in other neurological and psychiatric diseases (e.g. schizophrenia, attention-deficit hyperactivity disorder) are also related to abnormal levels of striatal dopamine (Frank, 2008). According to the model proposed by DeLong, Alexander, and Crutcher and updated by many others, the basal ganglia forms a circuitry specialized in action-selection (Alexander et al., 1986; Albin et al., 1989; Frank and Claus, 2006; Redgrave et al., 2008). The initiation of an action selected in the striatum depends on the removal of the inhibition that the output nucleus of the basal ganglia (e.g. the rat substantia nigra pars reticulata, SNr) exerts over the thalamocortical neurons that triggers that action. The striatum can either stimulate or inhibit the SNr through a direct and an indirect pathway, respectively (Alexander et al., 1986) The direct pathway is composed of striatonigral GABAergic neurons expressing mainly D1-like dopamine receptors (Surmeier et al., 2007), the activation of which promote synaptic plasticity thought to be necessary for facilitation of adaptive behaviors (Frank, 2005; Wickens et al., 2007).

In summary, we hypothesize that, in the two-way active avoidance, the avoidance behavior associated with positive outcomes (i.e., the avoidance of an aversive outcome) depends on D1 receptor activation in striatonigral neurons triggered by the warning stimulus, and resulting disinhibition of the thalamic neurons, thereby facilitating the crossing action. Since rats can readily learn this task (Da Cunha et al., 2001), we suppose that the amounts of dopamine released in the striatum are optimal for its learning and performance. Therefore, we predict that D1 receptor antagonists will impair learning and performance, whereas D1 receptor agonists will not further improve performance.

There is evidence that different parts of the striatum mediate different stages of instrumental learning (Atallah et al., 2007; Nicola, 2007; Yin et al., 2008). The precise role of the dorsal striatum (DS) and nucleus accumbens (NAc) is not clear, with different authors putting forth different hypotheses. A common view is that the NAc is needed for learning new S-R relations, when actions are sensitive to their consequent outcomes. In contrast, the DS is thought to be involved in the automation of the response so that it can be readily triggered by the stimulus, thus becoming more resistant to devaluations of the outcome (Yin and Knowlton, 2006; Nicola, 2007). A similar, but not identical view is that the NAc and the DS are respectively involved in acquisition and performance of an instrumental behavior, as in the "actor-director" model (Atallah et al., 2007). We propose that the S-R associations are reinforced by the outcome more slowly in the DS than in the NAc (Belin et al., 2009). This would result in the S-R trace being rapidly or slowly learned and extinguished in the NAc and DS, respectively. Based on this hypothesis, we predict that the infusion of D1 receptor antagonists in the NAc or in the DLS of rats will respectively impair the avoidance scores in an immediate or delayed session of the two-way active avoidance. The aim of the present study is to test these predictions.

2. Experimental Procedures

2.1. Animals

Adult male Wistar rats from our own breeding stock, weighing 280-310 g at the beginning of the experiments, were used. The animals were maintained in a temperature-controlled room (22 ± 2 ^{o}C) on a 12/12-h dark/light cycle (lights on at 7:00 a.m.), with food and water available ad libitum. All experiments and procedures adopted for the in vivo studies were previously approved by the Animal Care and Use Committee of the Federal

University of Parana State and were in compliance with the guidelines of the National Institutes of Health.

2.2. Materials

The drugs and other chemical compounds used in these experiments were purchased from the following sources: chloral hydrate (Reagen, Rio de Janeiro, Brazil), ethanol, penicillin G-procaine (Bristol-Myers Squibb, New York, NY, USA), magnesium sulfate, ascorbic acid and propylene glycol (Synth, São Paulo, Brazil), sodium thiopental (Abbott Laboratories, Abbott Park, IL, USA), atropine sulfate, SKF 81297 (Sigma Chemical Co., St. Louis, MO, USA), and SCH 23390 (Tocris Bioscience, US, USA).

2.3. Surgery

Seven days before the initiation of the behavioral experiments the animals received atropine sulfate (0.4 mg/kg, i.p.) to suppress salivation, penicillin G-procaine (20,000U in 0.1 ml, i.m.), and were anesthetized with 3 ml/kg equitesin (1% sodium thiopental, 4.25% chloral hydrate, 2.13% magnesium sulfate, 42.8% propylene glycol, and 3.7% ethanol in water). Next, stainless guide cannulae (1 cm long, 23 gauge) were implanted bilaterally aimed 2 mm above the DS or NAc, according to the following coordinates, respectively : AP, 0.0 mm from bregma; ML $\pm$3.8 mm from midline; DV, -2.8 mm from the skull or AP, +1.7 mm from bregma; ML $\pm$1.6 mm from midline; DV, -5.2 mm from the skull. DS and NAc coordinates were adapted from the Atlas of Paxinos and Watson (2005). The cannulae were fixed with polyacrylic cement anchored to the skull with stainless-steel screws and plugged with stainless-steel plugs. After surgery, the animals were allowed to recover from anesthesia in a temperature controlled chamber and then placed back in their cages.

2.4. Drug administration procedures

SKF 81297 and SCH 23390 were dissolved in saline (NaCl 0.9%) and administered 20 min (i.p.) or immediately before (intra-NAc or intra-DLS) the first session of training (Day 1). Intra-NAc and Intra-DLS drug administrations (0.4 µl/side) were done bilaterally through a pair of 30-gauge needles extending 2 mm beyond their tips that were gently inserted into each cannula while the animals were held. The injector was linked to a 10 µl Hamilton syringe and the drug solution was injected over 1 min. The needles were retained in place for an additional minute. Sham animals received saline instead of the drug solution. The number of animals per group is indicated in the figure legends.

2.5. The two-way active avoidance task

The active avoidance test apparatus is an automated 23x50x23 cm shuttle-box (Insight Instruments, Ribeirao Preto, Brazil) with a front Plexiglass and a floor made of parallel 5 mm caliber stainless-steel bars spaced 15 mm apart. The box was divided into two compartments of the same size by a wall with a door that remained open during the tests. The animals were trained in two sessions, the second one carried out 24 hr after the first (day 1 and day 2). In each session, after 10 min (Day 1) or 5 min (Day 2) habituation, 40 light cues (CS: maximum duration of 20 s) were paired with a subsequent 0.5 mA footshock (US: maximum duration of 10 s, starting 10 s after the CS onset) until the animal crossed to the other compartment. The light cue consisted in two 30 W light bulbs that were centered on each side of the rear of the chambers. The animal could turn off the light and avoid the shock by crossing to the other chamber during the presentation of the CS. If the animal did not avoid the shock, it could escape from it by crossing to the other chamber. The time between each trial (inter-trial crossing, ITC) varied randomly, ranging

from 10 to 50 s. The number of active avoidances, escapes, non-responses, and ITC were recorded automatically by the apparatus.

2.6. Histology

At the end of the experimental procedures, all rats were sacrificed with an overdose of pentobarbital. To check for cannula placement, the animals of all groups were transcardially perfused with a saline solution, followed by 10% paraformalin; the brains were removed and post-fixed in the same fixative containing 20% sucrose for 48 h before sectioning. The brains were then cut in the frontal plane in 30 μm thick sections with a vibrating blade microtome (Leica, VT1000 S, Bensheim, Germany). The sections were mounted on gelatin-coated slides and stained with thionin. Only the animals with lesions limited to the DLS and the NAc were included in the present analysis.

2.7. Statistical analysis

Data were analyzed by two-way ANOVA with repeated measures (training day). Differences among groups were further analyzed by the post-hoc Newman-Keuls test. Differences were considered to be statistically significant when $p<0.05$.

3. Results

Table 1 shows the two-way ANOVA of the scores of the rats that received i.p., intra-NAc, and intra-DLS injections of the D1 receptor agonist SKF 81297, or of the D1 receptor antagonist SCH 23390. The significant differences among the groups, as analyzed by the post-hoc Newman-Keuls test, are indicated in Figs. 1-3. Learning is evidenced by the significant increase in the number of active avoidance responses on Day 2 compared to

Day 1 in the saline groups. The animals that responded to the light cue avoided the footshock, which resulted in a significant reduction in the number of escape responses on Day 2 compared to Day 1.

None of the two-way avoidance scores were significantly altered by the i.p., intra-NAc, or intra-DLS injections of SKF 81297 in any of the doses (see the scores in the Figs. 1-3 and the injection site placements in Fig. 4). On the other hand, the administration of SCH 23390 impaired the learning of this task.

As shown in Fig. 1, the systemic administration of this D1 receptor antagonist significantly reduced the number of avoidances on days 1 and 2. On Day 1, some animals that did not cross to the other side of the chamber when the light cue was turned on did not cross it after the shock started either. This behavior resulted in a significant increase in the number of non-responses on Day 1. However, the i.p. administration of SCH 23390 barely altered the free locomotor activity of the rats in the inter-trial period (ITC). In contrast, the systemic effect of SCH 23390 on rat learning of the two-way active avoidance seems to have resulted from the combined effects of this drug on the NAc and DLS, the former affecting the scores on Day 1 and the latter the scores on Day 2, as can be seen in Figs. 2 and 3.

As shown in Fig. 2, the intra-NAc infusion of 0.2 and 0.4 μg SCH 23390 reduced the number of avoidances on days 1 and 2. No significant difference was observed between the scores of avoidance for the two sessions of the animals that received saline. The dose of 0.2 μg SCH 23390 also significantly increased the number of non-responses, but not of escape responses, on Day 1. Conversely, the higher dose (0.4 μg), significantly increased the number of escape responses, but did not significantly reduce the number of non-responses on Day 1.

As shown in Fig. 3, the bilateral infusion of 0.4 μg SCH 23390 into the DLS significantly decreased the number of avoidances and increased the number of escape responses on Day 2, but not on Day 1. This treatment did not significantly alter the number of non-responses in either session.

Table 1: Effect of the i.p., intra-NAc, and intra-DLS injections of the D1 receptor agonist, SKF 81297, or of the D1 receptor antagonist, SCH 23390 on the two-way active avoidance: two-way ANOVA statistics.

SKF81297

	Group		Session		Interaction*	
I.P.	*F* [*df* = 3;31]	p	*F* [*df* = 1;31]	p	*F* [*df* = 3;31]	p
Avoidance	0.160	0.921	63.876	**0.000**	2.097	0.120
Escape	1.651	0.197	113.24	**0.000**	1.362	0.272
No response	3.276	0.034	0.780	0.383	0.968	0.419
ITC	0.716	0.549	7.612	**0.009**	3.741	**0.021**
Intra-Nac	*F* [*df* = 3;36]	p	*F* [*df* = 1;36]	p	*F* [*df* = 3;36]	p
Avoidance	0.940	0.431	51.655	**0.000**	3.679	**0.020**
Escape	1.114	0.356	53.664	**0.000**	3.193	**0.034**
No response	0.552	0.649	0.045	0.833	1.863	0.153
ITC	1.269	0.299	0.106	0.746	0.389	0.761
Intra-DLS	*F* [*df* = 2;22]	p	*F* [*df* = 1;22]	p	*F* [*df* = 2;22]	p
Avoidance	0.808	0.458	44.995	**0.000**	0.099	0.906
Escape	0.946	0.403	42.737	**0.000**	0.259	0.773
No response	0.394	0.678	3.130	0.090	1.037	0.371
ITC	0.165	0.848	0.239	0.629	2.149	0.140

SCH23390

	Group		Session		Interaction*	
I.P.	*F* [*df* = 3;30]	P	*F* [*df* = 1;30]	p	*F* [*df* = 3;30]	p
Avoidance	15.845	**0.000**	137.866	**0.000**	1.942	0.143
Escape	2.419	0.085	0.029	0.864	3.912	**0.018**
No response	8.965	**0.000**	51.402	**0.000**	6.869	**0.001**
ITC	3.217	**0.036**	13.257	**0.001**	0.428	0.734
Intra-Nac	*F* [*df* = 2;25]	P	*F* [*df* = 1;25]	p	*F* [*df* = 2;25]	p
Avoidance	10.940	**0.000**	64.310	**0.000**	0.214	0.808
Escape	6.412	**0.005**	21.178	**0.000**	0.575	0.569
No response	3.979	**0.031**	3.302	0.081	1.489	0.244
ITC	3.320	0.052	7.272	0.012	0.469	0.630
Intra-DLS	*F* [*df* = 2;20]	P	*F* [*df* = 1;20]	p	*F* [*df* = 2;20]	p
Avoidance	6.073	**0.008**	21.686	**0.000**	2.389	0.117
Escape	8.978	**0.001**	16.142	**0.000**	2.002	0.161
No response	0.164	0.849	6.729	**0.017**	0.392	0.680
ITC	3.000	0.072	1.265	0.274	1.800	0.191

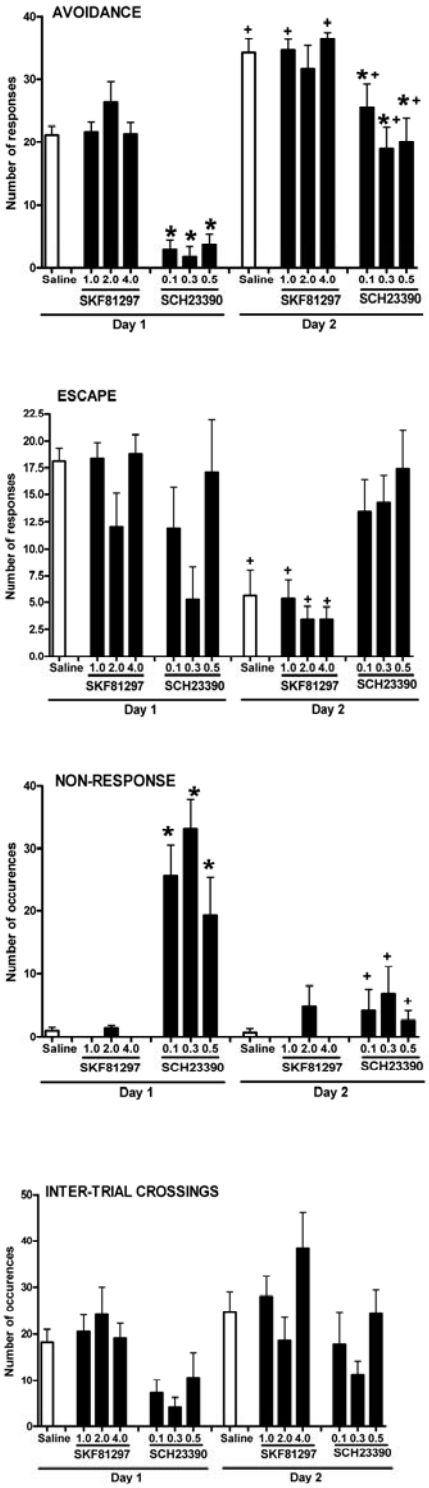

Figure 1. Effect of the i.p. infusion of the D1 dopamine receptor agonist, SKF 81297, and the D1receptor antagonist, SCH 23390, 30 min before the first session of training on learning of the two-way active avoidance task. The doses are expressed in mg/kg and the data are expressed as mean ± S.E.M. * $p<0.05$ compared to saline in the same day; + $p<0.05$ compared to the same group on Day 1. (N = 8-10 animals per group).

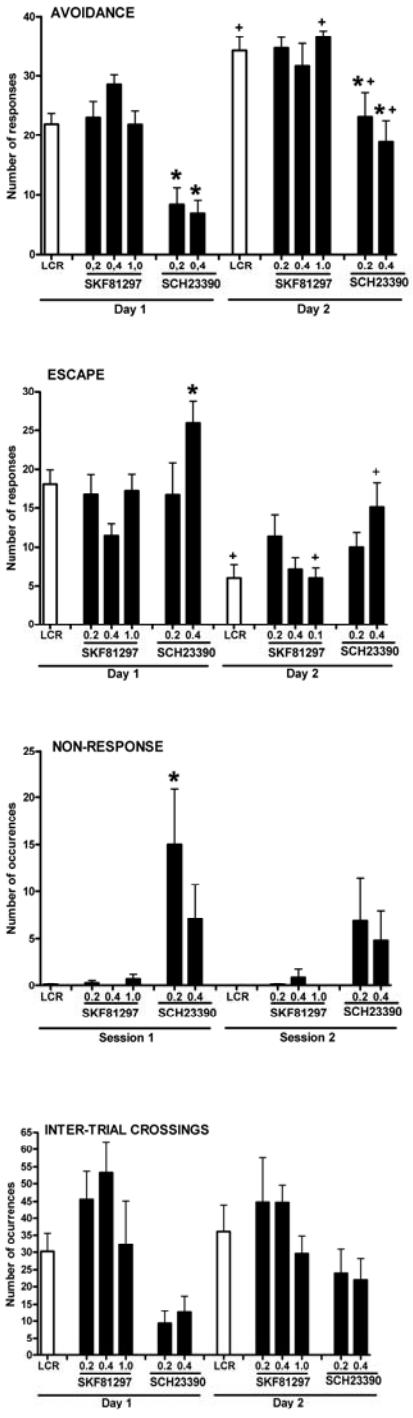

Figure 2. Effect of the pre-training infusion (Day1) of the D1 receptor agonist, SKF 811297, and the D1 receptor antagonist, SCH 23390, into the rat NAc on learning of the two-way active avoidance task. The doses are expressed in μg/side and the data are expressed as mean ± S.E.M. * $p<0.05$ compared to saline in the same day; + $p<0.05$ compared to the same group on Day 1. (N= 8-11 animals per group).

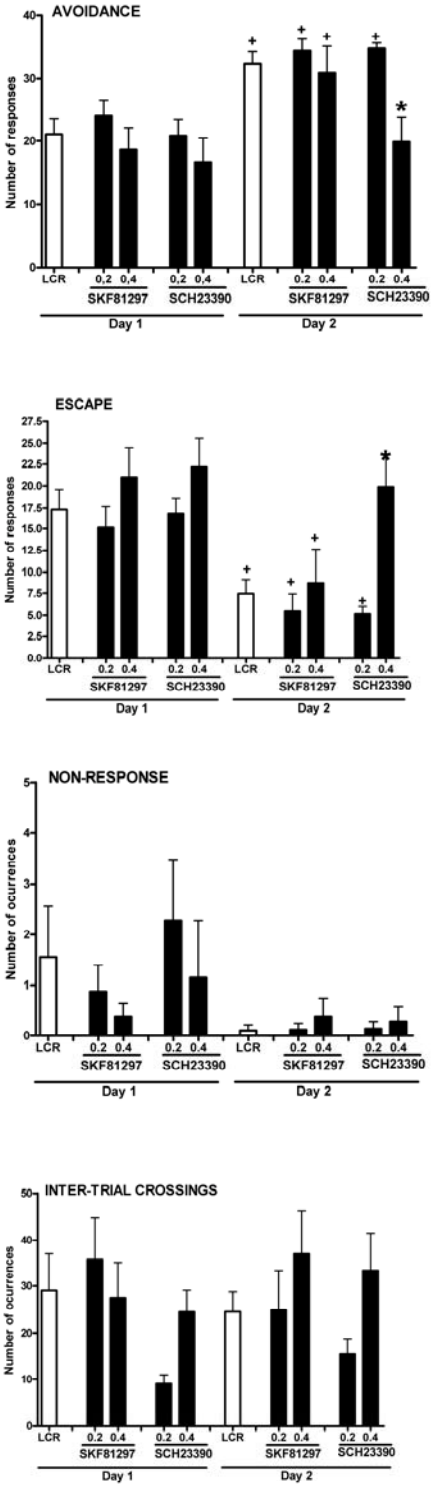

Figure 3. Effect of the pre-training infusion (Day1) of the D1 receptor agonist, SKF 811297, and the D1 receptor antagonist, SCH 23390, into the rat DLS on learning of the two-way active avoidance task. The doses are expressed in (g/side and the data are expressed as mean ± S.E.M. * $p<0.05$ compared to saline; + $p<0.05$ compared to the same group on Day 1. (N = 7-9 animals per group).

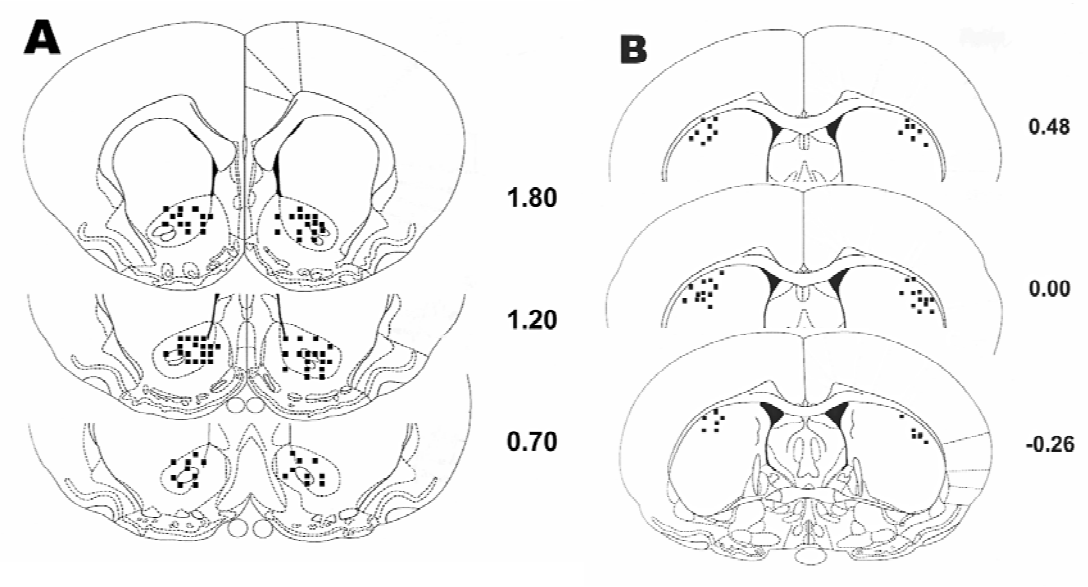

Figure 4. Schematic drawing of coronal sections indicating the injection site placements in the nucleus accumbens (A) and dorsal striatum (B). In the right of each section, the approximate distance (mm) from the bregma is indicated, according to the Paxinos and Watson Atlas (2005).

4. Discussion

The results of the present study are in agreement with our hypotheses that learning and performance of the two-way active avoidance depends on the activation of D1 receptors in the striatum and that their activation occurs at an optimum level by the endogenous release of dopamine. The phasic release of DA at an optimum level along the two-way avoidance sessions is supported by the lack of effect of systemic, intra-NAc, and intra-DLS administration of the D1 receptor agonist SKF 81297. The involvement of D1 receptor in the two-way avoidance is supported by the finding that systemic and intra-striatal administration of the D1 receptor antagonist, SCH 23390, impaired such learning.

These findings are in agreement with previous studies reporting that rats with a partial depletion of striatal dopamine induced by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are impaired to learn this task (Da Cunha et al., 2001; Gevaerd et al., 2001a; Gevaerd et al., 2001b). In addition, other studies reported that the systemic administration of SCH 23390 impaired CAR learning (Iorio et al., 1991; Ogren and Archer, 1994; Aguilar et al., 2000; Reis et al., 2004; Stuchlik and Vales, 2006). However, in contrast with the present results, (Stuchlik and Vales, 2006) reported that the systemic administration of the D1 receptor agonist A77636 in rats improved the learning of the active allothetic place avoidance, a CAR task in which the rats learn to avoid a room-frame-fixed shock sector on a continuously rotating arena. In another study such enhancement was not observed in pre-trained rats (Stuchlik, 2007).

In a recent review, (Nicola, 2007) summarized in 3 hypotheses the roles proposed for the striatal dopamine in learning: i) to facilitate the ability to respond to a predictable stimulus; ii) to facilitate the ability to respond to an unpredictable stimulus; iii) to participate in the action-selection in response to a stimulus through the direct and indirect pathways. The mosaic of broken mirrors model proposes that the striatum and the midbrain dopaminergic

neurons play a role in all of these processes at the same time (Da Cunha et al., 2009). According to this model, cortical representation of the stimulus and action are projected to the striatum forming repetitive units, some of them overlapping. When the novelty of a situation induces a phasic release of dopamine, the corticostriatal synapses of the units that are activated at the same time by the stimulus and by the response are reinforced. The occurrence of this synaptic potentiation in the direct pathway will make this action to be selected more easily in the presence of this stimulus. Further, the tonic release of dopamine facilitates the triggering of this action by the stimulus by acting on D1 receptors expressed in the striatonigral neurons, i.e., in the direct pathway. Our finding that a D1 receptor antagonist impaired both the learning and performance of the action of crossing the shuttle box, conditioned to the light stimulus, is in agreement with this model, and with computational versions thereof (Frank, 2005; Frank and Claus, 2006).

The increase in the number of non-responses in the animals treated with SCH 23390 strongly suggests the involvement of the direct pathway of the NAc in the initiation of the conditioned and unconditioned responses (see Fig. 2). Similar non-response effect of SCH 23390 in rodents has also been reported in previous studies (Morelli and Dichiara, 1985; Fletcher and Starr, 1988). However, this treatment did not cause immobility and did not significantly decrease the locomotion of the animals across the two chambers of the shuttlebox.

The results of the present study also support the hypothesis that the NAc mediates fast learning (potentially goal-directed or action-outcome), whereas the DLS mediates a slow learning of S-R associations. According to this hypothesis, the intra-NAc infusion of a D1 receptor antagonist would affect the learning scores of the two-way active avoidance in the first session mostly. This prediction was confirmed. As can be seen in Fig. 2, learning was profoundly impaired after the rats received an intra-NAc infusion of SCH 23390. However, the following day they learned this task normally – their scores were similar to those of the controls in the first training session. The

prediction that the infusion of SCH 23390 into the DLS would affect learning in the second, but not in the first training session, was also confirmed, as can be seen in Fig. 3.

These findings are in agreement with those reported in a recent study (Yin et al., 2009). They found an increase in the synaptic strength in the DMS, but not in the DLS, of mice after they had been trained to run in a rotarod. In contrast, after 8 days of training, the synaptic strength increased substantially in the DLS, but not in the DMS. They also found that the i.p. administration of SCH 23390 impaired the performance of mice in the early, but not in the late phase of the rotarod learning. Based on this finding, they proposed that, after extensive training, skill learning becomes independent of D1 receptor activation. Like our study, the Yin et al. study suggests that the activation of D1 receptors in the striatum is needed for the learning of many (if not all) kinds of procedural memory tasks. Taking both studies into account, one can say that the role of the DMS in the learning of these tasks is more related to the NAc than to the DLS. This conclusion is in agreement with the view that habit learning involves the sequential activation of striatal regions progressing from the ventral and medial to the dorsal and lateral parts (Yin and Knowlton, 2006; Nicola, 2007; Wickens et al., 2007; Belin et al., 2009). We did not test the lack of effect of the i.p. administration of SCH 23390 after 8 days of training in the two-way active avoidance task as (Yin et al., 2009) did for the training in the rotarod. Conversely, they did not test the effect of pretraining i.p. or intra-DLS administration of this D1 receptor antagonist on performance the following day. However, taken together, our results suggest that the activation of D1 receptors in the DLS is needed for the slow learning of a procedural memory task and that its performance may eventually become independent of such activation after extensive training. On the other hand, the activation of D1 receptors in the NAc seems to be needed for the rapid learning of these tasks.

In the review by (Nicola, 2007) mentioned above, it is proposed that “the DS controls action-selection in response to temporarily predictable stimuli

whereas the NAc controls action-selection in response to temporarily unpredictable stimuli". Our proposal that the NAc and the DLS mediates fast and slow learning of S-R associations can provide a mechanism to account for Nicola's hypothesis. If the learning and extinction of S-R associations mediated by the NAc is quick, it will constantly depend on new learning. This mechanism would support fast adaptation to environments that are constantly changing, being unpredictable in a long-term perspective. Such mechanism may also explain why learning mediated by the NAc and DMS is so sensitive to reward devaluation (Yin et al., 2005; Yin et al., 2008; Balleine et al., 2009). On the other hand, if the DS (and more strongly the DLS), mediates a slow learning and extinction of the S-R associations, it will produce more stable memories, proper for action-selection in stable environments, where the outcome of a chosen action can be predicted by their occurrence in the past. Due to the resistance of these memories to extinction, once the outcome of a response in the presence of a stimulus is learned, it can be more efficiently controlled by the warning stimulus. This may explain the resistance of this S-R habit to a devaluation of the outcome.

Another study by (Atallah et al., 2007) addressed the question of the differential roles for the NAc and DS in learning. They proposed that the NAc plays a role in learning and the DS in performance of instrumental learning, like in the actor-critic model of reinforcement learning (Sutton and Barto, 1998). Their hypothesis was supported by the finding that rats' performance in a test session of an instrumental task (entering a chamber with a particular odor to get a food reward) was impaired by the inactivation of the DS with the GABA-A agonist muscimol. However, the inactivation of the DS during the 3 previous training sessions did not affect the learning of this task, as revealed by their good performance in a subsequent drug-free session. On the other hand, the inactivation of the NAc during the training sessions affected the test scores, even when the animals had not received a pre-test infusion of muscimol in the NAc. Although this study did not discriminate between the DMS and the DLS, its results cannot be easily explained by the hypothesis

that the NAc mediates a fast and the DLS a slow learning. Neither can our data be explained by the Atallah et al. hypothesis that the NAc mediates learning and the DS the performance of procedural tasks, given that learning impairment on Day 2 was still observed in the DLS-infused rats despite the absence of drug on that day. This contradiction suggests that the D1 and GABA-A receptors in the striatum are involved in mechanisms that are more complex than the fast/slow learning and the actor-director hypotheses can explain.

In summary, in the present study we present evidence that the activation of D1 receptors in the striatum is needed for the learning of the two-way active avoidance, a task in which a rat learns to avoid a footshock by performing a crossing action in response to a warning stimulus. Our results also suggest a differential role for the D1 receptors of the NAc and DLS in the mediation of a fast and slow learning of S-R-O associations, respectively.

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5 PARTE 3

Nesse trabalho propomos validar o modelo animal de lesão unilateral com MPTP para o screening de drogas com potencial efeito para tratar a bradicinesia da fase inicial da DP. O comportamento rotatório em animais é uma ferramenta útil para testar drogas com ação sobre o sistema dopaminérgico. A infusão unilateral de 6-OHDA no feixe prosencefálico medial de ratos provoca morte de todos os neurônios dopaminérgicos da SNc, mimetizando o que ocorre em uma fase adiantada da DP. Esses animais quando desafiados com drogas agonistas dopaminérgicos diretos apresentam comportamento rotatório contraversivo a lesão, por outro lado, o desafio com agonistas dopaminérgicos indiretos causam rotações ipsiversivas. Por outro lado, a administração intra-nigral de MPTP na SNc dos ratos ocasiona morte parcial de neurônios dopaminérgicos, reproduzindo o estágio inicial da DP. Esses animais, ao oposto do que ocore com os animais lesados por 6-OHDA, apresentam rotações ipsiversivas após o desafio tanto com apomorfina, anfetamina e a maioria das drogas de uso clínico que foram testadas: levodopa + benserazida, levodopa + benserazida + entacapone, levodopa + benserazida + seleginina, pramipexol. Quando desafiados com amantadina, os ratos 6-OHDA apresentaram rotações ipsiversivas e os ratos MPTP rotações contraversivas. O fato de que as drogas que causaram alterações significantes no comportamento rotatório dos animais MPTP serem as mesmas usadas no tratamento da fase inicial da DP, valida este modelo para testar drogas antiparkinsonianas da fase inicial da DP.

PHARMACOLOGICAL VALIDATION OF THE 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) RAT MODEL OF THE EARLY STAGE OF PARKINSON'S DISEASE

Trabalho submetido para publicação na revista "The Journal of Pharmacology and Experimental Therapeutics", 2009.

Pharmacological validation of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) rat model of the early stage of Parkinson's disease

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The number of text pages (33)

Table (1)

Figures (4)

References (62)

The number of words in the Abstract (230)

Introduction (724)

Discussion (1500)

A list of nonstandard abbreviations used in the paper

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: MPTP

6-hydroxydopamine: 6-OHDA

Medial forebrain bundle: MFB

Parkinson's disease: PD

Substantia nigra *pars compacta*: SNpc

ABSTRACT

The present study aims to validate the use of rats treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) as a model for screening drugs to treat the early phase of Parkinson's disease (PD) and compare it with the 6-hydroxydopamine (6-OHDA) rat model. The unilateral infusion of 16 µg 6-OHDA into the medial forebrain bundle or of 100 µg MPTP into the *substantia nigra pars compacta* of rats caused the depletion of 98% and 70% striatal dopamine, respectively. Both groups presented dose-dependent turning behavior in response to systemic treatments with apomorphine and amphetamine and responded to entacapone, selegiline, pramipexole, or amantadine. Entacapone and selegiline were administered together with levodopa and benserazide. However, only the 6-OHDA rats responded to levodopa plus benserazide, and biperiden. In addition, except for amphetamine, the MPTP rats responded to the drugs mentioned above with turns in the opposite direction of those observed in 6-OHDA rats. This amazing result is suggestive that MPTP rats can model the neural alterations of the early phase of PD, that are different from those that occur in the end phase, as modeled by 6-OHDA rats. Furthermore, the fact that the drugs that caused significant alterations in the turning behavior of the MPTP rats are the same that have been effectively used to treat the early phase of PD, encourages the use of this model for the screening of drugs to treat PD patients in this condition.

Introduction

The discovery that most of the motor impairments observed in Parkinson's disease (PD) result from a deep depletion of dopamine in the striatum led to the development of effective dopaminergic drugs to treat this disease (1999). The antiparkinsonian effect of drugs can be predicted by their property to induce a turning behavior in rats with unilateral lesion of the *substantia nigra pars compacta* (SNpc) caused by intracerebral infusion of the neurotoxin 6-hydroxydopamine (6-OHDA) (Ungerstedt and Arbuthnott, 1970; Schwarting and Huston, 1996a; Schober, 2004).

The predictive basis of this turning behavior was better studied by challenging the animals with the dopamine receptors agonist, apomorphine, and the indirect dopamine receptor agonist, amphetamine. Amphetamine is considered an "indirect agonist" because it induces the release of dopamine (Schwarting and Huston, 1996b). In addition, the 6-OHDA rat model is effective in predicting the antiparkinsonian effects of practically all drugs that are currently being used, like the dopaminergic drugs levodopa, selegiline, pramipexole and even those acting through non-dopaminergic mechanisms, like the antimuscarinic agent biperiden and the NMDA receptor antagonist amantadine (Morelli, 1997; Ives et al., 2004; Poewe, 2004; Dekundy et al., 2006; Thobois, 2006). The unilateral infusion of 6-OHDA into the rat medial forebrain bundle (MFB) is considered to be a model of the end stage of PD (Deumens et al., 2002; Yuan et al., 2005). At this stage, patients have lost most of the dopamine neurons in the SNpc (Braak et al., 2003) and present

increased postsynaptic dopamine receptor density and/or supersensitivity in the putamen (Seeman and Niznik, 1990). Today, there is a great demand for drugs that can treat the impairments observed in the early stage of PD. In addition to their symptomatic effects, some of these drugs can prevent late dyskinesia (Wu and Frucht, 2005). It would be interesting to develop other sensitive and simple models that could be used for the screening of drugs effective during the early stage of PD. Here we propose that rats treated with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) can fulfill this demand.

MPTP was discovered after drug addicts were accidentally intoxicated with this drug and then presented symptoms clinically indistinguishable from idiopathic PD (Langston et al., 1983). In primates, this neurotoxin causes severe and selective loss of dopaminergic neurons in the SNpc (Chassain et al., 2001). Since then, MPTP-treated monkeys have been successfully used as a model of PD, while few studies have employed rats because these animals proved to be more resistant to the neurotoxic effect of MPTP (Giovanni et al., 1994; Smeyne and Jackson-Lewis, 2005). However, Harik et al. (1987) reported that the intranigral infusion of a high dose of MPTP into the rat SNpc caused its partial lesion and depletion of striatal dopamine in a more selective way than 6-OHDA. Later on, we have shown that, when MPTP is infused bilaterally into the SNpc, it causes the same memory impairments in the water maze test as observed in 6-OHDA-lesioned rats (Ferro et al., 2005). Compared to 6-OHDA rats, the tyrosine hydroxylase (TH)-immunostained cell loss in the SNpc, dopamine depletion in the striatum, and

animal mortality in MPTP rats were markedly lower. Since then, the MPTP rat model has been successfully used to study cognitive alterations qualitatively similar to those observed in the early stage of PD before the onset of motor impairments (Da Cunha et al., 2001; Gevaerd et al., 2001a; Gevaerd et al., 2001b; Da Cunha et al., 2002; Miyoshi et al., 2002; Da Cunha et al., 2003; Bellissimo et al., 2004; Braga et al., 2005; Perry et al., 2005; Kumar et al., 2008; Kumar et al., 2009).

The aim of the present study was to propose the use of the turning behavior test of rats with unilateral lesions induced by MPTP as a model of motor disabilities of the early stage of PD and validate it as a screening test for putative drugs to treat such disabilities. We compared the turning behavior response of the unilaterally MPTP-lesioned rats challenged with apomorphine, amphetamine or with some drugs used in the early and in the end phases of PD with the response of unilaterally OHDA-lesioned rats challenged with the same drugs. The qualitatively different and dose-dependent results presented in this study suggest that the MPTP model can be effectively used for the screening of drugs putatively useful to treat the motor impairments observed in the early stage of PD.

Methods

Animals. Adult male Wistar rats from our own breeding stock weighing 280-310 g at the beginning of the experiments were used. The animals were maintained in a temperature-controlled room (22 ± 2^{o}C) on a 12/12-h

dark/light cycle (lights on at 7:00 a.m.), with food and water available ad libitum. All the behavioral experiments were conducted between 7:00 a.m. and 1:00 p.m. All experiments and experimental procedures adopted for the in vivo studies were previously approved by the institution's ethics committee for research on laboratory animals and were in accordance with the standards of the European Community Council's directives (86/609/EEC).

Different sites of infusion were chosen for MPTP (SNpc) and 6-OHDA (MFB) based on previous findings showing that they result in more robust and reproducible SNpc lesions and turning behavior (Schwarting and Huston, 1996a; Deumens et al., 2002). In our experience, the infusion of MPTP into the rat MFB, compared to the infusion of 6-OHDA into the same structure, causes fewer loss of dopamine neurons in the SNpc or turning behavior in rats challenged with apomorphine or amphetamine (Tadaiesky et al., 2008). Previous findings also guided the choice of a higher dose of MPTP compared to 6-OHDA. Almost total lesion of the SNpc can be achieved by the infusion of 16 µg 6-OHDA into the MFB (Truong et al., 2006), whereas 10 µg MPTP causes minimal loss of SNpc dopamine neurons when infused directly into the SNpc daily for 5 days (Chiueh et al., 1984). However, a loss of 50-70% of dopamine neurons can be achieved when 100-200 µg MPTP is infused into the SNpc (Harik et al., 1987; Gevaerd et al., 2001a; Ferro et al., 2005).

In the present study, the animals received atropine sulfate (0.4 mg/kg, i.p.) to suppress salivation and penicillin G-procaine (20,000 U in 0.1 ml, i.m.) to avoid infection, and were anaesthetized with 3 ml/kg equitesin (1% sodium thiopental, 4.25% chloral hydrate, 2.13% magnesium sulfate, 42.8%

propylene glycol, and 3.7% ethanol in water). The MPTP-rats received 3 i.p. injections of 120 mg/kg acetaldehyde 10 min before, at the beginning, and immediately after surgery to increase the effectiveness of the neurotoxin. MPTP or 6-OHDA (Sigma Chemical Co., St. Louis, MO) was infused through a 30-gauge stainless needle at a flow rate of 0.25 µl/min. The needle was maintained in place for more than 2 min to avoid reflux. MPTP (100 µg, 1 µl in saline) was infused into the left, right, or both (bilateral) sides of the SNpc according to the following coordinates: anteroposterior (AP), -5.0 mm from the bregma; mediolateral (ML), ± 2.1 mm from the midline; dorsoventral (DV), -7.7 mm from the skull; nose bar, - 3.3 mm from the interaural line. 6-OHDA (16 µg in 2 µl saline supplemented with 0.2% ascorbic acid, 0.25 µl/min) was infused into the left MFB according to the following coordinates: AP, -1.9 mm; ML, - 1.9 mm; DV, -7.2 mm. The stereotaxic coordinates were adapted from the Atlas of Paxinos and Watson (2005). Sham-operated animals were submitted to the same procedure, but saline was infused instead of the neurotoxins. After surgery, the animals were allowed to recover from anaesthesia in a temperature-controlled chamber and were then returned to their home cage. The animals were fed a pasty diet consisting of a mixture of rats crumbled chow and water for the first 5 postoperative days. This procedure reduced body weight loss and, consequently, mortality.

Turning behavior test. One week after surgery, the animals were challenged with a subcutaneous injection of apomorphine (Sigma) or an i.p. injection of saline, amphetamine (Sigma) levodopa plus benserazide,

entacapone, selegiline, pramipexole, biperiden or amantadine. Selegiline and entacapone were administered together with levodopa and benserazide. The doses and number of animals per group can be seen in the figure legends. These chemicals were purchased from the following laboratories: Levodopa, benserazide (Roche, Palo Alto, CA), entacapone (Orion Corp., Espoo, Finland), selegiline (Biosintética, São Paulo, Brazil), pramipexole (Boehringer Ingelheim Pharma, Ingelheim am Rhein, Germany), biperiden (Cristália, São Paulo, Brazil) or amantadine (Eurofarma, São Paulo, Brazil). Immediately after the injection, the rats were individually placed in a round plastic container (28 cm in diameter and 25 cm high) and the number of 360º turns toward the side of the lesion (ipsiversive) and toward the opposite side (contraversive) was recorded for 2 h (Ungerstedt and Arbuthnott, 1970). On the next day, the same rats were challenged with 1 mg/kg (MPTP rats) or 0.1 mg/kg (6-OHDA rats) apomorphine and turning behavior was scored as described above. Data of animals that made fewer than 50 turns (ipsiversive for MPTP and contraversive for 6-OHDA rats) during the second session were excluded from the analysis. This criterion was adopted to avoid the inclusion of data from animals in which the neurotoxic lesion procedure was not effective (Schwarting and Huston, 1996b).

TH immunohistochemistry. After the behavioral tests, the animals were killed by decapitation and their dorsal striatum was removed for the

determination of dopamine concentration (see below). 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 with a sliding microtome on the frontal plane and collected from the caudal diencephalon to the caudal midbrain. The sections were immunostained with a monoclonal antibody against TH (diluted 1:5000, purchased from Incstar Corp., Stillwater, MN, USA). The antigen-antibody complex was localized with an ABC Elite kit (Vector Laboratories, Burlingame, CA, USA). Slides were then dehydrated and coverslipped with DPX.

Determination of dopamine by HPLC-electrochemical detection. Endogenous levels of dopamine were assayed by reverse-phase HPLC with electrochemical detection. The system consisted of a Synergi Fusion-RP C-18 reverse-phase column (150 x 4.6 mm i.d., 4-µm particle size, Phenomenex, Torrance, CA, USA), an L-ECD-6A electrochemical detector (Shimadzu, Kyoto, Japan), and an LC-10AD pump (Shimadzu).The column was maintained inside a temperature-controlled oven (30ºC, Shimadzu). The oxidation potential was fixed at + 0.80 V using an Ag/AgCl working electrode. The tissue samples were homogenized with a Vibra-Cell ultrasonic cell disrupter (Sonics, Newtown, CT, USA) in 0.1 M perchloric acid. After centrifugation at 15,000 x g for 30 min, 20 µl of the supernatant was injected into the chromatograph. The mobile phase, used at a flow rate of 1 ml/min, had the following composition: 15.7 g citric acid, 471.5 ml HPLC-grade water,

78 mg heptanesulfonic acid, 20 ml acetonitrile, and 10 ml tetrahydrofuran, pH 3.0. The peak areas of the external standards were used to quantify the sample peaks.

Data analysis. All results are reported as the mean ± S.E.M. Differences among groups were analyzed by one-way ANOVA, followed by the Newman-Keuls test. Data regarding the time course of apomorphine-induced turns are reported as the number of ipsiversive - contraversive turns and were analyzed by two-way ANOVA for repeated measures, followed by the Newman-Keuls test. Differences were considered to be statistically significant when $P < 0.05$.

Results

Rats unilaterally lesioned with either MPTP or 6-OHDA presented dose-dependent turning behavior when challenged with apomorphine or amphetamine (Fig. 1). Both MPTP and 6-OHDA rats showed ipsiversive turning behavior when challenged with amphetamine. However, apomorphine caused ipsiversive turning behavior in MPTP rats and contraversive turning behavior in 6-OHDA rats. Another difference was that higher doses of the challenging drug were required to cause the same scores of turns in MPTP

rats compared to 6-OHDA rats. The dose-effect range for apomorphine was 0.25-1.0 mg/kg for MPTP rats ($F(3,34) = 8.87$, $P < 0.001$) and 0.01-0.2 mg/kg for 6-OHDA rats ($F(4,42) = 28.39$, $P < 0.001$). When challenged with amphetamine, the doses ranged from 1 to 10 mg/kg for MPTP rats ($F(4,41) = 11.75$, $P < 0.001$) and from 0.1 to 1.0 mg/kg for 6-OHDA rats ($F(4,39) = 12.54$, $P < 0.001$). A small, but significant, decrease of ipsiversive turns was observed in 6-OHDA rats challenged with apomorphine compared to the saline group ($F(4,42) = 18.36$; $P < 0.001$, one-way ANOVA; $P < 0.001$, Newman-Keuls test). No significant effects were observed for contraversive turns made by MPTP rats challenged with apomorphine ($F(8,70) = 0.53$, $P = 0.82$) or amphetamine ($F(4,41) = 0.25$, $P = 0.90$), or for contraversive turns made by 6-OHDA rats challenged with amphetamine ($F(4, 39) = 1.93$, $P = 0.12$).

As illustrated in Fig. 2, the time courses of the effect of apomorphine on the turning behavior of MPTP and 6-OHDA rats presented opposite directions (Supplementary video 1) and were significantly different from sham-lesioned rats: toxin effect, $F(2,28) = 91.35$, $P < 0.001$; time interval effect, $F(11,308) = 4.23$, $P < 0.001$; interaction toxin x time interval effect, $F(22,308) = 4.47$, $P < 0.001$, two-way ANOVA). The Newman-Keuls test demonstrated significant differences between the scores of the three groups at all time intervals. The turning behavior remained almost constant for up to 35 min after the challenge of MPTP rats and for at least 60 min of 6-OHDA rats.

Turning behavior in MPTP and 6-OHDA rats was also observed after a challenge with some drugs used to treat PD (Fig. 3). However, as observed after the challenge with apomorphine, when administered in addition to levodopa and benserazide, those drugs caused turns in opposite directions in MPTP and 6-OHDA rats. Entacapone, selegiline and pramipexole caused ipsiversive turns in MPTP rats ($F(6,51) = 8.80$, $P < 0.001$, one-way ANOVA; see the figure legend for post-hoc comparisons) and contraversive turns in 6-OHDA rats $F(6,51) = 13.03$, $P < 0.001$, one-way ANOVA). The administration of levodopa and benserazide only induced contraversive turns in the 6-OHDA, but not in MPTP, rats. Amantadine caused ipsiversive turns in 6-OHDA rats ($F(6,51) = 10.85$, $P < 0.001$, one-way ANOVA) and contraversive turns in MPTP rats $F(6,51) = 4.55$, $P < 0.001$, one-way ANOVA). Biperidene induced ipsiversive turns in 6-OHDA, but not in MPTP, rats.

The analysis of immunostained sections by light microscopy revealed a smaller loss of TH-immunoreactive neurons in the SNpc of MPTP-lesioned rats compared to rats injected with 6-OHDA, as illustrated in Fig. 4. In addition, MPTP caused neuronal loss which was mainly restricted to the SNpc, spreading only modestly to the neighboring brain areas. On the other hand, 6-OHDA caused a massive loss of TH-immunoreactive neurons in the SNpc, ventral tegmental area, and retrorubral field (data not shown). Nissl staining showed that in both cases the *substantia nigra pars reticulata* and the surrounding non-dopaminergic neurons were not affected (data not shown).

The effect of these neurotoxins on the levels of dopamine is shown in Table 1. One-way ANOVA showed that MPTP caused a partial, but significant, loss of dopamine ($F(9,60) = 12.69$, $P < 0.001$) on the lesioned side compared to the other side of the striatum ($P < 0.05$, Newman-Keuls test) and compared to sham animals ($P < 0.05$, Newman-Keuls test). The same analysis showed that 6-OHDA caused an almost complete and significant loss of dopamine on the lesioned side compared to the other side of the striatum ($P < 0.05$, Newman-Keuls test) and compared to sham animals ($P < 0.05$, Newman-Keuls test).

The results in Supplementary Fig. 1 show that the ipsiversive turning behavior of MPTP rats challenged with apomorphine was independent of the lesioned side. Both left and right side MPTP-lesioned rats made turns toward the lesioned side (clockwise: $F(3,36) = 13.59$, $P = 0.001$; $P < 0.05$, post hoc Newman-Keuls test; counterclockwise: $F(3,36) = 12.24$, $P = 0.001$; $P < 0.05$, post hoc Newman-Keuls test). Bilaterally lesioned rats did not significantly differ from sham rats in terms of the number of clockwise or counterclockwise turns ($P > 0.2$, post hoc Newman-Keuls test).

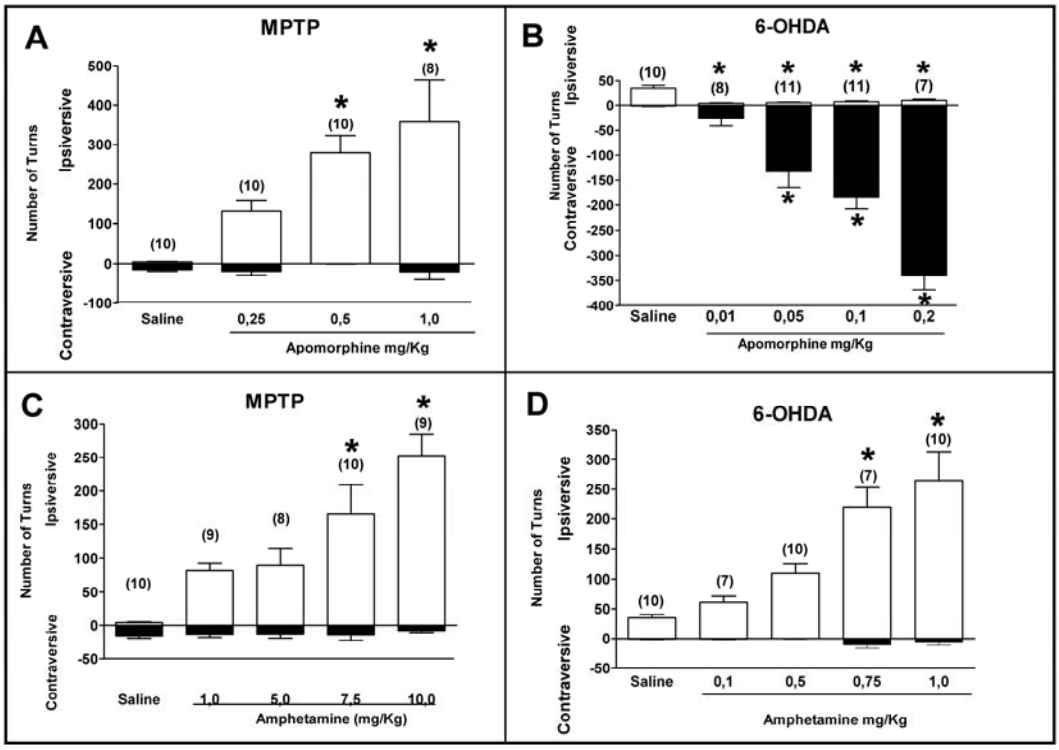

Fig. 1 Turning behavior of unilaterally MPTP- and 6-OHDA-lesioned rats challenged with apomorphine or amphetamine. Data are reported as the number of ipsiversive (positive scale) and contraversive turns (negative scale) counted over the first 30 min after the drug challenge. The number of animals per group are shown above the bars. * $P < 0.05$ compared to the saline group (Newman-Keuls test after one-way ANOVA).

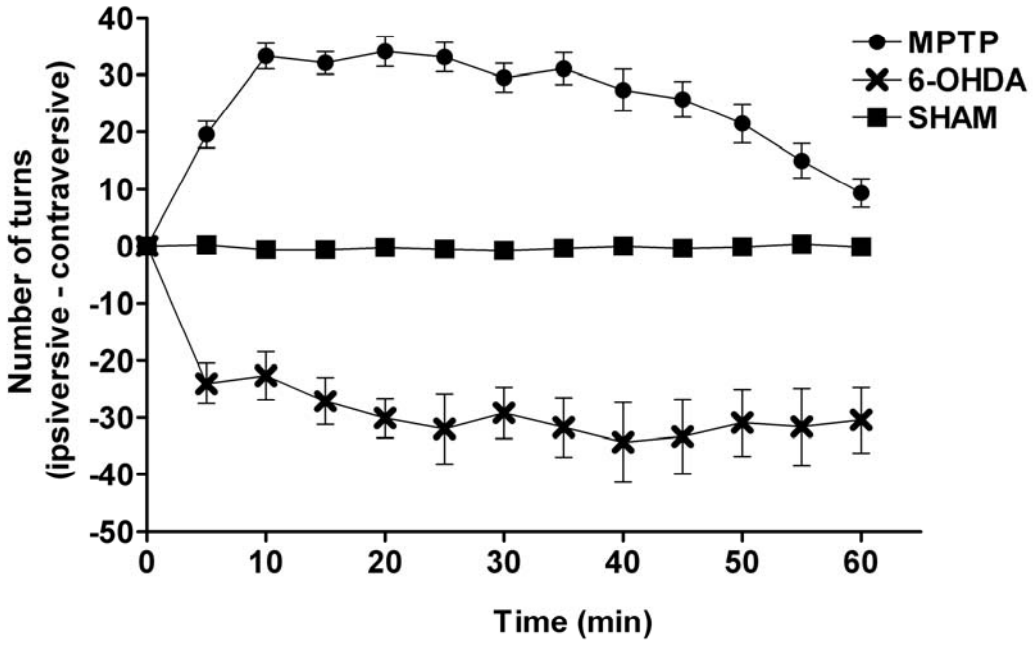

Fig. 2 Time course of the turning behavior of unilaterally MPTP- or 6-OHDA-lesioned rats after a challenge of 1 mg/kg apomorphine. Data are reported as the number of ipsiversive-contraversive turns counted at 5-min intervals. Number of animals per group: sham, n = 8; MPTP, n = 11; 6-OHDA, n = 12. Two-way ANOVA followed by the Newman-Keuls test demonstrated significant differences ($P < 0.05$) between the scores of the three groups at all time intervals.

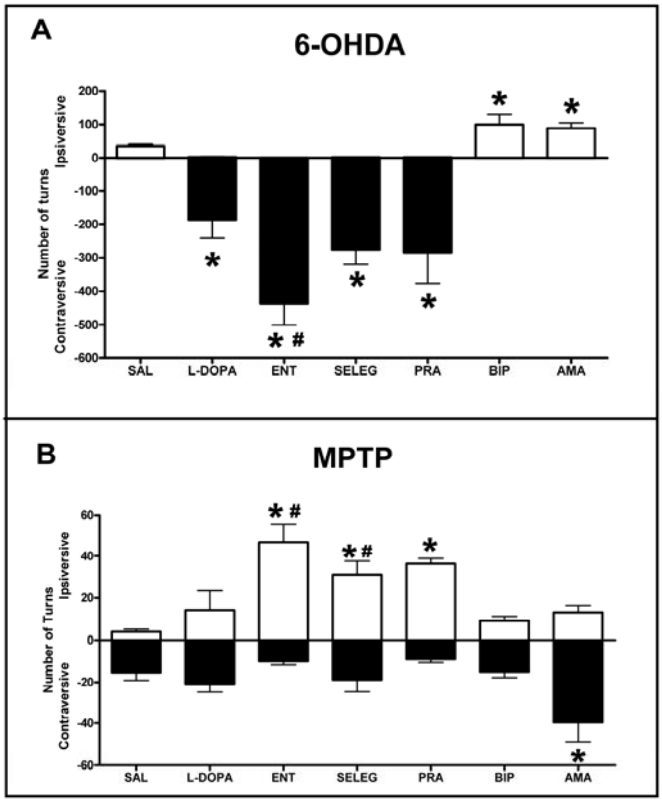

Fig. 3 Turning behavior of unilaterally MPTP- and 6-OHDA-lesioned rats. The 6-OHDA rats were challenged with the i.p. injection of saline (SAL), 50 mg/kg levodopa plus 12,5 mg/kg benserazide (L-DOPA), 50 mg/kg levodopa plus 12,5 mg/kg benserazide plus 30 mg/kg entacapone (ENT), 50 mg/kg levodopa plus 12,5 mg/kg benserazide plus 2 mg/kg selegiline (SELEG), 1 mg/kg pramipexole (PRA), 3 mg/kg biperidene (BIP) or 20 mg/kg amantadine (AMA). The MPTP rats were challenged with the double of the doses of the same drugs. Data are reported as the number of ipsiversive (positive scale) and contraversive turns (negative scale) counted over the first 2 h after the drug challenge. (N= 8 rats per group). * $P < 0.05$ compared to saline group, # $P < 0.05$ compared to L-DOPA group, Newman-Keuls test after one-way ANOVA.

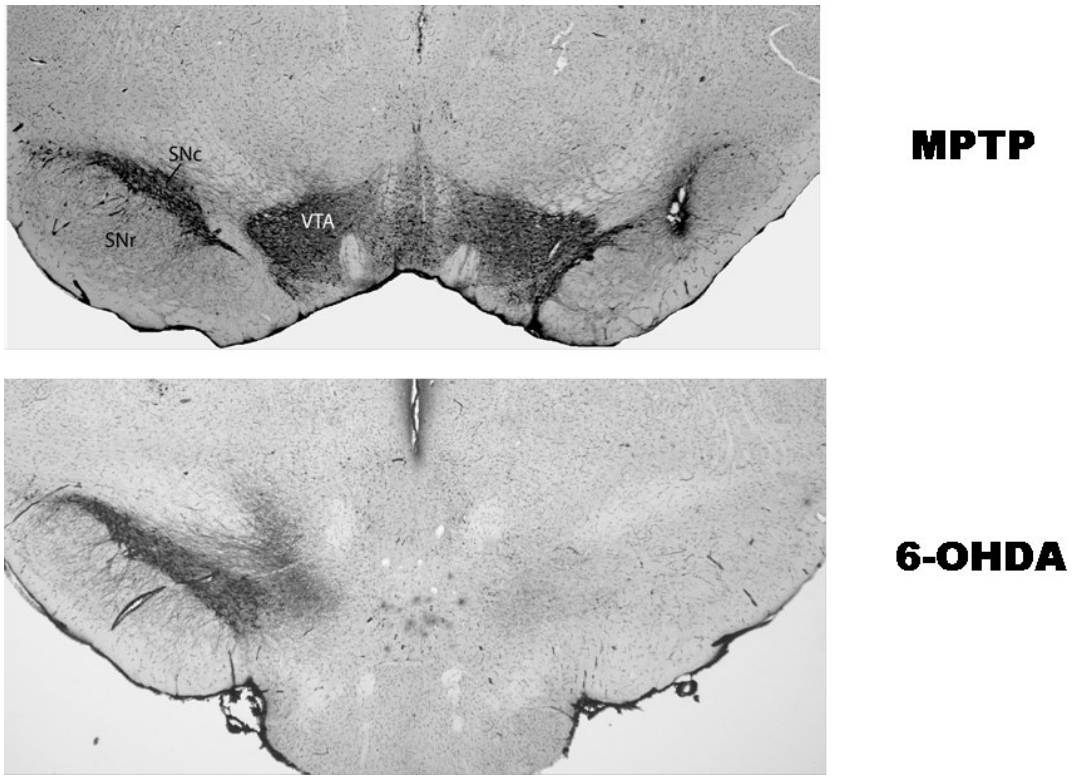

Fig. 4 Representative bright-field photomicrographs of tyrosine hydroxylase-immunostained sections illustrating the presence of unilateral 6-OHDA (upper panel) and MPTP (lower panel) dopaminergic cell lesions on the left side of the brain. MM = medial mammillary nucleus; SNpc = *substantia nigra pars compacta*; SNpr = *substantia nigra pars reticulata*; VTA = ventral tegmental area. Scale bars: 500 µm.

Table 1: Effect of the administration of MPTP into the SNpc (left, right, or bilateral), or of 6-OHDA into the left medial forebrain bundle of rats on the striatal levels of dopamine.

	Striatal dopamine (ng/g wet tissue)	
	Left	Right
Sham	5248.32 ± 333.32	5161.29 ± 369.06
MPTP, left	2558.74 ± 617.19 *#	4984.20 ± 1003.53
MPTP, right	5005.60 ± 1392.15	1698.26 ± 296.15 *#
MPTP, bilateral	995.74 ± 502.37 #	1016.26 ± 569.77 #
6-OHDA, left	127.47 ± 24.50 *#	6098.94 ± 414.38

Data are expressed as mean ± SEM. * $P < 0.05$ compared to the contralateral striatum; # $P < 0.05$ compared to the striatum of sham-operated rats (Newman-Keuls test after one-way ANOVA).

Discussion

The present results show that unilateral lesion of the rat SNpc with MPTP causes different effects when compared to 6-OHDA. In agreement with previous studies, the infusion of 6-OHDA into the rat MFB caused an almost complete loss of dopamine neurons in the midbrain and depletion of dopamine in the ipsilateral striatum (Schwarting and Huston, 1996a; Truong et al., 2006; Da Cunha et al., 2008; Gregorio et al., 2009). On the other hand, MPTP caused a partial loss of dopamine neurons and striatal dopamine, also in agreement with previous studies. The study of other behavioral and toxic effects of these two models was beyond the scope of the present investigation and a detailed description can be found elsewhere (Schwarting and Huston, 1996b; Ferro et al., 2005; Da Cunha et al., 2008).

The fact that 6-OHDA causes more dopamine neuron loss in rats than MPTP, has made it a much more popular rat model of PD (Kalaria et al., 1987; Schwarting and Huston, 1996a; Deumens et al., 2002; Ghorayeb et al., 2002). The lower neurotoxic potency of MPTP is possibly due to the fact that the rat brain capillaries contain exceptionally high levels of monoamine oxidase B, which represent an effective enzymatic blood-brain barrier (Kalaria et al., 1987; Riachi et al., 1988). Thus, systemic administration of MTPT to rats or the infusion of MPTP into the SNpc at the same dose usually used for 6-OHDA does not cause significant loss of dopaminergic cells. This low neurotoxic potency of MPTP has been the main reason for the preference of researchers to use 6-OHDA in rat studies. However, this feature can be

useful to model the early stage of PD. In addition, MPTP causes a lesion that more selectively affects dopaminergic neurons (Harik et al., 1987; Gevaerd et al., 2001a) and is more frequently located in the SNpc, sparing the ventral tegmental area (Ferro et al., 2005). Lesioning of the ventral tegmental area can be prevented in part by the infusion of 6-OHDA directly into the SNpc, but this protocol increases the variation in lesion size (Schwarting and Huston, 1996b; Deumens et al., 2002). In our experience, this protocol can result in animals with both small and large lesions which present ipsiversive and contraversive turning behavior, respectively, when challenged with apomorphine (see also Schwarting and Huston, 1996a). The same variation was not observed in rats in which 6-OHDA was infused into the MFB. All rats submitted to this protocol presented an almost total loss of midbrain dopaminergic neurons and contraversive turning behavior when challenged with apomorphine. Therefore, it seems more appropriate to use MPTP rats as a model of the early stage of PD and 6-OHDA (infused into the MFB) rats as a model of end stage of PD.

Recent studies from our laboratory suggest that rats treated with MPTP are a good model for the learning and memory impairments observed in the early stage of PD (Da Cunha et al., 2001; Gevaerd et al., 2001a; Gevaerd et al., 2001b; Da Cunha et al., 2002; Miyoshi et al., 2002; Da Cunha et al., 2003; Bellissimo et al., 2004; Braga et al., 2005; Perry et al., 2005; Kumar et al., 2008; Kumar et al., 2009). The most useful characteristic of these bilaterally lesioned MPTP rats for cognitive studies was the lack of gross motor alterations.

The present study showed that unilaterally MPTP rats also present motor alterations in response to challenges with some antiparkinsonian drugs, and that this effect is dose-dependent. Recent studies suggest that the contraversive turning behavior of 6-OHDA rats is more correlated with the dyskinetic than with the antiakynetic effect of the challenging drug (Konitsiotis and Tsironis, 2006; Lane et al., 2006). Regarding dopaminergic drugs, this makes sense since 6-OHDA causes an almost total loss of presynaptic dopamine terminals and postsynaptic overexpression of dopamine receptors (Ungerstedt, 1971; Thal et al., 1979; Da Cunha et al., 2008). Indeed, the contraversive turning behavior is related to a higher stimulation of these dopamine receptors by direct agonists, such as apomorphine, in the ipsilateral striatum of 6-OHDA rats (Thal et al., 1979). On the other hand, dopamine neurotransmission is hypofunctional, but not absent, in the ipsilateral striatum of MPTP rats (Da Cunha et al., 2001). Therefore, a direct dopamine agonist will have an additive effect to the dopamine released in both the ipsi- and contralateral striatum, resulting in a higher concentration of dopamine in the contralateral striatum and, consequently, in ipsiversive turning behavior (see Da Cunha et al., 2008). An amphetamine challenge causes the release of endogenous dopamine (Schwarting and Huston, 1996b) which will be higher in the contralateral striatum of both 6-OHDA and MPTP rats, with both types of animals thus presenting ipsiversive behavior. The higher potency of apomorphine, amphetamine, pramipexole, entacapone, and selegiline (the later two administered in addition to levodopa and benserazide) to induce turning behavior in 6-OHDA rats

compared to MPTP animals is probably due to the upregulation of dopamine receptors observed in 6-OHDA (Ungerste.U, 1971; Thal et al., 1979), but not in MPTP, rats (Perry et al., 2005).

Seen from this perspective, the ipsiversive turning behavior of MPTP rats would be modeling a phenomenon that occurs when early stage PD patients are treated with dopaminergic drugs. This hypothesis is consistent with our finding that only the drugs that are more effectively used to treat these patients, causing lower dyskinetic effects were effective to cause ipsiversive turns in MPTP rats. The early phase of PD is an important period in patients life, when their pharmacological treatment is initiated. Nowadays, some drugs used in the end stage of PD, such as levodopa, are avoided in this early stage (Lees, 2005) because they can induce dyskinesias and aggravate the time course of the disease (Bonuccelli et al., 2002; Nagatsu and Sawada, 2009).

Note that, while the 6-OHDA rats presented contraversive turns in response to levodopa, a feature also shown in other studies (Schwarting and Huston, 1996a), MPTP rats did not respond to levodopa and biperidene. On the other hand, they responded to pramipexole with ipsiversive turns. Direct dopamine agonists, like pramipexole and ropinirole, can be used instead of levodopa to treat early phase PD patients. Unlike levodopa, they do not require metabolic conversion, do not compete with dietary amino acids, and can reduce the risk of dyskinesias if used as monotherapy (Pahwa et al., 2006). The 6-OHDA rats also responded to pramipexole, but with contraversive turns. The MPTP rats also presented ipsiversive turns in

response to a challenge with entacapone, a COMT inhibitor, another drug used in the early phase of PD. It can reduce fluctuations in the activation of dopamine receptors induced by levodopa (Gallagher and Schrag, 2008; Nagatsu and Sawada, 2009). On the other hand, 6-OHDA rats responded to entacapone with contraversive turns, as shown in this and in previous studies (Tornwall and Mannisto, 1993; Gerlach et al., 2004).

Great efforts have been made in the search for neuroprotective drugs to be used in the early stage of PD (Wu and Frucht, 2005; Gallagher and Schrag, 2008). Selegiline, and more recently rasagiline, has been proposed as monotherapy in early PD, as well as adjuvant therapy in levodopa-treated patients with mild motor complications (Linazasoro, 2008). The MPTP rats also responded to a selegiline challenge with ipsiversive turns. 6-OHDA rats responded with contraversive turns as reported in this and in a previous study (Prat et al., 2000).

Amantadine is a drug that has been used to reduce dyskinesias induced by levodopa (Metman et al., 1998; Wolf et al., 2008). It is interesting that MPTP and 6-OHDA rats responded to a challenge to this drug with turns in the opposite direction of that observed in response to other antiparkinsonian drugs that also induce dyskinesias: the MPTP rats presented contraversive turns and the 6-OHDA rats presented ipsiversive turns. Ipsiversive turning behavior in 6-OHDA rats and mice in response to amantadine was also reported previously (Vonvoigt and Moore, 1973; Hesselink et al., 1999) and may have been caused by the release of dopamine in the striatum (Arai et al., 2003). However, this effect cannot

explain the contraversive turning behavior of the MPTP rats, that may be predictive of an antidyskinetic effect of the drug. This may be the reason why the MPTP rats did not respond to levodopa, which is in the drug that, at the same time, is more effective to produce antiakinetic and dyskinetic effects (Nutt, 1990).

In a study by Dekundy et al. (2007), they reported that many antidyskinetic drugs did not increase (e.g. amantadine, buspirone, riluzole, fluoxetine, propranolol) or even decreased (e.g. yohimbine, clozapine, clonidine) the locomotion with contralateral side bias of 6-OHDA rats. According to their view, the turning behavior of 6-OHDA rats in response to a challenging drug cannot discriminate between dyskinetic or antiakinetic effects and they claim for more selective animal models. The turning behavior of the MPTP rats can be the response to this claim, consisting in a simple and promising model for the screening of antiakinetic drugs useful to treat the early phase of PD and also for the study of the neural alterations related to this phase.

Acknowledgements

This research was supported in part by CNPq, CAPES, FAPESP, Institutos do Milênio, and Fundação Araucária. The skillful assistance of Maria Ines Bellissimo with some of the HPLC assays and the valuable comments of Dr. Susan Wonnacott are acknowledged.

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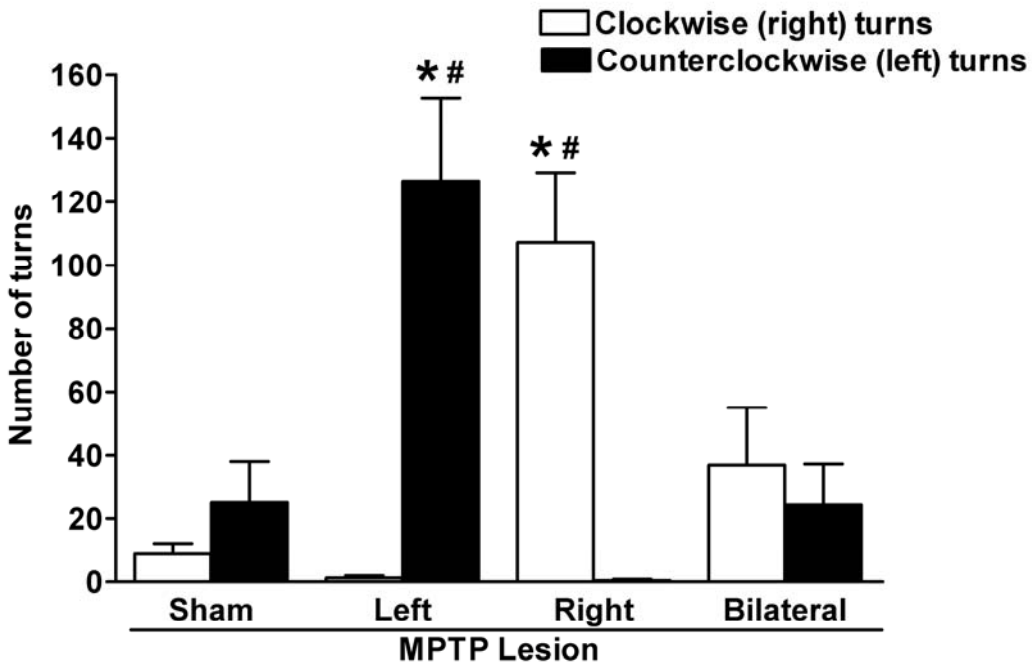
SUPPLEMENTAL DATA:



Supplementary Fig. 1. Turning behavior of unilaterally MPTP-lesioned rats challenged with 1 mg/kg apomorphine. Data are reported as the number of clockwise and counterclockwise turns counted over the first 30 min after the drug challenge. Number of animals per group: sham, n = 12; right, n = 10; left, n = 10; bilateral, n = 8 bilateral. * $P < 0.05$ compared to sham rats; # $P < 0.05$ compared to rats lesioned on the contralateral side; Newman-Keuls after one-way ANOVA.

Supplementary video 1. The movie shows two rats with a lesion in the left SNc induced by MPTP (left rat) or 6-OHDA (right rat). Both were challenged with apomorphine. Note that the MPTP rat presents ipsiversive- and the 6-OHDA rat contraversive-turning behavior.

6 CONCLUSÕES

- O envolvimento da via nigroestriatal e da via direta nos no aprendizado da esquiva ativa e no comportamento rotatório de ratos pode ser explicado pelo modelo mosaico dos espelhos quebrados.
- Os resultados desta tese mostram que a ativação da via direta, tanto no NAc como no estriado dorsolateral, é crítica para o aprendizado da esquiva ativa de duas vias. Segundo o modelo do mosaico dos espelhos quebrados a ação da dopamina nos receptores D1 da via direta promove um fortalecimento das sinapses que associam os neurônios corticais que representam o CS (luz) e daqueles que desencadeiam a resposta de cruzar para o outro lado da caixa e que projetam para os mesmos neurônios estriatais. Em situações normais de aprendizagem estes receptores são ativados pela liberação fásica de dopamina deflagrada pela novidade da conseqüência (evitar o choque). Por isso o bloqueio destes receptores pelos antagonistas D1 prejudicou o aprendizado. Ainda segundo o modelo do mosaico dos espelhos quebrados, após o aprendizado, a escolha da resposta de cruzamento depende da ativiação da via direta pela dopamina liberada de forma crônica. Esta seria a razão pela qual o antagonista D1 aumentou o número de não-respostas.
- Os recepetores dopaminérgicos D1 presentes no NAc e no estriado dorsolateral medeiam um aprendizado associativo rápido e lento, respectivamente.
- O comportamento rotatório ipsiversivo de ratos com lesão por MPTP pode modelar o que ocorre nos estágios iniciais da DP em pacientes tratados com drogas dopaminérgicas. No modelo de lesão unilateral por MPTP, os agonistas dopaminérgicos direto e indireto (apomorfina e anfetamina, respectivamente), além das drogas de uso clínico induzem comportamento rotatório dose-dependente ipsiversivo à lesão. De acordo como o modelo dos mosaicos dos espelhos

quebrados, os animais MPTP perdem a capacidade de escolher virar para o lado não-lesado quando estimulados por agonistas dopaminérgicos.

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